Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700007

Development of robust, scalable and synthetic systems for the maintenance of pluripotency and subsequent differentiation

T. Gaskell[1], S. McRae[1], M. Jones[1]

Abstract

Overview:

In driving hES cell technology towards widespread applications considerable effort has been focused on the improvement of culture conditions and on enabling efficient differentiation. We have established two technologies which will better enable researchers to achieve these aims.

Technology:

The use of feeder based protocols for the creation, expansion and banking of hES cell lines are well established. The advances in technology for feeder free culture have predominantly relied on the cultivation of pluripotent cells in colony based systems, commonly used in conjunction with ill-defined matrices. We have developed a process to allow enzymatic single cell passaging in a medium in which Wnt3a and bFGF are interchangeable by a method utilising small molecule control of beta-catenin interaction with its binding partners in concert with additional growth factor supplementation. This medium can be partnered with chemically defined or synthetic matrix components to provide exemplary consistency of phenotype within and between passages. Following extended passage in the Pluripro® P300 system cells retain the undifferentiated phenotype, as evidenced by expression of markers of the undifferentiated state, and capacity for in-vitro differentiation into the three germ lineages. In addition we have formulated a range of growth factors (including FGF2) with enhanced efficacy at a level of multiple orders of magnitude, when comparing equivalent molar concentrations, through multivalent conjugation to carrier substrates (STAR technology).

Conclusion:

The development of improved culture systems for pluripotent cells and the increased efficacy of growth factors in inducing and establishing differentiated progeny are essential requirements if stem cell technology is to fulfil its potential and overcome technical and economic barriers. Taken together, these two advancements have the potential to transform stem cell research and the development of cellular therapeutics.

1 Cell Guidance Systems, Cambridge, United Kingdom

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700008

Rat embryonic fibroblasts improve reprogramming of human keratinocytes into induced pluripotent stem cells

A. Kleger[1,2], L. Linta[3], M. Stockmann[3], K.N. Kleinhans[2], A. Boeckers[3], A. Storch[4], H. Zaehres[5], Q. Lin[6], G. Barbi[5], T. Boeckers[3], S. Liebau[3]

Abstract

Patient specific human induced pluripotent stem (hiPS) cells not only provide a promising tool for cellular disease models in general, but also open up the opportunity to establish cell-type specific systems for personalized medicine. One of the crucial prerequisites for these strategies, however, is a fast and efficient reprogramming strategy from easy accessible somatic cell populations. Keratinocytes from plucked human hair had been introduced as a superior cell source for reprogramming purposes compared to the widely used skin fibroblasts. The starting cell population is however limited and thereby further optimization in terms of time, efficiency and quality is inevitable. Here we show that rat embryonic fibroblasts (REFs) should replace mouse embryonic fibroblasts as feeder cells in the reprogramming process. REFs enable a significantly more efficient reprogramming procedure as shown by colony number and total amount of SSEA4-positive cells. We successfully produced k-hiPS cells from various donors. The arising k-hiPS cells display the hallmarks of pluripotency such as expression of stem cell markers and differentiation into all three germ layers. The increased reprogramming efficiency using REFs as a feeder layer occurred independent of the proliferation rate in the parental keratinocytes and acts, at least in part, in a non-cell autonomous way by secreting factors known to facilitate pluripotency such as Tgfb1 Inhba and Grem1. Hence, we provide an easy to use and highly efficient reprogramming system which could be very useful for a broad application to generate human iPS cells.

[1]University Ulm, Department of Internal Medicine 1, Ulm, Germany, [2]Institute of Molecular Medicine and Max-Planck-Research Department on Stem Cell Aging, Ulm, Germany, [3]University Ulm, Institute for Anatomy & Cell Biology, Ulm, Germany, [4]Dresden University of Technology German Center for Neurodegenerative Diseases, Research Site Dresden, Dresden, Germany, [5]Max Planck Institute for Molecular Biomedicine, Cell and Developmental Biology, Münster, Germany, [6]University Medical School, Institute for Biomedical Engineering, Department of Cell Biology, Aachen, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research; Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700009

Advances in reprogramming efficiency and culture of iPS cells

M. Lu1, V. Chu1, C. Moore1

Abstract

The generation of induced pluripotent cells (iPS) from adult somatic cells provides tremendous advantages for the study of disease specific models and in the future may become a source of cells for regenerative therapies. Many methods of developing iPSs are currently in use and each possesses inherent difficulties resulting in low frequencies of fully reprogrammed cells. We will present a brief review of epigenetic processes involved in reprogramming followed by a description of easy and efficient lentiviral methods which incorporate a single polycystronic vector and use a Cre-mediated excision process to eliminate the exogenous transgenes. We will further discuss culture protocols we have developed that optimize generation and characterization of iPS cells.

1EMD Millipore, Temecula, United States

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700010

Human tendons harbour a population of non tumorigenic, perivascular embryonic stem cell-like cells

H. Tempfer1, C. Lehner2, R. Gehwolf1, A. Wagner3, F. Rivera4, L. Aigner4, H. Resch2, H.-C. Bauer1,3

Abstract

Tendon-derived stem cells are commonly considered to be of mesenchymal origin, having the capacity to differentiate into adipocytes, chondrocytes, osteoblasts and tendon cells. Earlier on we have shown that human tendon perivascular cells express markers associated with neural stem cells such as Nestin and Musashi1.

Here we describe a so far unrecognized type of human tendon perivascular stem cells (hTPSC) expressing markers commonly associated with embryonic stem cells (ESC). Tissue samples from intact human biceps-, supraspinatus and semitendinosus tendons were obtained with patients´ informed consents, tissue donors were aged from 18-73 (n=10) years. By immunohistochemistry and single cell PCR we demonstrate that hTPSCs express Oct4, Nanog, Klf4, Cmyc and Sox2 in vivo. In cell culture, these cells give rise to clonal spheroid cell aggregates harbouring cells expressing the stem cell markers mentioned and markers associated with all three embryonic germ layers, such as Insulin and Glucagon (endoderm), Collagens type 1 and 3 (mesoderm) and GFAP and Galactosyl ceramidase (ectoderm). In differentiation experiments, hTPSC can give rise to adipocytes, osteoblasts, oligodendrocytes, astrocytes and insulin producing cells.osteoblasts, oligodendrocytes, astrocytes and insulin producing cells.experiments, hTPSC can give rise to adipocytes, osteoblasts, oligodendrocytes, astrocytes and insulin producing cells.Collagens type 1 and 3 (mesoderm) and GFAP and Galactosyl ceramidase (ectoderm). In differentiation experiments, hTPSC can give rise to adipocytes, osteoblasts, oligodendrocytes, astrocytes and insulin producing cells.Despite their ESC-like marker expression and their mulitpotency, these cells do not form tumors upon injection into immunodeficient mice.

Interestingly, these cells seem to persist up to an old age. They could even be detected in tendon tissue from a 73 year old donor.

These findings suggest that TPSC represent a more undifferentiated cell type than mesenchymal stem cells.

An important issue that will have to be addressed in the future is to learn more about the nature of the niche that keeps these cells undifferentiated throughout life. Also the role of these cells in tendon degeneration and healing needs to be examined.

Moreover, hTPSC may be a valuable source for future applications in tissue engineering and cell therapy.

1Paracelsus Medical University, Applied Cell Biology Unit, Salzburg, Austria, 2University Hospital Salzburg, Department of Trauma Surgery and Sports Medicine, Salzburg, Austria, 3University of Salzburg, Department of Organismic Biology, Salzburg, Austria, 4Paracelsus Medical University, Institute for Molecular Regenerative Medicine, Salzburg, Austria

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research; Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700011

Targeting the minor subset of melanoma stem cells eradicates established melanoma lesions

P. Schmidt[1], C. Kopecky[1], A.A. Hombach[1], P. Zingrino[1], C. Mauch[1], H. Abken[1]

Abstract

Current paradigms in cancer therapy attempt to eliminate all malignant cells of a tumor lesion. The cancer stem cell (CSC) paradigm, however, predicts that tumors are initiated by a minor subset of cancer initiating cells and are maintained by a few, so far less identified cancer maintaining cells. In this contribution, however, we demonstrate that specific elimination of a less than a 2% subset of melanoma maintaining cells eradicates established melanoma lesions without targeting the tumor cell mass. The tumor stem cell subset is selectively eliminated from tumor lesions by adoptive transfer of cytotoxic T cells redirected by an engineered chimeric antigen receptor. Targeted elimination of the minority of tumor cells which co-express HMW-MAA (MCSP) and CD20 eradicated established melanoma lesions in long-term despite the bulk of tumor cells. Targeting of any random cancer cell subset was not effective. HMW-MAA+ CD20+ melanoma subset cells were found in about 4 out of 5 melanoma biopsies of different histology and clinical grade. Our data provide first evidence that progressing melanoma is maintained by a minority of cells, the targeted elimination of which results in tumor eradication

1University of Cologne, Center for Molecular Medicine Cologne, Köln,

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700012

Progenitor cell dynamics of sebaceous gland development in mammalian skin

D. Frances[1], M. Petersson[1], C. Niemann[1]

Abstract

Mammalian epidermis consists of the interfollicular epidermis (IFE) with associated hair follicles (HF) and sebaceous glands (SG) (Blanpain and Fuchs, 2009).
Functional SGs are important for barrier acquisition and protection against pathogens. Upon maturation, cells of the SG (sebocytes) disintegrate and release sebum (Niemann, 2009). This requires constant replenishment of differentiated cells and implies stem or progenitor cells to be involved in SG-renewal.
Previously, our lab has demonstrated that individual bulge stem cells and their descendants constantly regenerate the SG under homeostatic conditions in adult skin (Petersson et al, 2011).
In contrast, little is known about the cellular origins and molecular mechanisms crucial for the development of this organ and the entire pilosebaceous unit.We have investigated the cellular processes during SG formation in more detail. Analysis of the spatio-temporal organisation of stem and progenitor compartments was performed during morphogenesis of the pilosebaceous unit. Our results suggest a dynamic expression of distinct HF stem cell markers during the formation of the HF and the SG.For instance, progenitor markers Lrig1 and Sox9 are first coexpressed by epidermal progenitor cells and become confined to distinct compartments as soon as the SG starts to form. We provide evidence that proliferation of Lrig1 positive progenitor cells drives SG development at the upper part of the HF. In contrast, the MTS24/Plet1 progenitor cell pool is not likely to contribute to the initial stages of SG formation. Our data clearly demonstrate that different stem and progenitor compartments within the HF are established at different time-points during development.
Furthermore, by studying the process of SG morphogenesis in more detail, we found that in HFs of mouse tail skin, two prominent SG arise from one single cluster of precursor cells.
Finally, to identify the cellular origin of the SG, we have performed lineage tracing experiments during epidermal morphogenesis. First results indicate that SGs originate from IFE.
Further analysis will help to unravel the cellular and molecular signals governing establishment of distinct stem cell compartments and SG formation.

1CMMC, University of Cologne, Köln, Germany Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700013

Notch1 regulates chondrogenic and neuroectodermal differentiation via the novel target gene Sox9

R. Schwanbeck[1], S. Martini[1], R. Haller[2,3], K. Bernoth[1], J. Rohwedel[2], U. Just[1]

Abstract

Objectives: Notch signaling is a crucial cell-cell communication pathway affecting cell-lineage decisions, proliferation, apoptosis, self-renewal and differentiation processes. In the canonical pathway, Notch is thought to mediate its function via the transcription factor RBP-J mainly by increasing the expression of the target proteins of the Hes/Hey family. Recently, we described novel cell context dependent Notch1 target genes that comprise a high percentage of transcription factors. Among these we found Sox9 to be regulated under various differentiation conditions.

Methods/Results: Here, we describe that during mesodermal differentiation as embryoid bodies this Notch1 induced upregulation of Sox9 had its maximum at day 4 and diminished at day 10. Furthermore, by inhibition of protein synthesis as well as luciferase experiments with RBP-J binding site mutants of Sox9 promoter reporter constructs, Sox9 was shown to be a direct target gene. To further investigate the role of Sox9 for Notch1 mediated effects, we employed a siRNA strategy to specifically quench the Sox9 peak induced by Notch1 induction without affecting induction of other Notch1 target genes. For chondrogenic differentiation we found that a temporary activation of Notch1 during the early stages of EB formation resulted in a strong increase in chondrogenic differentiation during later stages. This increase in cartilage development could be entirely reversed by the application of Sox9 siRNA, whereas the known blockage of cardiac differentiation by Notch1 was unaffected by the reduced Sox9 levels. In another set of experiments we used the same Sox9 siRNA strategy to investigate the role of Sox9 in Notch-mediated cell lineage decisions during neuroectodermal development. Notch is known to play a role in the decision between glial and neuronal cells as well as for the induction of neural crest differentiation, though the molecular basis if these effects are not understood. Our experiments revealed that in neuroectodermal differentiation conditions Sox9 is also a direct target gene of Notch1. Notch1 induction led to a strong increase in glial cell formation while inhibiting the generation of neurons. Quenching of the Sox9 peak induced by Notch1 signaling using Sox9 siRNA led to a significant decrease of glial cells, demonstrating the pivotal role of Sox9 in mediating Notch1 signals in this context.
Conclusion: In summary, our data indicate that the effects of Notch signaling are not only mediated through the well-described Hes/Hey family. The novel direct Notch1 target gene Sox9 described here plays an essential role, both, in chondrocytic development as well as in neuroectodermal differentiation, thus emphasizing the importance of alternative mechanisms in Notch signal transduction.

1University of Kiel, Institute of Biochemistry, Kiel, Germany, 2University of Lübeck, Dept. of Virology and Cell Biology, Lübeck, Germany, 3Medizinische Hochschule Hannover, Leibniz Forschungslaboratorien für Biotechnologie und künstliche Organe, Hannover, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700014

Positive impact of sitagliptin on cardiac function and survival after acute myocardial infarction

L. Krieg[1], H.D. Theiss[1], G. Assmann[2], J. Mueller-Hoecker[2], W.-M. Franz[1]

Abstract

Background:

This study analyzes the effects of sitagliptin +/- G-CSF treatment on survival and myocardial regeneration after myocardial infarction in a mouse model. The SDF-1-CXCR4 axis is a key mechanism of cardiac homing of stem cells. G-CSF is known to mobilize bone-marrow-derived stem cells into peripheral blood, whereas sitagliptin is a DPP-IV (dipeptidylpeptidase IV)-inhibitor- Sitagliptin therefore prevents the essential stem cell homing factor SDF-1 from being cleaved.

Methods:

Acute myocardial infarction was induced by surgical occlusion of the left descending artery in 10-11 weeks old male C57BL/6 mice. Sitagliptin was administered per os in a titrated dose regimen with blood levels measured by LC-M/M. DPP-IV activity was analyzed by enzyme activity assays. Saline and G-CSF were injected intraperitoneally for 6 days following myocardial infarction. The effects of the dual therapy as well as the effects of sole sitagliptin treatment on cardiac stem cell mobilization and homing was measured by flow cytometry. The impact on neovascularization and cell proliferation was analyzed by immunohistochemistry and the treatment benefits on infarct size was assessed by histology. The treatment impact on cardiac function and survival was analyzed by millar tip catheterization and the Kaplan-Maier-method.

Results:

Enzyme activity assays revealed a significant decrease in DPP-IV enzyme activity after sitagliptin application.

Results:

Sitagliptin+G-CSF as well as sole sitagliptin therapy Enzyme activity assays revealed a significant decrease in DPP-IV enzyme activity after sitagliptin application. Sitagliptin+G-CSF as well as sole sitagliptin therapy enhanced both mobilisation and cardiac homing of BMCs. Cell proliferation (Ki67+) and neovascularization were increased in both treatment groups, resident cardiac stem cells were stimulated and cardiac remodelling was significantly decreased. Dual therapy consisting of sitagliptin and G-CSF as well as sole sitagliptin application significantly reduced infarct size, had a positive impact on myocardial function and improved survival compared to sole G-CSF or saline application. The beneficial effects seen were most remarkable for the dual therapy group, but also significant for sole sitagliptin administration. Additional application of the CXCR4-antagonist AMD3100 reversed the beneficial treatment effects of both treatment regimens back to baseline. This suggests specificity of the treatment effects to the CXCR4-axis.

Conclusions:

This is the first study showing that combined application of G-CSF and Sitagliptin and yet sole sitagliptin administration increases cardiac homing of stem cells, induces neovascularization, reduces cardiac remodelling, enhances cell proliferation, has a positive impact on cardiac function and improves survival after acute myocardial infarction. Combined administration of sitagliptin and G-CSF and even sole sitagliptin application has beneficial effects on cardiac regeneration beyond its known anti-diabetic potential and may be a new therapeutic regimen after myocardial infarction.

1Ludwig-Maximilians-University, Medical Department I, Muenchen, Germany, 2Ludwig-Maximilians-University, Institute of Pathology, Muenchen, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700015

MMP-8 impairs adhesion of hematopoietic stem cells in the endosteal niche

C. Steinl[1], M. Essl[1], T.D. Schreiber[2], L. Prokop[3], S. Stevanovic[3], W.K. Aicher[4], G. Klein[1]

Abstract

The endosteal hematopoietic stem cell (HSC) niche consists of cellular components including osteoblasts, osteoclasts and mesenchymal stromal cells as well as extracellular matrix components (ECM) and chemokines. HSCs reside in this niche in close contact to their surroundings. In the process of stem cell mobilization, a proteolytic microenvironment contributes to the release of hematopoietic stem and progenitor cells (HSPCs) from their niches, the precise molecular mechanism, however, is still not known. In the present study we characterized the expression and secretion of a member of the matrix metalloproteinase (MMP) family, the collagenase MMP-8, in the context of the endosteal stem cell niche identifying a new component of the proteolytical environment during mobilization.

We analyzed blood serum samples of HSPC donors treated with G-CSF for several days. The elevated number of cycling CD34+ HSPCs correlated very strongly with an increased serum concentration of MMP-8. Circulating CD34+ HSPCs can also be found physiologically in umbilical cord blood. An ELISA analysis of umbilical cord blood serum showed also elevated MMP-8 serum concentration. Next, we investigated the influence of MMP-8 on the adhesive interaction of HSPCs with osteoblasts. After binding of fluorescently labeled CD34+ HSPCs to primary osteoblasts, activated MMP-8 was added. The assay revealed a strong reduction of HSPC attachment to osteoblasts after incubation with activated MMP-8 compared to non-activated MMP-8. We hypothesized that this reduction in cell attachment was due to the degradation of cell adhesion and/or extracellular matrix (ECM) molecules. A strongly adhesive ECM component synthesized by human osteoblasts is laminin 511 (LM-511). MMP-8 is able to proteolytically digest recombinant LM-511 as shown by silver staining after SDS-PAGE, but this degradation had no impact on cell adhesion. A detailed analysis of the cleavage products using peptide mass fingerprint analysis showed that the important integrin-binding sites are still intact. CXCL12α, a chemokine with an important role in HSPC migration, was also tested as a potential substrate for MMP-8. MALDI-TOF analysis revealed that MMP-8 can process the N-terminal end of CXCL12α. In cell migration assays using non-degraded and MMP-8 processed CXCL12α as a stimulus, a strong inhibitory effect on HSPC migration through the degradation by MMP-8 was exhibited.

In summary, we provide strong evidence that the matrix metalloproteinase MMP-8, which can be released from granulocytes, can drastically reduce adhesion of HSPCs to osteoblasts. Furthermore, we suggest that the proteolytic degradation of cell-cell interactions and the important chemokine CXCL12α by MMP-8 can support mobilization of HSPC out of their niche.

1 University of Tübingen, Center for Medical Research, Tübingen, Germany, 2 University of Tübingen, Naturwissenschaftliches und Medizinisches Institut, Reutlingen, Germany, 3 University of Tübingen, Department of Immunology, Tübingen, Germany, 4 University of Tübingen, Center for Regenerative Medicine, Tübingen, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research: Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700016

A novel cardioprotective mechanism mediated by bone marrow c-kit+mERα+ cell via paracrine IL-6

S. Svetlana[1], A. Skorska[2], A.-X. Wassim[2], S. von Haehling[3], J. Dong[4], C. Curato[1], S. Gustav[2], T. Unger[1], J. Li[1,2]

Abstract

Cardioprotective actions of estrogen are well recognized for many years. Recent studies indicate a novel role of estrogen receptors (ER) in stem/precursor cell-involved cardiac repair. Taking into account that cardiac c-kit+ precursor cells are mainly recruited from bone marrow (BM) c-kit+ cell populations, we aimed here to elucidate the functional importance of ERα in BM c-kit+ precursor cells after ischemic heart injury. The c-kit+ cells were isolated from femurs and tibias of male wistar rats 7 days after myocardial infarction (MI) by magnetic activated cell sorting in combination with fluorescent activated cell sorting (FACS). After MI, the percentage of BM c-kit+ cells increased by 2.11 fold. BM c-kit+ cells, which expressed both intracellular and membrane (m)ERα, were shown to inhibit apoptosis of co-cultured cardiomyocytes in a paracrine manner. In addition, ERα stimulation could improve paracrine cardioprotection by BM c-kit+ cells. According to the expression of mERα, BM c-kit+ cells were further sorted using FACS into c-kit+mERα+ and c-kit+mERα- cell populations. Notably, BM c-kit+mERα+ cells were more potent in supporting paracrine cardioprotection than c-kit+mERα- cells both in vitro and in vivo. Futher analysis revealed that BM c-kit+mERα+ cells were characterized by increased production of cardioprotection cytokines including IL-6 and IL-10. Importantly, blocking IL-6, but not IL-10, by neutralizing antibody abolished the protective effect of BM c-kit+mERα+ cell in supporting cardiomyocytes, indicating IL-6 was responsible for BM c-kit+mERα+ cell-mediated paracrine cardioprotection. Finally, the c-kit+mERα+ cell population was verified in peripheral blood of patients with heart failure. Thus, this work explains a novel cardioprotective mechanism mediated by BM c-kit+mERα+ cells via paracrine IL-6.

1Center for Cardiovascular Research and Institute of Pharmacology, Charité - Universitätsmedizin Berlin, Berlin, Germany, 2Reference and Translation Center for Cardiac Stem Cell Therapy (RTC), University of Rostock, Rostock, Germany, 3Department of Cardiology, Charité - Universitätmedizin Berlin, Berlin, Germany, 4German Rheumatism Research Centre, Berlin, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research: Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700017

Desmin promotes Nkx2.5 expression during early cardiomyogenesis via temporal restricted interaction with the minimal cardiac specific enhancer of the Nkx2.5 gene

S. Gawlas[1], C. Fuchs[1], P. Heher[1], G. Weitzer[1]

Abstract

Desmin, a type three intermediate filament protein is expressed in all types of muscle cells and contributes to homeostasis in the adult heart. Knock out of the desmin gene lacks a clear embryonic phenotype; however, upregulation of desmin expression resulted in an increased number of developing cardiomyocytes and a temporal restricted upregulation of the cardiogenic transcription factor Nkx2.5 in cardiac progenitor cells during a small window in time. Thus we hypothesize that desmin may directly promote cardiogenic commitment and myocardial differentiation. To test whether desmin influences transcriptional regulation of the Nkx2.5 gene during cardiomyogenesis we choose embryoid bodies as a model system to study the short-lived function of desmin in nascent cardiogenic cells. Desmin indeed interacts with regulatory DNA elements of the Nkx2.5 gene, is present in nuclei of cardiac progenitor cells, activates the Nkx2.5 gene via the cardiac specific enhancer element in fibroblasts, myoblasts, and cardiomyocytes, and rescues Nkx2.5 related haplo-insufficiency during cardiomyogenesis. These results attribute a new dimension to the role of desmin in commitment and differentiation of progenitor cells to cardiomyocytes, by demonstration of its participation in the transcriptional regulation of the Nkx2.5 gene.

1Medizinische Universität Wien, Max f. Perutz Laboratorien, Wien, Austria

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700018

In vitro modeling of ryanodine receptor 2 dysfunction using induced pluripotent stem cells

A. Fatima[1], G. Xu[1], S. Kaifeng[1], S. Papadopoulos[2], M. Lehmann[1], J.-J. Arnaiz Cot[3], A. O. Rosa[3], F. Nguemo[1], S. Dittmann[4], M. Matzkies[1], S.L. Stone[3], M. Linke[5], U. Zechner[5], V. Beyer[5], H.-C. Hennies[6,7,8], S. Rosenkranz[7,9], B. Klauke[4], M. Farr[4], A.S. Parwani[10], W. Haverkamp[10], L. Cleemann[3], M. Morad[3], H. Milting[4], J. Hescheler[1], T. Šarić[1]

Abstract

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited cardiac disorder characterized by emotional and physical stress-induced ventricular tachyarrhythmia, syncope and sudden cardiac death in children and young adults. In many cases, CPVT has been linked to mutations in the cardiac ryanodine receptor type 2 gene (RYR2) encoding a Ca2+ channel in the membrane of the sarcoplasmic reticulum (SR) leading to excessive Ca2+ release from the SR during diastole upon catecholaminergic stimulation. Here we report the generation of induced pluripotent stem cell (iPSC) lines from a CPVT patient carrying the novel heterozygous autosomal dominant mutation p.F2483I in the RYR2 gene. Single cardiomyocytes derived from CPVT iPS cells revealed arrhythmias and delayed after depolarizations (DADs) after isoproterenol stimulation. Arrhythmic response to adrenergic stimulation was also observed in multicellular beating clusters. Patient iPS derived cardiomyocytes exhibit higher amplitudes and longer durations of spontaneous Ca2+ release events at basal state when compared to control cardiomyocytes. In addition, the Ca2+-induced Ca2+ release events continued after repolarization and were abolished by increasing the cytosolic cAMP levels with forskolin. This study demonstrates the suitability of iPSCs in modeling RYR2-related inherited cardiac disorders in vitro and opens new opportunities for drug development, to optimize patient treatment, and pinpoint the role of different RYR2 mutations in disease pathogenesis.

1University of Cologne, Institute for Neurophyisology, Cologne, Germany, 2University of Cologne, Institute for Vegetative Physiology, Cologne, Germany, 3Medical University of South Carolina, Cardiac Signaling Center, Charleston, United States, 4Heart and Diabetes Center NRW,University Hospital of the Ruhr-University of Bochum, Erich and Hanna Klessmann-Institute for Cardiovascular Research and Development, Bochum, Germany, 5Johannes Gutenberg University, Mainz, Institute for Human Genetics, Mainz, Germany, 6University of Cologne, Cologne Center for Genomics, Cologne, Germany, 7University of Cologne, Center for Molecular Medicine Cologne, Cologne, Germany, 8University of Cologne, Cluster of Excellence on Cellular Stress Responses in Aging-associated Diseases, Cologne, Germany, 9University of Cologne, Clinic III for Internal Medicine, Heart Center, Cologne, Germany, 10Campus Virchow Klinikum, Charité-Universitaetsmedizin, Medical Department, Division of Cardiology, Berlin, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research; Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700019

Intra-myocardial homing of adult stem cells in a goat model: Glandular vs. mesenchymal stem cells

N.W. Guldner[1], T. Hardel[1], P. Rumpf[1], R. Noel[2], H.-H. Sievers[1], C. Kruse[3]

Abstract

Objectives:

Stem cell therapy is a promising approach treating end-stage heart failure. Mesenchymal stem-cells (MSCs) injected intra- myocardially differenciate into capillaries while glandular stem cells, derived from pancreas, parotis or submandibularis, transform into cardio- myocytes. Major questions of applying stem cell therapy in a failing myocardium are the intra-myocardial homing and the development of gap junctions. The following study will deal with these 2 questions.

Methods:

Glandular stem cells were characterized by red PKH26 and MSCs by green PKH67 makers. A mix of one million of each cell type was injected into three locations of the goat's myocardium of the left ventricle. Intra-myocardial homing of glandular stem cells and MSCs (CD133+) were evaluated in 6 female goats after 1 and 3 hours after intra-myocardial injection. Additionally from 6 female goats myocardium was harvested with injected stem cells after 6 weeks. Furthermore glandular stem cells of goats were co-cultured with goat´s myocardium for 48 h and kept in culture for 3 weeks. An immune-histological staining of connexin 43 (gap junctions) was performed on these cultured glandular stem cells.

Results:

Having used a mix of intra-myocardial injection of GSCs and MSCs, MSCs showed a significant cell migration into the surrounding myocardium, more expressed after 3 hours than after one hour. After 6 weeks, within the frozen myocardial slices 76,4% of the marked stem cells were identified as GSCs (red) but only 23,6% as green MSCs (P≤0,05). Additionally in cell cultures glandular stem-cells being in contact with myocardium developed connexin 43 mainly in that part of the cell membrane being in cell to cell contact. These primarily results of course need more research work concerning connexin 43 expression after an intramyocardial injection in a big animal model.

Conclusion:

Due to a significant better intra-myocardial residence of GSCs in comparison to MSCs combined with the ability expressing connexin 43 (gap junctions), glandular stem cells might become a very promising treatment option for the repair of irreversible damaged myocardium.

1Universitätsklinikum Schleswig-Holstein-Campus Lübeck, Klinik für Herz- und thorakale Gefäßchirurgie, Lübeck, Germany, 2Universitätsklinikum Schleswig-Holstein-Campus Lübeck, Department of Animal Care, Lübeck, Germany, 3Fraunhofer IBMT, Arbeitsgruppe Zelldifferenzierung und Zelltechnologie, Lübeck, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700020

Cardiovascular stem cell biology and development

R. David[1]

Abstract

The proliferative potential of pluripotent stem cell derived cardiomyocytes is limited and reasonable yields for novel therapeutic options have yet to be achieved. In a first attempt of such “cardiovascular forward programming” using pluripotent stem cells, we have previously shown that MesP1 represents a master regulator sufficient to induce cardiovasculogenesis (David et al., Nat Cell Biol, 2008). In ES cells MesP1 overexpression resulted in significantly increased numbers of beating cardiomyocytes and of endothelial cells. Our experiments revealed a prominent function of MesP1 within a gene regulatory cascade causing Dkk-1 mediated blockage of canonical wnt-signalling. Our findings suggest a mechanism for cardiovascular specification highly conserved in vertebrates initiated via MesP genes with prominent factors such as Nkx acting further downstream. Detailed patch clamping analyses showed electrophysiological characteristics corresponding to all subtypes of cardiac ES cell differentiation in Nkx2.5 as well as MesP1 programmed embryoid bodies (EBs) but fractions of cardiomyocytes had distinct characteristics: MesP1 forced the appearance of early/intermediate type cardiomyocytes (~60%) in comparison to control cells whereas Nkx2.5 led to preferentially differentiated ventricular cells (~80%) (David et al., Cardiov Res, 2009). In order to unravel the regulation of MesP1 expression we have now analysed Eomes and Brachyury(T) as its potential inducers. We demonstrate that the MesP1 positive cell population is derived from the Brachyury(T) positive fraction in the embryo as well as in ES cells. Likewise, loss of Brachyury(T) causes a dramatic decrease of MesP1 expression accompanied by reduced cardiac markers. Using EMSA, ChIP and reporter assays we found a 3.4 kb proximal MesP1 promoter fragment, directly bound and activated by Brachyury(T) via a T responsive element (David et al., Cardiov Res, 2011). To characterize the cellular progeny eliminating an overexpression situation we used this promoter fragment for isolating MesP1 positive cells from differentiating pluripotent stem cells via magnetic cell sorting based on a deleted CD4 surface marker. This yielded a highly pure common cardiovascular progenitor population with the potential to form all three cardiovascular lineages: cardiomyocytes, endothelial cells and smooth muscle cells. Electrophysiological and pharmacological parameters of the derived cardiomyocytes affirm the pivotal role of MesP1 during the earliest cardiovasculogenic events: by far most of the cardiomyocytes (~94%) corresponded to the desirable multipotent early/intermediate type highly exceeding the numbers achieved by the above described forward programming via MesP1 (~60%) (David et al., in revision).
Finally, I will address ongoing work transferring our forward programming approach described above to the enrichment of pacemaker cells, which may become useful for biological treatment of the “sick sinus syndrome”.

1LMU Munich - Großhadern, Munich, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700021

Micro-Raman spectroscopy for stem cell research

S. Koch[1], M. Pudlas[2], A. Knopf[2], H. Walles[2], K. Schenke-Layland[2]

Abstract

Objectives:

The ability of stem cells to differentiate into various somatic cell phenotypes makes them an attractive source for tissue engineering and regenerative medicine. Focusing on a potential therapeutic application of these cells, a non-invasive and rapid method for the characterization of the cell's differentiation state is needed. The aim of this study was to determine, if Raman-spectroscopy is a suitable, tool to identify different cell types, cellular activity or even to detect the differentiation states of stem cells in a non-invasive and label-free manner.

Methods:

To determine differences in the biochemical component patterns, Raman-spectra of the cell groups were obtained and analyzed with principal component analysis (PCA) and the support vector machine (SVM) using the Opus and Unscrambler software. To verify the actual cells and cell states biological tests were used, like immunofluorescence labeling and flow cytometry. For cell viability analysis we combined a temperature induction of apoptosis and necrosis in the cell lines SAOS-2 and SW-1353 with flow cytometry and fluorescence labeling for apoptosis and necrosis. For cell identification of MSC and fibroblasts flow cytometry and cell culture for cellular differentiation was used to reference to the Raman spectroscopic results. For the analysis of stem cell differentiation we used undifferentiated murine ESCs (feeder - free cell line CCE), differentiated embryoid body (EB) - derived cells and ESC - derived cardiovascular cells. Murine embryonic fibroblasts (MEFs) served as controls. The differentiation states of the cells were confirmed by immunofluorescence staining employing antibodies against pluripotency markers (Oct4 and SSEA) and CD31, a marker that labels cells, which are committed to the cardiovascular lineage.

Results and conclusions:

The results show that the Raman spectroscopy can be used to determine small differences in the cells, which can be correlated to cell viability, differentiation of cell types, for example MSC and fibroblasts and to analyze stem cell differentiation.

1CellTool GmbH, Tutzing, Germany, 2Fraunhofer IGB, Cell systems, Stuttgart, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700022

Effects of bacteria and bacterial compounds on differentiation of mesenchymal stem cells in vitro

K. Peters[1], A. Salamon[1], S. Adam[1], J. Taubenheim[2], S. Thümecke[1], J. Otto[1], B. Kreikemeyer[2], C. Prinz[3], H.-G. Neumann[3], J. Rychly[4], T. Fiedler[2]

Abstract

Introduction:

Staphylococcus aureus (S. aureus), Streptococcus pyogenes (S. pyogenes) and enterobacteria such as Escherichia coli (E. coli) belong to the most frequent initiators of wound infection. It is known that wound healing involves specific differentiation of resident mesenchymal progenitor cells. Since it is unknown which effects bacteria and bacterial compounds exert on differentiation of these stem/progenitor cells, we investigated the influence of the above-listed bacteria on the differentiation state of mesenchymal stem cells from adipose tissue (adipose tissue-derived stem cells/ASC) in vitro.

Materials and methods:

S. aureus, S. pyogenes, and E. coli (isolated from infected wounds) were exposed to ASC (isolated from liposuction-derived adipose tissue) in passage 4 under standard cell culture conditions. Cytotoxicity was tested by live/dead staining, whereas adherence and internalization of bacteria on or in ASC were tested in an antibiotic protection assay. Furthermore, effects of vital and heat-inactivated bacteria and bacterial cell wall compounds were monitored by assays for metabolic activity (MTS), proliferation and osteogenic and adipogenic differentiation. Statistics were performed by Mann-Whitney-U-Test.

Results:

Co-incubation of ASC with bacteria adherence and internalization of the bacteria. Gram-positive species (S. aureus, S. pyogenes) were much more effective in adherence and internalization than the Gram-negative E. coli. In the short run (up to 24 h), none of the bacterial species tested executed cytotoxic effects. In long term experiments (14 d), adipogenically or osteogenically stimulated ASC were exposed to heat-inactivated bacteria, bacterial lysates, and bacterially conditioned cell culture media. Exposure of inactivated E. coli or their membrane compound lipopolysaccharide (LPS) to ASC induced an increase in proliferation, reduction of metabolic activity, increased osteogenic and decreased adipogenic differentiation. In contrast, inactivated Gram-positive bacteria and their cell wall component LTA did not induce clear effects.

Discussion:

The effects observed may be mediated by Toll-like receptors belonging to the innate immune system and serving for the recognition of pathogenic structures, among them bacterial compounds. ASC express toll like receptors TLR1 - TLR6 and TLR9. The recognition of LPS is by TLR4, recognition of LTA is by TLR2. Thus, specific stimulation of ASC by E. coli or LPS is presumably mediated by activation of TLR4. Since Gram-positive bacteria and LTA did not induce clear effects, although the receptor is expressed, further studies are necessary.

This work is financially supported by the EU, the Federal State of Mecklenburg-Vorpommern and the research funding FORUN of the Medical Faculty, University of Rostock.

1University of Rostock/Medical Faculty, Department of Cell Biology/Junior Research Group, Rostock, Germany, 2University of Rostock/Medical Faculty, Institute of Medical Microbiology, Virology and Hygiene, Rostock, Germany, 3DOT GmbH, Rostock, Germany, 4University of Rostock/Medical Faculty, Department of Cell Biology, Rostock, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700023

Transfection behaviour of human bone marrow mesenchymal stem cells from patients

W. Li1, W. Wang[1], L. Ou[1], P. Mark[1], C. Nesselmann[1], C.A. Lux[1], A. Kaminski[1], A. Liebold[2], K. Lützow[3], A. Lendlein[3], R.-K. Li[4], G. Steinhoff[1], N. Ma[1]

Abstract

Transplantation of mesenchymal stem cells (MSCs) derived from adult bone marrow has been proposed as a potential therapeutic approach for postinfarction left ventricular (LV) dysfunction. However, age-related functional decline of stem cells has restricted their clinical benefits after transplantation into the infarcted myocardium. The limitations imposed on patient cells could be addressed by genetic modification of stem cells. This study was designed to improve our understanding of genetic modification of human bone marrow derived mesenchymal stem cells (hMSCs) by polyethylenimine (PEI, branched with Mw 25 kDa), one of non-viral vectors that show promise in stem cell genetic modification, in the context of cardiac regeneration for patients. We optimized the PEI-mediated reporter gene transfection into hMSCs, evaluated whether transfection efficiency is associated with gender or age of the cell donors, analyzed the influence of cell cycle on transfection, and investigated the transfer of therapeutic vascular endothelial growth factor gene (VEGF) hMSCs were isolated from patients with cardiovascular disease aged from 41 to 85 years. Optimization of gene delivery to hMSCs was carried out based on the particle size of the PEI/DNA complexes, N/P ratio of complexes, DNA dosage and cell viability. The highest efficiency with the cell viability near 60% was achieved at N/P ratio 2 and 6.0μg DNA/cm2. The average transfection efficiency for all tested samples, middle-age group (< 65y), old-age group (>65y), female group and male group was 4.32%, 3.85%, 4.52%, 4.14% and 4.38%, respectively. The transfection efficiency didn't show any correlation either with the age or the gender of the donors. Statistically, there were two subpopulations in the donors; and transfection efficiency in each subpopulation was linearly related to the cell percentage in S-phase. No significant phenotypic differences were observed between the two subpopulations. Furthermore, PEI-mediated therapeutic gene VEGF transfer could significantly enhance the expression level.

1University of Rostock, Rostock, Germany, 2Universitätsklinikum Ulm, Klinik für Herzchirurgie, Ulm, Germany, 3GKSS Research Center, Institute of Polymer Research, Teltow, Germany, 4Toronto General Hospital and the University of Toronto, Division of Cardiovasculary Surgery, Toronto, Canada

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research: Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700024

Using a novel bioreactor to sneak a peek at the odd behavior of Endothelial Progenitor Cells (EPCs) - Are they the right cell source for tissue engineering?

S. Olszewski[1,2,3], S. Wirz[1], C. Weber[4], T. Schmitz Rode[1], F. Vogt[5], M. Post[2], J. Bernhagen[6], S. Jockenhoevel[1]

Abstract

Objectives:

Recent findings indicate that Endothelial Progenitor Cells (EPCs) differentiate towards an endothelial like cell type and accelerate re-endothelialization of blood vessels. Healthy veins and arteries are usually lined by a single layer of endothelial cells. This cellular coating, called endothelium, has to be intact for hemocompatibility and prevention of thrombi formation. In order to create intact endothelium within autologous tissue engineered vessels patient's own healthy veins have to be sacrificed to harvest endothelial cells (ECs). Hence EPCs could become an important substitute for ECs in tissue engineering of grafts. Anyhow there is still an ongoing discussion about endothelial progenitor cells and their phenotypes. On the other hand it has already been shown that late outgrowths EPCs are very similar to ECs. Thus we exposed late outgrowth EPCs from human peripheral blood to defined shear stress within our bioreactor and examined them.

Methods:

We developed a novel bioreactor system that provides defined levels of shear stress. Basically the device consists of two coaxial cylinders with different sizes. The inner one rotates and impels medium in the gap between both cylinders. This results in shear stress for the cells that are seeded on the inner wall of the outer cylinder. EPCs were exposed to laminar shear stress of 0.45 Pa for 24h. Then expression of endothelial specific proteins PECAM-1 and VEGF-R2 was estimated by immunocytochemistry and quantitative real-time PCR. Also we monitored the shape of the cells with the systems own bright field microscope.

Results:

EPCs were influenced by the shear stress treatment. To our surprise bright field microscopy images revealed that late outgrowth EPCs being exposed to shear stress did not orientate in direction of flow within 24h. Interestingly, they rather flattened themselves. PECAM-1 and VEGF-R2 expression of EPCs was lower after shear stress. In addition these results were underscored by comparative real-time PCR data.

Conclusions:

Our new bioreactor system is a suitable device for investigation of cells under defined shear stress conditions. Observation of EPCs revealed that they flatten under the influence of shear stress instead of aligning in direction of flow. However taking the drop of PECAM-1 and VEGF-R2 expression into consideration that was noticeable at both translation and transcription levels, doubts arouse about the endothelial character of used EPCs. Therefore undeniably more investigation will be necessary in order to judge the future role of human peripheral blood EPCs in tissue engineering.Without doubt our system can match all needs of future experiments as shear stress can be adjusted to any desired level. Moreover the device permits long time cultivation as medium exchange can be done via incorporated ports very easily. To be brief we proved the feasibility of our bioreactor and demonstrated an odd behavior of heatedly discussed EPCs under shear stress.

1RWTH Aachen - Applied Medical Engineering AME, Tissue Engineering & Biomaterials, Aachen, Germany, 2University Maastricht, Department of Physiology, Maastricht, Netherlands, 3University Hospital Aachen, Institute for Molecular Cardiovascular Research (IMCAR) and Department of Cardiology, Pneumology, Angiology and Intensive Care (Internal Medicine I), Aachen, Germany, 4University Hospital Aachen, RWTH Aachen, Institute for Molecular Cardiovascular Research (IMCAR), Aachen, Germany, 5University Hospital Aachen, RWTH Aachen, Department of Cardiology, Pneumology, Angiology and Intensive Care (Internal Medicine I), Aachen, Germany, 6University Hospital Aachen, RWTH Aachen, Institute of Biochemistry and Molecular Cell Biology, Aachen, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research; Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700025

Fibrin hydrogel - an alternative scaffold for respiratory tissue engineering

C.G. Cornelissen[1,2], S. Krüger[1], T. Schmitz-Rode[3], S. Jockenhoevel[2]

Abstract

Introduction:

Fibrin gel has proven a valuable scaffold for Tissue Engineering. Complex geometries can be produced by injection moulding, it offers effective cell seeding and can be produced autologously. In order to evaluate its suitability for respiratory Tissue Engineering, we examined proliferation and differentiation of respiratory epithelial cells on fibrin gel in comparison to culture on collagen-coated, microporous membranes.

Methods:

Respiratory epithelial cells were isolated from ovine tracheae using published protocols and expanded to passage two. Consequently, cells were seeded onto fibrin gels and collagen coated polyethylene membranes, Proliferation was assessed every 24 hours using a Casy Cell Counter for 6 days. Differentiation was evaluated by measurement of transepithelial electrical resistance, live-microscopy for ciliary motion analysis and histology. Cells were kept in culture for 4 weeks on the respective surfaces.

Results:

Respiratory epithelial cells formed a confluent layer by day 4 on both surfaces as revealed by live microscopy. Proliferation showed no significant difference with respect to surface. The transepithelial electrical resistance increased during the first 6 days of culture, falling to a steady level by day 10. No significant differences could be shown with respect to resistance between the two groups. Live-microscopy revealed the development of ciliae by day 18 with ciliary motion being observed by day 24 in both groups. Histology showed a differentiated respiratory epithelium.

Conclusion:

We hypothesized that respiratory epithelium might proliferate and differentiate as well on fibrin gel as it does on collagen-coated, microporous membranes. In our study, we could not demonstrate any difference in cell proliferation or differentiation when grown on either surface. Thus, we concluded that fibrin gel might prove a suitable scaffold for respiratory Tissue Engineering and merits further investigation.

1University Hospital Aachen, Medical Clinic - Pneumology, Aachen, Germany, 2RWTH Aachen University, Helmholtz Institute, Applied Medical Engineering - Tissue Engineering & Textile Implants, Aachen, Germany, 3RWTH Aachen University, Helmholtz Institute, Applied Medical Engineering, Aachen, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research: Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700026

A novel cardiac assist device (Engineered Heart Tissue) improved cardiac function and restored β-adrenergic responsiveness in dilative

F. Schlegel[1], S. Leontyev[2], C. Spath[1], M. Nichtitz[1], R. Schmiedel[2], F.W. Mohr[2], S. Dhein[1]

Abstract

Aim:

We investigated the effect of implantation of engineered heart tissue (EHT) in heart failure in vivo.
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Methods:

EHT was created from neonatal rat cardiomyocytes, collagen, matrigel and media. After cultivation time (14 days) electrically stimulated EHT started to contract spontaneously and developed force (0.44 ±0.13mN). Histological analysis revealed the presence of troponin I and connexin 43 positive, cross striated cardiomyocytes, besides pre-formed vessels and connective tissue.
We induced DCM by application of doxorubicin in rats for 6 weeks. Cardiac function was controlled by echocardiography during the experiment. On 80 days animals were separated into 3 groups: 1. Day 80-group with animals to examine the status of DCM on day 80 (n=6), 2. EHT-group (n=13), 3. Sham-group (n=12).

In EHT-group we implanted EHT around the beating heart and in Sham-group animals underwent the same surgery without EHT-implantation.
One month after operation hemodynamic measurements were performed (Millar catheter). We examined the LV (left ventricular) +dp/dt max as measurement for contractility, under control conditions and under dobutamine (0.2mg/kg) for stimulation of β-adrenergic receptors (β-AR). Subsequently the hearts were prepared for epicardial electrical mapping analysis and finally for histological analysis.

Results:

Echocardiography revealed a significant impairment of heart function on day 80 (33.1±0.7%) measured as fractional shortening (FS) as compared to healthy animals (41.9±0.9%, p< 0.05). After surgery in EHT-group FS increased by +4.6±1.3% . In comparison, Sham-group exhibited further decrease in FS (-7.5±3.7%, p< 0.05). Hemodynamic measurements indicated a decrease in LV dp/dt max in Day 80-group (4698±370mmHg) and in Sham-group (5336±723mmHg) compared to healthy animals (6470±246mmHg, p< 0.05), but not in EHT-group (7840±672mmHg). Contractility analysis revealed that the dobutamine-induced increase in contractility was abolished in DCM (healthy: 12350±1619mmHg/s vs. Day 80: 7050±1045mmHg/s, p< 0.05), but was restored in EHT-group (12579±2892mmHg/s), while it further declined in Sham-group (5824±543mmHg/s). The restored dobutamine response indicated that the long-term hemodynamic situation in DCM was improved by EHT-implantation. Additionally, mapping analysis exhibited electrical coupling of EHT with the recipient heart. EHT was tightly ingrown into the native myocardium and was connected to the coronary system. In vivo EHT showed organised collagen structure, elastic fibres, and troponin I and connexin 43 positive cardiomyocytes.

Conclusion:

The restored dobutamine response indicated that DCM-induced increase in sympathic activity with consequent β-AR down-regulation can be reversed by EHT-implantation, which demonstrated an improvement in hemodynamic regulation and cardiac function. (BMBF: 0313909)

1Herzzentrum Leipzig, Forschung und Lehre/Chirurgie, Leipzig, Germany, 2Herzzentrum Leipzig, Chirurgie, Leipzig, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700027

Bioengineering of a semiautologous arterial vessels with reconstructed media and intima, longtime tested in vivo

T.A. Sagban[1]

Abstract

Objective:

Goal was to engineer biological, arterial grafts with antithrombotic, autologous endothelial luminal surface combined with extraluminal smooth vascular muscle layer (VSMC) and to test invivo.

Methods:

(1) Different decellularisation methods described in literature were compared to identify the most suitable one with focus on the preservation of extracellular fibre matrix.

(2) Endothelial precursor cells (EPC), isolated from bone marrow and VSMC from small venous segments of donor animals were cultivated. Cells were seeded sandwich-like on homologous decellularized venous scaffolds and conditioned under pulsatile circulation in a bioreactor.

(3) The semiautologous grafts were implanted in carotidal position on both sides in five Beagle dogs (n=10; group 2) as interposition

. A group of five animals, receiving only acellular grafts in both carotid positions (n=10; group 1) served as controls.

Results:

Comparison of four in literature described decellularisation methods showed different preservation of elastic and collagen fibres compared with native veins, whereas decellularity was similar in all methods. This forced us to choose a decellularization protocol with the best preservation of the extracellular matrix. The invivo experiments showed in group 1 (control) already after one week a complete thrombotic occlusion of the decellularized implants, whereas in group 2 9/10 semiautologous grafts were patent after 98±4 days in ultrasound, angiography and histology (p=0,0001)..

Conclusion:

A complete incorporation of semiautologous grafts in the surrounding tissue could be shown. The seeding with two different cell types preserved an aneurysmatic degeneration under arterial conditions with patency without anticoagulation.

1University of Düsseldorf, Dept. of Vascular Surgery and Kidney Transplantation, Düsseldorf, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700028

About cells and scaffolds - the BioStent in the field of tissue engineering

L. Rongen[1], S. Weinandy[1], T. Deichmann[2], S. Olszewski[1], A.H. Mahnken[3], T. Gries[2], T. Schmitz-Rode[1], S. Jockenhövel[1,2]

Abstract

Objective:

The percutaneous stent angioplasty of peripheral vessels is well established as clinical routine. Unfortunately the patency rates of small-calibre grafts (< 6mm) are still unsatisfying, especially in the lower limb reg ion. The aim of the BioStent concept is to overcome restenosis. The basic approach is total separation of atherosclerotic plaques from the blood stream. The second and essential approach is to form an intact, functional and active endothelial cell layer. The proposed concept bases on the combination of a self-expanding nitinol stent with the principles of vascular Tissue Engineering: The moulding process of vascular grafts, based on a fibrin gel scaffold, allows complete integration of a self-expanding stent structure within the tissue-engineered vessel. With this completely new principle the major causes of restenosis (1.) the foreign body reaction, (2.) the cell proliferation with ingrowths in the lumen and (3.) acute thrombosis by hemo-incompatibility will be prevented. The reason is total exclusion of the atherosclerotic surface from the blood stream and the coating of the neolumen with a functional endothelial cell layer, including antithrombotic function of the endothelial cells..

Methods:

Small-calibre (6mm) BioStents were made by combining a self-expanding nitinol stent with a thin fibrin-based tissue-engineered blood vessel.

Remodelling of the fibrin scaffold with mature autologous proteins was tested by histological analyses.A confluent endothelial cell monolayer lining the luminal surface of the Biostent was shown by scanning electron microscopy.

Results:

A thin coverage of about 200µm completely wrapping the stent structure was achieved. Scanning electron microscopy revealed that the total surface was covered by a confluent layer of endothelial cells.

Conclusion:

The present feasibility study shows successful combination of a self-expanding nitinol-stent with a fibrin-based tissue-engineered blood vessel. The next step in this project will be animal studies in a sheep model .
Thus first stents have already been implanted and we are looking forward to the results. Based on the results of this study the system will be improved. Our current stent construct has a diameter of 6 mm and is supposed for peripheral blood vessels.
Aim of further studies will be to reduce the diameter of the stent for coronary stent application. Finally, our tissue engineered stent platform is well suited to be used in other applications.
Recently, we already started projects based on the BioStent concept that aim to improve endobronchial and urogenital stenting.

1Institute of Applied Medical Engineering Helmholtz Institute of RWTH Aachen University & Hospital, Department of Tissue Engineering & Biomaterials, Aachen, Germany, 2Institut für Textiltechnik, RWTH Aachen University, Aachen, Germany, 3Department of Diagnostic and Interventional Radiology, University Hospital Aachen, Aachen, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700029

A novel electrospun nanofiber scaffold with bio-magnetic property augments hepatic differentiation of human mesenchymal stem cells

D.K. Bishi[1,2], S. Guhathakurta[1], R.S. Verma[2], K.M. Cherian[1]

Abstract

Objective:

Soft tissue regeneration by mesenchymal stem cells and biomaterial scaffolds has been an emerging technology in regenerative medicine today. In the present study, we have evaluated the hepatomimetic potential of a novel biodegradable, biocompatible, polymeric nanofiber scaffold made up of PLLA (Poly-L-Lactide)-collagen and fibrin clot extracted from blood. Such a novel scaffold fabricated by electrospinning technique was evaluated for fiber morphology, composition, mechanical property, biomagnetic property, biocompatibility and hepatic differentiation potential in vitro.

Methods:

The nanofibers were fabricated by electrospinning and their morphology was observed under a scanning electron microscope. The tensile properties of nanoscaffold were evaluated with a mechanical testing system. Elemental composition of the nanofiber scaffold (fibrinogen, iron content, albumin) was assessed biochemically. Biomagnetic behaviour of nanoscaffold was studied using a vibrating sample magnetometer.

The proliferation of MSCs seeded on electrospun nanofiber was assessed quantitatively by MTT assay. Hepatic differentiation was carried out with a novel hepatogenic conditioning media, derived from goat liver extract, with EGF and FGF-4 pre-induction. Differentiation of MSCs to hepatocyte like cells on nanoscaffold was confirmed by immunofluorescence and electron microscopy.

Results:

Morphological analysis reveals the blended nanofibers have a fiber diameter of 381± 0.171 nm. The amount of iron and fibrinogen present in nanofiber scaffold was significant compared to original electrospinning solution. SDS PAGE analysis of nanofiber components reveals two bands of 57 kDa and 48 kDa, which represents the fibrinogen. The magnetic behavior of the electrospun nanofiber reveals a significant magnetic hysteresis loop to a varying magnetic field (-2000 to 3000 G) at room temperature. This is the first report describing biomagnetic property of a nanoscaffold derived from blood components. Morphological characterization of the MSCs seeded on nanoscaffolds by scanning electron microscopy shows better cell entrapment and infiltration onto nanofibers and their enhanced proliferation. The morphology of cells on nanofibrous scaffold is more of hepatocyte like and it was confirmed by immunofluorescence analysis for the albumin expression. Cells on nanoscaffold showed higher expression compared to that on TCP.

Conclusion:

All the above results support enhanced morphological and functional hepatic differentiation of MSCs on a novel bio-magnetic and biocompatible nanoscaffold, making its future therapeutic application widespread. This study indicates that in hepatic failure, autologous blood clot derived similar nanoscaffold impregnated with autologous bone marrow derived mesenchymal cells may be a therapeutic option for augmenting liver regeneration, if implanted at orthotopic site.

1Frontier Lifeline & Dr. K. M. Cherian Heart Foundation, Tissue Engineering and Regenerative Medicine Laboratory, Chennai, India, 2Indian Institute of Technology, Madras, Stem Cells and Molecular Biology Laboratory, Bhupat and Jyoti Mehta School of Biological Sciences, Chennai, India

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700030

Stem cells in cardiac drug discovery and safety pharmacology

U. Kraushaar[1], E. Guenther[1], T. Meyer[2]

Abstract

Cardiomyocytes are an important target in drug Discovery and Safety Pharmacology. Many drugs can interfere with human cardiac ion channels and trigger fatal arrhythmia. However, as animal cells do have a different set of cardiac ion channels than human cells the aim for a long time has been to establish a human cell based assay. Here we compare the properties of iPS cell derived cardiomyocytes, ES cell derived cardiomyocytes with a non mammalian primary cardiomyocyte based assay and heterologous expression systems.

We compare results from cardiac tissue recordings and Langendorff hearts with our datasets obtained from a variety of stem cell derived cardiomyocytes for a panel of reference compounds.

1Natural & Medical sciences Institute at the Tübingen University, Electrophysiology, Reutlingen, Germany, 2Multi Channel Systems MCS Gmbh, Reutlingen, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700031

Presenting a new international standard - Application of risk management and requirements for processing practices for medical products containing viable human cells

S. Kloth[1]

Abstract

Currently the International Organization for Standardization (ISO) is developing a new standard document (ISO 13022) which will address the requirements for risk management for medical products based on viable human cells.
By definition of the relevant European Directives and Regulations products including human tissues and cells are currently considered as medicinal products in Europe, and standards are not commonly used for medicinal products in the European Community. However, there is an obvious benefit in a close coordination of the work on regulatory guidelines in Europe and internationally harmonized standards for all manufacturers of these products who are focusing on the international market.
The standard ISO 13022 specifies a procedure to identify the hazards and hazardous situations associated with such products, to estimate and evaluate the resulting risks, to control these risks, and to monitor the effectiveness of that control. Furthermore, it outlines the decision process for the residual risk acceptability, taking into account the balance of residual risk, and expected medical benefit as compared to available alternatives. It will cover viable human materials of autologous origin as well as allogeneic human material.

The document is intended to provide requirements and guidance on risk management related to the hazards typical of medical products manufactured utilizing viable human materials such as:

a) contamination by bacteria, moulds or yeasts and parasites,
b) contamination by viruses,
c) contamination by agents causing Transmissible Spongiform Encephalopathies (TSE),
d) contaminating material responsible for undesired pyrogenic, immunological or toxicological reactions,
e) decomposition of the product and degradation products caused by inadequate handling including procurement, packaging, storage, transport and application, and
f) complications resulting from the mix up of human raw materials.

These considerations apply to all stages from donor selection to application of the product.

Information on the status of the standard document and the potential for participation in the developing process will be further topics of the presentation.

1TÜV SÜD Product Service Gmbh, Clinical Affairs, München, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700032

MicroRNA regulation pathway study in the case of cardiomyocyte differentiation of murine ESCs

L. Gan[1], S. Schwengberg,[2], B. Denecke[1]

Abstract

Differentiation of embryonic stem cells (ESCs) is a comprehensive biological process controlled by many regulators. A better understanding of differentiation process is an essential base for development research, tissue engineering and pharmaceutical oriented ESC research. Recent studies demonstrated that microRNAs (miRNA) play a central role in the regulation of ESC differentiation. In the present study, a transgenic murine ESC line was applied as a model for miRNA regulation study in cardiomyocyte specific differentiation. miRNA expression was profiled using high throughput microarray platforms for samples obtained at undifferentiated stage (day0) and different time points during cardiomyocyte specific differentiation and maturation (day12, day19 and day26). 50 miRNAs were identified as plausibly different expressed during the process from ESCs to mature cardiomyocytes and therefore recognized as candidate regulator miRNAs involved in cardiomyocyte specific differentiation procedure. Parallel to miRNA profiling, a genome wide transcriptome analysis
was performed for the same samples. This analysis discovered regulation effects between undifferentiated ESCs and cardiomyocytes on transcriptional level. Functional analysis of regulated genes and miRNA target genes reveals possible regulatory pathway of candidate miRNAs. Although it is widely believed that miRNA regulators act on both transcriptional and translational levels,
this study concentrated on finding correlation between miRNA regulator and target gene transcript. Regulated miRNA target genes showed enrichment in important pathways involved in cardiomyocyte specific differentiation process.

Further studies can surely complete the scenario of cardiomyocyte specific ESC differentiation.

1RWTH Aachen University, Aachen, Germany, 2Axiogenesis AG, Cologne, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700033

Ca2+-activated K+-channels - New tools to initiate cardiac commitment towards pacemakers-like cells in human Induced

R. Russell[1], M.M. Müller[2], M.S. Stockmann[3], C.W. Weidgang[2], A. Wobus[4], G.V.W. von Wichert[2], B.F. Fleischmann[5], S.L. Liebau[3], A.K. Kleger[2,6]

Abstract

Rationale:

Embryonic stem (ESC) and induced pluripotent stem (iPSC) cells are attractive sources for ex vivo generation of cardiomyocytes. These systems are well-suited for developmental studies, high throughput drug screenings and cell replacement approaches. Activation of calcium-activated potassium channels (SKCas) via the small molecule 1-EBIO (1-Ethyl-2-benzimidazolinone) leads to an induction of mesodermal differentiation and an enrichment of cardiac pacemaker cells. To date, these findings were restricted to mouse embryonic stem cells, thus limiting a broad applicability.

Objective:

It is obvious that this strategy could be a powerful approach for clinical and research applications.
Therefore, we have validated our previous findings using human induced pluripotent stem cells derived from plucked human hair.

Methods and results:

We assessed SKCa-function via the small molecule 1-EBIO in terms of cardiac differentiation in human pluripotent cells and provide the following results: (i) the generation of virus-free human iPS cells as a potential source for patient-specific iPS cells and (ii) the successful transfer of the SKCa activation assay to the human system in terms of rapid remodeling of the actin cytoskeleton, inhibition of proliferation and induced commitment to the cardiac lineage upon SKCa activation.

Conclusion:

Our data clearly demonstrate the generation of SKCa-induced cardiac pacemaker cells from human iPSCs and overcomes specific limitations for potential applications. With our system using human iPSCs we further identify new strategies for the generation of autologous cells for cell replacement applications, thereby, providing a key milestone on the path to making cardiac subtypes in men.

1University Ulm, Ulm, Germany, 2University Ulm, Department of Internal Medicine I, Ulm, Germany, 3University Ulm, Institute for Anatomy & Cell Biology, Ulm, Germany, 4Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany, 5University of Bonn, Institute of Physiology I, Life and Brain Center, Bonn, Germany, 6Institute of Molecular Medicine and Max-Planck-Research Department on Stem Cell Aging, Ulm, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700034

Implementing the sinuses of Valsalva in fibrin-based tissue engineered heart Valves

A. Borgmann[1], M. Weber[1], J. Frese[1], T. Schmitz-Rode[2], S. Jockenhoevel[1], P. Mela[1]

Abstract

Objectives:

The implementation of the sinuses of Valsalva for both aortic and pulmonary position is a crucial step towards the development of functional tissue engineered heart valves with optimal hemodynamic performance and reduced risk of thrombi formation. However, the implementation of these features is not standard in tissue engineered heart valves. In our laboratory we aim at the realization of autologous heart valves starting from materials isolated from the patient (fibrinogen and cells) and shaped into 3D geometries by moulding techniques. We present a new fabrication method that results in the realization of a heart valve scaffold reproducing the complete complex geometry of a semilunar valve. Our concept consists of a mould in two parts: a ventricular part and a vascular part which contains three removable bulbs representing the sinuses of Valsalva (Figure 1).

Methods:

The moulds were designed with the 3D CAD software Pro/Engineer (PTC, Needham, MA, USA) and manufactured by rapid prototyping. The cell embedded fibrin gel valves were produced by polymerizing a fibrinogen solution in TBS (10 mg/ml) with CaCl2, thrombin and ovine umbilical cord derived fibroblasts (10x106 /ml) suspended in TBS. Afterwards, the tissue engineered heart valve was placed in a static bioreactor to be cultured on the mould for 14 days in order to avoid cell-mediated tissue contraction before transferring it to a bioreactor for dynamic cultivation of a duration of 30 days.

Results:

After the polymerization of the fibrin gel had occurred, first the vascular part and subsequently the three removable sinuses of Valsalva were removed. The construct was successfully released without any tearing despite the poor mechanical properties of the hydrogel. We obtained a conduit presenting the complex geometry of a semilunar valve with a three leaflet valve and the sinuses of Valsalva cast. The scaffold consisted of a single piece without the need for suturing any of the parts together. After static and dynamic cultivation the tissue was well developed and the conduit demonstrated a good compliance.

Conclusions:

The presence of the sinuses of Valsalva in the aortic and the pulmonary root is fundamental for the correct functioning of semilunar heart valves. The implementation of the sinuses in tissue engineered valves will lead to an improvement of the valve function and an increased durability. Ongoing research focuses on the culturing, mechanical conditioning in bioreactors and evaluation of the scaffolds in vitro. Further evaluation will include immunohistology and determination of hydroxyproline and DNA content.

Figure 1: Mould with three removable sinuses of Valsalva

1Institute for Applied Medical Engineering, Helmholtz Institute for Biomedical Engineering; RWTH Aachen, Tissue Engineering & Textile Implants, Aachen, Germany, 2Institute for Applied Medical Engineering, Helmholtz Institute for Biomedical Engineering; RWTH Aachen, Aachen, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700035

Myocardial tissue engineering (past, present and future)

A. Arti[1], A. Bader

Abstract

Tissue engineering is a multidisciplinary science that merges different fields of sciences like cell biology, physics, chemistry, material sciences, genomics, proteomics, engineering and medicine together to create biological substitutes of native tissues for doing research in lab or to use for medical application.

Tissue engineering is a promising approach to overcome the problems of shortage of organs and tissues for replacing or repairing damaged tissues or organs. Although the history of tissue engineering goes back into the last century but it is nearly 3 decades since accelerating into research in this field especially with the experimental and clinical goals.

The main progress in this field arise from 1990s that since that time tissue engineering turns from just a laboratory research field into a rapidly growing industry. With industry application of tissue engineering more research funds invests into the research labs and therefore this field of science due to earlier mentioned reasons and especially due to regenerative medicine and clinical application needs has an extremely promising future.

In the past tissue engineering considered as a part of biomedical sciences but it is now more considered as a multidisciplinary field of science. Tissue engineering is now used in many fields of biological sciences and medical sciences and has many experimental and clinical applications (e.g. to replace or repair organs or tissues like bone and cartilage,…).

In this presentation the past of tissue engineering and its application in regenerative medicine will briefly discussed and then I spend more time on the myocardial tissue engineering (MTE) as the main scope of this presentation.

Cardiovascular diseases (CVDs) and especially myocardial infarction (MI) are the number one cause of mortality and morbidity in the developing countries. It is also one of the main cause of mortality and morbidity in the developing and rest of the world countries.

There are many routine therapy methods following MI like oxygen therapy, beta -blockers medication therapy or injection of Heparin or to administrate anti platelet medications like Aspirin or Clopidogrel to inhibit blood clots in the coronary artery to restore blood flow in the blocked coronary .

Myocardial scar formation and myocardial infarct size are large and the damages are irreversible. To help the damaged myocardial tissue back to normal function also seems need new therapy approaches in addition to previous routine therapies that earlier mentioned.

Myocardial tissue engineering needs some constructs that they should have those like biocompatibility, Mechanical integrity, biodegradability, being cell friendly, biomimetic and also have fabrication capability.

In this presentation after this introduction I discuss advantages and disadvantages and success and failures of MTE in details. The goal of this presentation is to give a better idea on the past, present and the future of the MTE.

1University of Leipzig, Cell Techniques and Applied Stem Cell Biology, Leipzig, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700036

Differentiation stage limits cellular reprogramming in murine liver

A. Kleger[1,2], P. Mahaddalkar[2], A. Lechel[2], H. Schöler[3], S. Liebau[4], K.L. Rudolph[2]

Abstract

Background and aims:

Ectopic expression of certain transcription factors can reprogram somatic cells to a pluripotent state. It has been shown that hematopoietic stem cells can be reprogrammed with a higher efficiency than differentiated blood cells. Similar findings have not been demonstrated in other primary organ systems. Moreover, molecular characteristics in the cellular hierarchy of tissues that influence reprogramming capacities need to be delineated. Here, we analyzed the influence of the differentiation stage of freshly isolated, murine liver cells on the reprogramming efficiency.

Results and methods:

Liver progenitor cell (LPC)-enriched cell fractions from fetal and adult liver showed a significantly increased reprogramming efficiency compared to unsorted fetal liver cells, when 3 or 4 reprogramming factors were transduced for the generation of iPS cells.

The reprogramming efficiency of fetal LPCs reached up to 30% in the 4-factor experiments.LPC-derived iPS cells showed all hallmark features of pluripotency. Increased reprogramming efficiencies of LPCs correlated with the expression of reprogramming factors (Klf4 and c-Myc in fetal LPCs and c-Myc in adult LPCs) as well as with an elevated expression of BAF-complex members (Baf155, Brg1) in adult and fetal LPCs. The increased reprogramming efficiency of LPCs compared to differentiated liver cells occurred independent of the proliferation rate in the freshly isolated cell populations.

Conclusions:

The current study provides the first experimental evidence that the differentiation stage impairs reprogramming efficiency in freshly isolated cells from mouse liver. The data indicate that LPCs carry intrinsic, cell proliferation-independent characteristics allowing highly efficient reprogramming. LPCs could serve as a novel cell source for fast and efficient iPS formation.

1University Ulm, Department of Internal Medicine I, Ulm, Germany, 2Institute of Molecular Medicine and Max-Planck-Research Department on Stem Cell Aging, Ulm, Germany, 3Max Planck Institute for Molecular Biomedicine, Cell and Developmental Biology, Münster, Germany, 4University Ulm, Institute for Anatomy & Cell Biology, Ulm, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research: Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700037

Liver cell transplantation - a new therapeutic option for children with urea cycle defect

W. Rüdinger[1], S. Kafert-Kasting[1]

Abstract

Aims:

Urea Cycle Disorders (UCD) are inherited errors of metabolism with deficiencies of one of the six enzymes involved in the urea cycle. Patients suffering from UCDs cannot detoxify nitrogen and have a poor prognosis, especially if the onset of the disease occurs in the neonatal period. Mortality reaches about 85% after 10 years in these children, most of the survivors showing severe neurological impairment. The current therapeutic concepts include dietary restrictions and treatment with ammonia scavenger drugs, which are, however, often not sufficient to prevent UCD patients from recurrent hyperammonemic crisis. Early orthotopic liver transplantation (OLT) is a definite cure of the metabolic disease but a risky procedure in small children. Liver cell transplantation aims to provide an additional therapeutic option especially for the group of very young patients.

Methods:

Liver cell transplantation is applied to children with UCD in a clinical trial in Germany, after previous experience gained from individual therapeutic attempts. The children treated so far received 1-6 intraportal infusion of liver cell suspension after surgical catheter placement, with concomitant immunosuppression. The liver cells were isolated from donated organs (donor age, 5 days to 55 years) in a GMP compliant manufacturing procedure.

Results:

Intraportal liver cell infusion was well tolerated in all nine study patients and four cases of therapeutic attempts treated so far. The procedure was feasible even in neonates (n = 4) and no complications were noted during the infusions, which were carried out with ongoing control of peripheral oxygen saturation and portal blood circulation. In most patients the clinical situation stabilised after treatment over a period of up to 21 months or until OLT. Further efficacy evaluation using laboratory, biochemical, and immunohistological methods is currently still ongoing in order to quantify the engraftment of donor hepatocytes in the recipient's liver.

Conclusions:

Intraportal liver cell transplantation is feasible and safe in children with UCD. In parallel to a broader clinical use of LCT based on clinical studies, research on suitable liver stem cells should be promoted to overcome the limited availability of adult hepatocytes, with concomitant improvement of repopulation.

1Cytonet GmbH & Co. KG, Weinheim, Germany

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700038

Correlation of HGF and SDF-1 with peripheral mobilisation of CD133+/CD45+ bone marrow stem cells (BMSCs) after hepatectomy and chemotactic effects

C. Duhme[1], M. Wildner[1], M. Schmelzle[2], A. Krieg[1], G. Fürst[3], K. Raba[4], J.C. Fischer[4], S. Topp[1], N.H. Stoecklein[1], W.T. Knoefel[1], J. Schulte am Esch[1]

Abstract

Objectives:

Hematopoietic BMSCs are involved in hepatic regeneration after liver resection. We previously demonstrated peripheral mobilisation of CD133+/CD45+ hematopoietic BMSCs after extended forms of clinical hepatectomy. In this study we correlated peripheral CD133+/CD45+cell mobilisation with the extent of resected liver volume and its regain, with paracrine factors participating in hepatic regeneration like hepatic growth factor (HGF), CXCL12 (SDF-1) and alpha feto protein (AFP). Additionally the potential mobilising capacity of HGF and SDF-1 for these stem cells was analyzed.

Methods:

Peripheral progenitor mobilisation was investigated by FACS analyses in 30 hepatectomy patients. Exact extend of liver volume loss and regain by day 21 after hepatic resection was determined by CT scan volumetry. 20 patients with resection volume of less than 20% (group A; n=20) were compared to 10 patients with a resection extend of 30-67% (group B; n=10). HGF, SDF-1 and AFP levels in patient's serum were determined by ELISA technology. Mobilising capacity of HGF and SDF-1 for CD133+/CD45+ BMSCs was investigated in in-vitro Transwell Chemotaxis Assays (Boyden Chamber).

Results:

In group B we observed increased serum levels of HGF, SDF-1 in the first 6h and of AFP beyond 24h if compared togroup A. Beyond an augmented peripheral mobilisation of CD133+/CD45+ cells from day 2 on in the large resection group, levels of early CD133+/CD45+ stem cell mobilisation correlated directly with the level of hepatic volume regain by day 21.
The mobilisation of CD133+/CD45+ cells on day 4 after extended liver resection in group B was directly correlated with the levels of AFP serum levels on day 6 after hepatectomy.
In in-vitro migration assays human BM derived CD133+ cells isolated by MACS showed a specific target-directed migration towards recombinant HGF and SDF-1 gradients in concentration-dependant manner.
The observed mobilising capacity of HGF and SDF-1 for CD133+/CD45+ BMSCs was partly dependent on the specific receptors c-Met and CXCR4.

Conclusions:

These data suggest HGF and SDF-1 to play a role for mobilisation of CD133+ stem cells from BM in the liver regenerative scenario subsequent to hepatic resection. The increase of AFP serum levels supports the activation of stem cells after extended liver resections since AFP is considered to be a marker of “stemness” in hepatic regeneration. Whether peripheral mobilisation of CD133+ BMSCs that correlates with levels of liver regeneration after hepatectomy results in integration of those cells in intra-hepatic stem cell compartments, as possibly suggested here by AFP expression data, needs to be further explored.

1Heinrich-Heine-University and University Hospital Düsseldorf, Department of General-, Visceral- and Pediatric Surgery, Duesseldorf, Germany, 2Beth Israel Deaconess Medical Center, Harvard Medical School, Department of Medicine, Liver Center and Transplantation Institute, Boston, United States, 3Heinrich-Heine-University and University Hospital Düsseldorf, Department of Diagnostic Radiology, Duesseldorf, Germany, 4Heinrich-Heine-University and University Hospital Düsseldorf, Institute of Transplantation Diagnostics and Cell Therapeutics, Duesseldorf, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research; published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700039

Heterogeneous roles of Caspase8-signalling in hepatocytes and non-parenchymal cells in a model of liver stem cell activation and sclerosing cholangitis

K. Chaudhary[1], C. Liedtke[2], C. Trautwein[2], K. Streetz[2]

Abstract

Background and aim:

Caspase-activation plays a fundamental role in the maintenance of tissue homeostasis by clearing injured cells to maintain tissue homeostasis through receptor mediated apoptosis. An important role of caspase8 during immune-mediated liver injury and regeneration was previously demonstrated by our laboratory. We aim to examine its role during hepatic stem cell (Oval cells) activation, a process closely related to liver regeneration..

Materials and Methods:

For this approach, hepatocyte specific conditional caspase8 knockout (casp8ΔHepa) animals and mice with an ubiquitous deletion of caspase8 (casp8ΔMx) were compared in the DDC (3,5-diethocarbonyl-1,4-dihydrocollidine) model of oval cell activation and secondary sclerosing cholangitis. Oval cells were isolated and characterized by flow cytometry (FACS) and real time PCR. Liver tissue was further analysed by IHC.

Results:

Higher transaminases and bilirubin levels were observed in Casp8ΔMx levels compared to wild type (WT) and control, while casp8ΔHepa animals were protected after 4-weeks of DDC-feeding. Additionally, histological analysis revealed reduced liver injury and immune-cell infiltration in Casp8ΔHepa mice

Thereby caspase3 and -8 activities were reduced in both knockout strains as demonstrated by caspase3 and -8 assay, suggesting other mechanisms being responsible for the phenotype. Correlating to the stronger liver injury, casp8ΔMx mice displayed more proliferation in periportal areas where LPC emerge and reside as indicated by 5-fold higher BrdU incorporation rate and significantly higher CD133, Cyclin A, D and E mRNA expression. The following analysis of hepatic progenitor cells by flow cytometry (sca-1, OC-1, -2 and -3) as well as by immunohistochemistry (CK-19) unrevealed a significant stronger (5-fold) oval cell activation in casp8ΔMx mice, while casp8ΔHepa had significantly less then WT mice after 4-weeks of DDC-feedinig. Deletion of caspase8 itself was not evident in isolated progenitor cells. Preliminary data now point to an additionally enhanced infiltration of immune-cells (CD45, CD11b, CD4) in casp8ΔMx mice. This finally resulted in a stronger fibrosis progression of the underlying sclerosing cholangitis induced by DDC in casp8ΔMx mice, as evidenced by an enhanced expression of collagen and α-SMA.

Conclusions:

Our data suggest a differential role of death receptor mediated liver injury through caspase8 activation in individual liver cell types. While hepatocyte specific knockout provided protection from liver damage an ubiquitous deletion of caspase8 triggered more injury and inflammation. This was finally related to a significantly stronger activation of the liver progenitor cell compartment and more tissue remodelling.

1University Hospital Aachen, RWTH Aachen, Department of Internal Medicine III, Aachen, Germany, 2University Hospital Aachen, RWTH Aachen, Department of Internal Medicine, Aachen, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700040

Estradiol modulates membrane linked ATPases , antioxidant enzymes, membrane fluidity, lipid peroxidation and lipofuscin in aged rat liver

P. Kumar[1], R.K. Kale[1], N.Z. Baquer[1]

Abstract

Free radical production and oxidative stress are known to increase in liver during aging, and may contribute to the oxidative damage, which plays an important role in the aging process. These changes increase during menopausal condition in females when the level of estradiol is decreased. The objective of this study was to observe the changes in activities of membrane linked ATPases (Na+K+ ATPase, Ca2+ATPase), antioxidant enzymes (superoxide dismutase, glutathione-S-transferase), lipid peroxidation levels, lipofuscin content and membrane fluidity occurring in livers of female rats of 3, 12 and 24 months age groups, and to see whether these changes are restored to 3 months control levels rats after exogenous administration of17-β-estradiol (E2).

The aged rats (12 and 24 months) were given subcutaneous injection of E2 (0.1µg/g body weight) daily for one month. After 30 days of hormone treatment, experimental animals of all the groups were sacrificed and livers were isolated for further study. The results obtained in the present work revealed that normal aging was associated with significant decrease in the activities of membrane linked ATPases, antioxidant enzymes, membrane fluidity and an increase in lipid peroxidation and lipofuscin content in livers of aging female rats.

The present study showed that E2 treatment reversed the changes to normal levels. E2 treatment may be beneficial in preventing some of the age related changes in the liver by increasing antioxidant defenses.

1Jawaharlal Nehru University, School of Life Sciences, New Delhi, India

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research; Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700041

Stem cells in orthopaedics - retrospective analysis of 10 year translational research

M. Jäger[1], M. Herten[2], S. Landgraeber[1], R. Krauspe[2]

Abstract

More than 40 years have gone since Friedenstein described the so called colony forming unit fibroblasts. At least in the late 1990ties, a boom of stem cell research aiming for clinical application was trigger mainly but not only by publications of the groups of Pittenger, Caplan, Bruder and Prokop et al.. These publications include information of the last 20 years about the multipotency of mesenchymal progenitor cells and emphasize the significance of the human bone marrow as a cellular source for tissue regeneration. At those days “mesenchymal stem cells” (MSCs) seems to be a promising candidate to regenerate almost every local musculo-skeleteal defect. Getting more precise and detail data about the stem cell biology including differentiation potential, intracellular signaling, homing behavior and immunosuppression stem cell research was strongly promoted by industrial grants but also by public funding. There was great euphoria and hope to treat even systemic diseases such as osteoporosis, osteogenesis imperfecta and others diseases sufficiently by cell therapeutics.

In contrast to the rapid increase in knowledge and a spirit of optimism in MSC research, the regulatory authorities observe the clinical application of autologous progenitor cells more critically and sometimes with suspect. This skepticism seems to be based on the indistinct use of the word “stem cell” in many publications but also on a lack of clinical reliable data justifying a broad application besides healing attempts. In addition, the use of and research on embryonic stem cells was controversly discussed in public leading to new regulations and laws on the national and European level. Third, due to a lack of money in the health care systems insurance companies did not promote or reimburse new treatment concepts including tissue regeneration by MSCs.

Especially during the last few years controversy has arisen regarding the role and relevance of MSC in orthopaedic surgery. In contrast to the limited results in cartilage regeneration, bone regeneration was successful as shown in several clinical case series and is therefore one major focus for potential clinical application.Some investigators as the authors of this abstract suggested that the application of bone marrow aspiration concentrate (BMAC) is a valuable tool to stimulate local bone formation.

This hypothesis is based on recent data which demonstrate that a) MSCs are not the exclusive source of osteoblast, b) hematopoietic cells promote osteoblastic differentiation and c) both cell lineages are located next to each other in their physiological niches.

However, it is questionable if the relatively small number of living spongious MSC is sufficient to induce relevant bone regeneration in vivo.

Therefore osteoconductive or osteoinductive bone substitutes are essential mimicking as a scaffold and osteopromoting microenvironment. In a review of the literature and the presentation of own in vitro but also in vivo data it is demonstrated that especially progenitors from the adult bone marrow are still promising for the treatment of defined musculoskeletal disorders. Compared to tissues with a complex three dimensional histoarchitecture such as cartilage or muscle, bone regeneration seems to be most effective. However, controlled and prospective clinical trials are strongly required to establish orthopaedic cell therapy as a standard procedure in the near future.

Key words:

osteoblast, mesenchymal stem cell, bone regeneration, bone marrow

1University Hospital Essen, Department of Orthopaedics, Essen, Germany, 2University Hospital Düsseldorf, Department of Orthopaedics, Düsseldorf, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700042

Survival of mesenchymal stem cells in collagenase induced tendonitis in an ovine model

A. Crovace[1], L. Lacitignola[1], G. Rossi[2], E. Francioso[1]

Abstract

The pourpose of this study was to evaluate the efficacy and the survival of local injection of allogenic MSC marked with Red Fluorescent Protein (RFP) (Lentigen-Italy) in collagenase induced tendonitis in the ovine Achille's tendon.The study was performed after the approval by the National Animal Care and Use Committee

Four sheep (2 years old, female, 45 bwt) have been enrolled in the study. After some days for the acclimatation, the sheep have been investigated to exclude any previous Achilles' tendon lesion. Three weeks before starting of the study one sheep was randomly selected for Bone Marrow harvesting for MSCs cultivation. The MSCs obtained has been trasfected with a lentivirus for integration of a gene for expression of Red Fluorescent Protein (RFP). After a week the other 3 sheep was injected in both Achilles' tendon with 400 U.I. of Collagenase IA of Cl. hystoliticum (Sigma-Aldrich-Italy). After two weeks the left Achilles' tendon of each sheep was injected with a solution of 6x106 RFP-MSCs (MSCRFP) in 1 ml of fibrine glue (TISSUCOL , Baxter). The remaining tendons were used as negative control and received the same volume of saline solution as placebo. At 3-4-6 weeks from the treatment the tendons were harvested and evaluated for morphology, collagen I and III expression, and visualized at fluorescence microscope to assess RFP expression of the grafted MSCRFP. The results of these investigations evidenced the presence of MSCRFP in the treated tendons respect to the control ones at 3, 4 and 6 weeks after the treatment. Moreover, the RFP positive tissue showed high expression of collagen I and low collagen III with good morfphology in comparison to the lesions treated with placebo.The presence of high expression of collagen I and low collagen III with good morfopholgy, in term of restored tendon architecture, can be related to the MSCRFP injected into tendon lesions, as a large number of cells can survive in the site of injection.

These results showed that intralocal administration of MSCs into the tendon lesion can lead to a good effect on injured tendon . The local infusion delivery entails injecting MSCs directly into the tissue of interest and guarantees a higher number of engrafted cells and optimal therapuetic effect.

Besides the survival of high numbers of positive RFP -MSCs in treated samples have been demonstrated at 3, 4 (Fig.1) and 6 weeks from the treatment. We have evaluated also that quality of tendon healing in MSCRFP treated tendons has been based on a better architecture of collagen fibers and high expression of collagen I respect to collagen III (Fig.2), related to the control tendons.

The data obtained in this study confirm that MSCs allograft have a positive effect on tendon healing, its lack of significant immunogenicity permitting allogenic transplantation without immunosuppressive drugs and that the local injection in the tendon allows the survival of MSCs with good engraftment effincency.

1University of Bari Aldo Moro, D.E.T.O., Valenzano, Italy, 2University of Camerino, Department of Veterinary Science, Matelica, Italy

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700043

Wnt5a and Wnt3a alternate to steer embryonic stem cell differentiation into osteoblasts

H. Ding[1], A. Seeliger[1], K.C. Keller[2], N.I. zur Nieden[1,2]

Abstract

Objectives:

Understanding pathways that control normal bone development is imminent for deducing novel therapeutic targets, which could be aimed at disease intervention in the clinic. The Wnt signaling pathway for instance plays a central role in osteogenesis and human disease including bone cancer, with β-catenin (CatnB) being a key mediator of the canonical signaling cascade. In contrast to the classic canonical Wnt ligand Wnt3a, non-canonical Wnt5a may trigger inactivation of CatnB transcriptional activity in a cell context specific manner. While older evidence places canonical Wnt signaling at the regulation of bone formation, newer studies suggest an involvement of Wnt5a in the regulation of mesenchymal commitment. Here, we use murine embryonic stem cells (ESCs), an extremely versatile stem cell that undergoes all developmental stages from an unspecified cell to a mature matrix calcifying osteoblast when differentiated in vitro, as a model to investigate how canonical and non-canonical Wnt ligands affect CatnB levels and regulate proper osteogenic progression.

Results:

Using stable T-Brachyury::GFP and LEF/TCF::GFP ESC lines and recombinant Wnt ligands, we were able to show that CatnB transcriptional activity was up-regulated during early mesendoderm formation concomitantly with T-Brachyury expression (differentiation day 3-5) carried by Wnt3a. In contrast, addition of Wnt5a between differentiation days 5 to 7 led to enhanced matrix calcification and expression of bone specific genes. Western blot analysis of fractionated protein lysates revealed diminished nuclear CatnB levels and decreased LEF/TCF transcriptional activity upon Wnt5a supplementation.
A stable ESC line encoding for a GFP reporter combined with a Wnt5a promoter was then used for FACS sorting to confirm the role of Wnt5a in osteogenic commitment. We were able to show that Wnt5a::GFP+ progenitors sorted on day 7 after differentiation initiation were characterized by an enhanced mineralization capacity when compared to GFP- cells. In addition, a second Wnt3a responsive phase was detected that correlated with subsiding Wnt5a expression and enhanced expression of genes specific for mesenchymal cells.

Conclusions:

In summary, different members of the Wnt family seem to function at different time points during differentiation to enhance osteogenesis.

1Fraunhofer IZI, Department of Cell Therapy, Leipzig, Germany, 2University of California Riverside, Department of Cell Biology and Neuroscience, Riverside, United States

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700044

Expression analysis of MHC molecules and immunosuppressive factors in jaw periosteum-derived cells

D. Alexander[1], M. Rieger[1], N. Ardjomandi[1], S. Reinert[1]

Abstract

For tissue engineering applications it is essential that the used stem cells elicit immunomodulatory functions. It is well known that bone marrow mesenchymal stem cells avoid immune responses by the release of different immunosuppresive factors.
Since jaw periosteal cells (JPCs) represent a suitable stem cell source for bone regeneration in the oral and maxillofacial surgery we analysed the expression of MHC molecules and immunosuppressive factors in untreated, osteogenic and adipogenic differentiated JPCs.
By FACS analysis undifferentiated JPCs were shown to be MHC class I positive, MHC class II negative and they expressed intracellularly intermediate levels of HLA-G being a MHC class I-like protein. After induction of osteogenesis JPCs maintained basically their MHC expression whereasinduction of adipogenesis led to decreased levels of MHC class I and HLA-G expression. By microarray analysis we detected elevated expression levels of immunosuppressive factors such as Galectin-1 (Gal-1) and -3, prostaglandin E2 receptor and synthase, semaphorin 3A and hepatocyte growth factor (HGF) during JPC osteogenesis. These expression patterns were validated by quantitative RT-PCR, proteome arrays and/or western blot.
Our preleminary data show that JPCs might elicit immunosuppressive functions by the release of Gal-1, -3, HGF and HLA-G. Moreover, they maintain their MHC expression during in vitro expansion and osteogenesis but not during adipogenesis.

1University of Tübingen, Oral and Maxillofacial Surgery, Tübingen, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700045

Mesenchymal stem cell - dependent formation of tenton-bone-insertions

V. Seiffart[1], S. Shahab-Osterloh[1], F. Witte[2], A. Hoffmann[1,3], S. Laggies[1], B. Neumann[1], G. Gross[1]

Abstract

Ligaments link bone to bone, whereas tendons connect muscles to bone. Both tissues transmit the forces developed by muscle contractions across joints, stabilize these or produce motion. Tendons originate in muscle (musculo-tendinous junction) and insert into bone forming an osteotendinous junction (OTJ, enthesis). Ligament-to-bone and tendon-to-bone interfaces (entheses, OTJs) serve to dissipate stress between soft tissue and bone. Surgical reconstruction of these interfaces is an issue of considerable importance as they are prone to injury and the integration of bone and tendon/ligament is in general not satisfactory. Soft tissues like cartilage, tendons and ligaments are poorly vascularized and heal slowly. Tendon-to-bone healing is a rather slow process and involves complex biological activities between these nonhomogenous soft and hard tissues.

As a novel mean, to regenerate tendon-bone attachment sites we report here the stem cell-dependent spontaneous formation of fibrocartilaginous and fibrous entheses in heterotopic locations of the mouse if progenitors possess a tenogenic and osteo-/chondrogenic capacity. The tenogenic and osteo-/chondrogenic competence was mediated by viral expression of the biologically active Smad8 signaling mediator (Smad8ca) and of Bone Morphogenetic Protein 2 (BMP2) in murine and human mesenchymal stem cells (MSCs). Modified MSCs were implanted in subcutaneous or intramuscular sites of the mouse.

The stem cell-dependent enthesis formation was characterized histologically by immunohistological approaches and by in situ hybridization. We have already described the Smad8-dependent formation of tendon-like structures before (Hoffmann et al., J. Clin. Invest., 2006). Using this system, we were able to initiate the MSC-dependent generation of fibrocartilaginous tendon-bone junctions of murine MSCs after heterotopic implantations of modified stem cells. Viral modification of human MSCs (hMSCs) exhibited a similar result: hMSCs expressing both the biologically-active Smad8ca and BMP2 generate ectopic tendon-bone insertions, however, without fibrocartilaginous elements. Moreover, it could be demonstrated that Smad8ca expression alone was sufficient for the formation of tendon/ligament-like structures without bony elements.

We conclude that the Smad8/BMP2-dependent modification of MSCs may contribute to regenerative therapies for the stem cell-dependent establishment of tendon-bone attachment sites destroyed in accidents or by chronic inflammatory diseases

1Helmholtz Centre for Infection Research, Signaling and Gene Regulation, Braunschweig, Germany, 2Medical School Hannover, Department of Orthopaedic Surgery, Hannover, Germany, 3Medical School Hannover, Department of Trauma Surgery, Hannover, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research; Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700046

Adipose derived stem cells and Nitinol - a bridge between bone tissue engineering and complete implants

S. Strauß[1], S. Dudziak[2], R. Hagemann[2], K. Reimers[1], S. Barcikowsi[2], D. Kracht[2], P.M. Vogt[1]

Abstract

Aim:

As in vitro creation of large pieces of bone still remains a challenge, use of hybrid-implants offers a bridge between tissue engineering and complete metal or ceramic implants. Regarding flexibility and stability Nitinol is a promising material for bone implants. Adipose derived stem cells (ASCs) carry the potential for osteogenic differentiation and offer an alternative to mesenchymal stem cells as they are easy available from every patient. Pre settlement of an implant with autologous ASCs might prevent inflammatory reactions on the implant and might shorten the time to osseointegration.
The implant structure should allow ingrowth of ASCs and vessels, but still has to fulfill its stabilization duty. So the implant needs a high-resolved inner structure. Selective laser melting (SLM) offers a high-resolution manufacturing process for individual implant design.

Material and methods:

Meshes and cage-like endostructured implants were processed by SLM with web width down to 50µm. Mesh width varied between 250 and 500µm. The implants were colonized with ASCs and cultured for 24h up to 6 weeks. Osteogenic differentiation was induced chemically (by culture media) or mechanically (by application of pressure on the cages in customized clamps). Cells on the Nitinol structures were analyzed by immunfluorescence, histology and scanning electron microscopy.

Results:

ASCs adhere, proliferate and differentiate osteogenically on Nitinol structures. Osteogenic differentiation can be induced chemically or mechanically, which was demonstrated by expression of osteogenic factors as BMP-6. Furthermore, calcium inclusions can be detected. Visual appearance and measured vitality of cells are normal.

Conclusion:

Whether used as full or foamy implant the general problems of Nitinol are the same as with other implant materials:
Long time of osseointegration or insufficient integration, inflammatory reactions, cell death in sponge forms because of insufficient cell supply (lack of blood vessels). These problems could be solved by the combination of a cellular bioactivation of the surface with an alternative implant structure.
As the work at hand shows, osteogenic differentiation of ASCs can be induced by mechanical stress. No predifferentiation in vitro or the use of growth factors in vivo is needed. This might shorten the time to osseointegration in vivo.
Cells show high vitality as the open structure allows optimal nutrition and oxygen supply.
Autologous ASCs and Nitinol are a promising combination for processing customized osteoimplants with a cage-like structure.

1Hannover Medical School, Department for Plastic-, Hand- and Reconstructive Surgery, Hannover, Germany, 2Laserzentrum Hannover e.V., Material and Process Technology, Hannover, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research; Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700047

How the nervous system uses substrate elasticity as a control element to modulate the hematopoietic system during mobilization

C. Lee-Thedieck[1,2], N. Rauch[3,] R. Fiammengo[1], G. Klein[4], J.P. Spatz[1,2]

Abstract

Objectives:

In the bone marrow, hematopoietic stem cells (HSC) reside in endosteal and vascular niches. Their interactions with the niches are essential for the maintenance of HSC number and properties. The molecular composition and the matrix elasticity of the microenvironment play important roles in the differentiation of adult and embryonic stem cells. However, the mechanisms responsible for how elasticity plays a role in the HSC niche are still unclear. Osteoblasts, the major cellular component of the endosteal HSC niche, flatten during HSC mobilization when stimulated by the nervous system. We hypothesized (I) that a flat osteoblast during mobilization has different elastic properties from a “normal” osteoblast under steady-state conditions and (II) that the HSC in contact with the osteoblasts can react after sensing this change in elasticity during mobilization.

Material and methods:

KG-1a cells were obtained form DSMZ and human hematopoietic stem and progenitor cells were isolated from umbilical cord blood after informed patients consent. Confocal and atomic force microscopy were applied in a simplified in vitro model of the endosteal stem cell niche in order to investigate the cell height and elasticity of osteoblasts. Polyethylene glycol diacrylate hydrogels with tunable elasticity were developed and applied for cell adhesion and migration studies.

Results:

We could show, that the osteoblast flattening during mobilization is accompanied by osteoblast stiffening, demonstrating that during mobilization not only biochemical but also mechanical properties of the niche are modulated. HSC are able to sense differences in the elasticity of their underlying substrate and therefore respond with altered adhesion and migration, which could facilitate the exit of HSC from the niche.

.**Conclusion:**

Concluding from our data, we propose the following model for how the nervous system uses the elasticity of the osteoblasts in the endosteal HSC niche to modulate HSC adhesion and migration: During mobilization, signals from the nervous system lead to osteoblast flattening and stiffening. The HSC in the niche respond to the change in elasticity of their cellular support with increased adhesion and migration, which facilitates the egress of HSC from their niche. Thus, matrix elasticity is an important factor in regulating the retention of HSC in the endosteal niche and should be considered in attempts to propagate HSC in vitro for clinical applications.

1Max Planck Institute for Intelligent Systems, Department of New Materials and Biosystems, Stuttgart, Germany, 2University of Heidelberg, Department of Biophysical Chemistry, Heidelberg, Germany, 3South Westphalia University of Applied Sciences, Iserlohn, Germany, 4University of Tübingen, Center for Medical Research, Section for Transplantation Immunology and Immunohematology, Tübingen, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research; Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700048

Neuroprotective role of 17β-estradiol administration on altered age related neuronal parameters in female rats

P. Kumar[1], R.K. Kale[1], N.Z. Baquer[1]

Abstract

Objectives:

During normal aging, brain experiences structural, molecular, and functional alterations. Aging in females and males is considered as the end of natural protection against age related diseases like osteoporosis, coronary heart disease, diabetes, Alzheimer's disease and Parkinson's disease. Protection from age-related disorders is provided by several factors, including estrogens. These changes increase during menopausal condition in females when the level of estradiol is decreased.
The objective of this study was to observe the changes in activities of superoxide dismutase (SOD), glutathione S-transferase (GST), Ca2+ATPase, intracellular calcium levels, DNA degradation and glucose transporter 4 (GLUT4) occurring in brains of female albino Wistar rats of 3 months (young), 12 months (adult) and 24 months (old) age groups, and to see whether these changes are restored to normal levels after exogenous administration of estradiol.

Methods:

The aged rats (12 and 24 months old) (n= 8 for each group) were given subcutaneous injection of 17-β-estradiol (0.1µg/g body weight) daily for one month. Controls animals received The aged rats (12 and 24 months old) (n= 8 for each group) were given subcutaneous injection of 17-β-estradiol (0.1µg/g body weight) daily for one month. Controls animals received an equal volume of vehicle. After 30 days of hormone treatment experimental animals of all the groups were sacrificed and brains were isolated for further study.

Results:

The results obtained in the present work revealed that normal aging was associated with significant decrease in the activities of Na+K+ ATPase, SOD, GST, Ca2+ATPase and GLUT4 levels in the brains of aging female rats, and an increase in DNA degradation and intracellular calcium levels. Administration of E2 brought these changes to near normalcy.

Conclusions:

It can therefore be concluded that E2's beneficial effects seemed to arise from its antioxidant and antilipidperoxidative effects, implying an overall neuroprotective and anti-aging action. The results of this study will be useful for pharmacological modification of the aging process and applying new strategies for control of age related disorders.

1Jawaharlal Nehru University, New Delhi, India

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research; Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700049

Fish stem cell biology a feed back from applied research

P. Stahlschmidt-Allner[1], B. Allner[1], D. Steinhagen[2], M. Proepsting[2], M. Latz[1]

Abstract

Objectives:

Seasonal spawning and ambisexual (sex changing) teleost fish species show postembryonic stem cell based differentiation processes which are thought to be strictly related to foetal (intraovum and intrauterine) development of homeothermic vertebrates. Seasonal gonadal recrudescens and the initial steps of heterologous gonadal tissue development in the course of sex change share common features. Both processes are based on motile stem cells. These cells can be isolated from fish brain and seem to be of neuroectodermal origin.

Methods and results:

Histological examination of fish during the gonadal recrudescent or sex inverting period and primary cell cultures derived from fish of the corresponding status show that migrating stem cells are connected to their origin via tunnelling nanotubes / filodia in the 10 mm range. In appropriate cell culture systems an intra syncytial transport of granula and a extra cellular transport of cytoplasm components originating from the primary cells along the tunnelling nanotubes is recognizable.Although these cells are derived from somatic adult fish transcription of genes which are thought to be strictly related to embryonic development of oviparous vertebrates could be observed in stem cell bearing tissues and corresponding tissue cultures. Parallel investigation of the Koi Herpes Virus (Cy HV- 3) disease of carp suggests that stem cells under investigation are the primary target and virus depot in latent infected fish.

Conclusions:

We conclude that postembryonic proliferation of stem (germ) cells is under the control of a long distance signalling pathway. The results implicate that spatial and temporal separation of gene amplification -transcription and -translation could play a role in control of adult stem cell and stem cell transmitted viral infections. The robustness and reproducibility of our tissue culture systems represent a promising and innovative tool to investigate the physiological integration of stem cells with embryonic characteristics on the organisational level of a somatic adult organism.

1Institut für Gewässerökologie und angewandte Biologie GOBIO-GmbH.de, Zellbiologie, Aarbergen, Germany, 2University of Vet. Medicine, Centre for Infection Medicine, Fish Disease Research Unit, D- Hannover, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research; Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700050

Human skin-derived mesenchymal stem cells induce the differentiation of naive helper T cells into FoxP3+ regulatory T cells without CD28-costimulation

K. Pfisterer[1], C. Vaculik[1], D. Strunk[2], A. Elbe-Bürger[1]

Abstract

We have found that phenotypically-defined, plastic-adherent human dermal mesenchymal stem cells (MSCs) have immunomodulatory capacities. To unravel, whether regulatory T cells are involved, total adherent dermal cells, adherent dermal MSC subsets and, as a control, bone marrow-derived (BM-) MSCs, which all lack the expression of CD80/86, were subjected to functional in vitro assays with CD25-depleted naive helper T (Th) cells and stimulation with αCD3 or αCD3/CD28 mAbs. Using flow cytometry, T cell proliferation was visualized via carboxyfluorescein succinimidyl ester dilution and the differentiation of dividing cells into regulatory T cells was evaluated by counterstaining of the transcription factor FoxP3. Stimulation of naive Th cells with αCD3/CD28 and coculture with dermal cells or BM-MSCs led to a marginal increase of FoxP3-expressing T cells, while stimulation with αCD3alone significantly increased the FoxP3 expression from a value of 4.09%±1.26 in stimulated T cells alone, to 15.36%±1.81 after coculture with dermal cells ($P < 0.003$, n=7) and 10.98%±2.0 with BM-MCSs ($P < 0.02$, n=6). Interestingly, CD90+ dermal cells induce more FoxP3 expression in Th cells than CD90- cells (19.14%±4.02 vs. 8.11%±1.70, n=3). In line with this observation, a higher percentage of FoxP3-expressing Th cells were detected upon coculture with CD271- dermal cells (> 90% coexpressed CD90) compared with CD271+ cells (50-70% coexpressed CD90; 14.56%±2.54 vs. 9.62%±2.56, n=3).
Our data suggest that skin-resident MSCs may coordinate the interactions that are necessary to initiate the induction and proliferation of regulatory T cells to maintain the immune homeostasis in human skin and to prevent excessive immunopathology.

1University of Vienna, Department of Dermatology, Division of Immunology, Allergy and Infectious Diseases (DIAID), Vienna, Austria, 2Medical University of Graz, Department of Hematology, Graz, Austria

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research; Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700051

Umbilical cord blood derived very small embryonic-like stem cells lack stem cell properties

R. Danova-Alt[1,2], A. Heider[2], D. Egger[1], M. Cross[3], R. Alt[1,2]

Abstract

Very small embryonic-like (VSEL) cells have been described as putatively pluripotent stem cells present in murine bone marrow and human umbilical cord blood (hUCB) and as such are of high potential interest for regenerative medicine. However, there remain some questions concerning the precise identity and properties of VSEL cells, particularly those derived from hUCB. For this reason, we have carried out an extensive characterisation of purified populations of VSEL cells from a large number of UCB samples. Consistent with a previous report, we find that VSEL cells are CXCR4+, have a high density, are indeed significantly smaller than HSC and have an extremely high nuclear/cytoplasmic ratio. Their nucleoplasm is unstructured and stains strongly with Hoechst 33342 . A comprehensive FACS screen for surface markers characteristic of embryonic, mesenchymal, neuronal or hematopoietic stem cells revealed negligible expression on VSEL cells. These cells failed to expand in vitro under a wide range of culture conditions known to support embryonic or adult stem cell types and a microarray analysis revealed the transcriptional profile of VSEL cells to be clearly distinct both from well-defined populations of pluripotent and adult stem cells and from the mature hematopoietic lineages. Finally, we detected an aneuploid karyotype in the majority of purified VSEL cells by fluorescence in situ hybridisation. These data support neither an embryonic nor an adult stem cell like phenotype, suggesting rather that hUCB VSEL cells are an aberrant and inactive population that is not comparable to murine VSEL cells.

1Vita34 AG, Leipzig, Germany, 2Universität Leipzig, Translationszentrum für Regenerative Medizin, Leipzig, Germany, 3Universität Leipzig, Abteilung für Hämatologie, Leipzig, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research; Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700052

Human platelet lysate gel provides a novel 3D-matrix for enhanced culture expansion of mesenchymal stromal cells

G. Walenda[1], H. Hemeda[1], R. K. Schneider[2], W. Wagner[1]

Abstract

Cell culture protocols have specific requirements in regenerative medicine. Human platelet lysate (HPL) has proven as effective substitute for fetal calf serum (FCS) without the risk of xenogeneic immune reactions or transmission of bovine pathogens. Heparin needs to be added as anticoagulant before addition of HPL to culture medium - otherwise HPLmedium forms a gel within few hours. Here, we demonstrated that such HPL-gels provide a suitable 3D-matrix for cell culture which - apart from heparin - consists of the same components as the over-layered culture medium.

Mesenchymal stromal cells (MSC) grew in several layers at the interface between HPL-gel and HPL-medium without contact to any artificial biomaterials. Notably, proliferation of MSC was much higher on HPL-gel compared to tissue culture plastic (TCP).

Furthermore, the frequency of initial fibroblastoid colony forming units (CFU-f) was increased on HPL-gel. The viscous consistency of HPL-gel enabled passaging with a convenient harvesting and re-seeding procedure by pipetting of cells together with their HPL-matrix - this method does not require washing steps and can easily be automated. Alternatively, plasmin could be added to dissolve HPL-gels. The immunophenotype and in vitro differentiation potential of MSC were not affected by culture-isolation on HPL-gel. Taken together, HPL-gel has many advantages over conventional plastic surfaces: it facilitates enhanced CFU-f outgrowth, increased proliferation rates, higher cell densities and non-enzymatic passaging procedures for culture expansion of MSC.

1Helmholtz-Institute for Biomedical Engineering; Stem Cell Biology and Cellular Engineering, RWTH Aachen University Medical School, Aachen, Germany, 2Institute of Pathology, RWTH Aachen University Medical School, Aachen, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research; Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700053

Expansion of cord blood-derived hematopoietic stem cells with different polymers

M.V. Ferreira[1], N. Labude[1], G. Walenda[2], W. Wagner[2], D. Piroth[3], T. Hieronymus[4], M. Zenke[4], W. Jahnen-Dechen[5], S. Neuss[1,5]

Abstract

Over the last decade, cord blood (CB) has become a viable option for haematopoietic stem cell (HSC) transplantation. The use of CB is hampered by the insufficient number of SC per graft required for adult patients. Therefore, several strategies have been followed in order to ex vivo expand the number of HSC.

Here we use biomaterials for CB-HSC expansion. First, seventeen degradable biopolymers and synthetic polymers from our biomaterial bank (Neuss et al., Biomaterials 2008) were assessed for basic compatibility. Viability, cytotoxicity and apoptosis were analyzed using a standardized method (Ferreira MV et al., Journal of Biomolecular Screening 2011).

A group of six compatible materials was selected further. PVDF, Texin, Resomer LR704, Resomer RG503, PCL and Fibrin were used to culture HSC during 7 days. Long-term viability, cytotoxicity and apoptosis were measured. CFSE staining was used to evaluate cell proliferation on the material surfaces. Together with CFSE, the immunophenotype of the cultured cells was analyzed by using the progenitor markers CD133, CD38 and CD34. Colony-forming unit assays and morphology (SEM) were also evaluated. Hematopoietic reconstitution of NOD/SCID mice is currently under assessment (Ferreira MV, Biomaterials 2011, submitted).

To evaluate the contribute of each biomaterial to the potential of expansion, proliferation curves (CellTiter-Blue® Cell Viability Assay) were created for HSC cultured over a period of 17 days in each of the materials, either using a standard culture cocktail or a previously optimized cocktail (Ferreira MV, Journal of Tissue engineering and Regenerative Medicine 2011, in revision). So far, HSC cultured in fibrin proliferate better and exhibit a more primitive phenotype in comparison to the control (TCPS). In the future, 3D scaffolds will be tested for HSC expansion.

1RWTH Aachen University, Institute of Pathology, Aachen, Germany, 2RWTH Aachen University, Helmholtz Institute for Biomedical Engineering, Aachen, Germany, 3RWTH Aachen University, Department for Gynecology, Aachen, Germany, 4RWTH Aachen University, Helmholtz Institute for Biomedical Engineering, Department of Cell Biology, Aachen, Germany, 5RWTH Aachen University, Helmholtz Institute for Biomedical Engineering, Biointerface Group, Aachen, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research; Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700054

Ex-vivo large scale expansion of human RBCs from hematopoietic progenitors on a novel nanofiber scaffold mimicking bone marrow milieu

B. Ramesh[1], C. Kotturathu Mammen[2], S. Guhathakurta[3]S. Neuss[1,5]

Abstract

Introduction:

Various transmittable diseases from donor blood led to explore in-vitro manufacturing, cost effective, clinically applicable RBCs in large scale with several defined protocols worldwide. Commercially available alternative blood substitutes and medicines to increase the blood constituents are unstable with less oxygen carrying capacity and have not been successful in replacing donor derived RBCs. Large scale production of RBCs in-vivo depends on potential cell sources, bio-physiological parameters, erythroid inducers and sterile, non reactive, non -tumorogenic expansion media, to produce stable and functionally efficient RBCs. We formulated an efficient bone marrow milieu considering the physiological factors, favouring the differentiation of haematopoietic progenitors to clinically applicable and pathogen free mature RBCs.

Method:

The mononuclear cells were isolated from cord blood and bone marrow of living human donor with a pre-informed consent. The cells were nourished with media derived from human plasma of same blood group, bone marrow milieu and erythropoietic differentiation inducers (following laboratory proprietary protocol). The Temperature, Oxygen concentration, pH, Osmolarity of the cultures were maintained optimally. Finally, the cultured RBCs were subjected to various assays for cell proliferation, differentiation, haemoglobin content, and structural and functional properties of cultured RBCs such as oxygen affinity and dissociation ability etc.

Results:

The day 4 onward burst colony forming units were observed proving the erythroid lineage , which was monitored and documented on daily basis till the transformation happened from nucleated to enucleated adult RBC formation on 17th day. The scalability of the functional RBCs appeared to be 15 times of the originally seeded mononuclear cell number (2x106/ml of culture media) in the culture flask and it was reproducible. The cells were in majority mature RBCs (95% by FACS) with adult haemoglobin, with trivial presence of other lineages. The adult haemoglobin had been proved by haemoglobin electrophoresis. Cultured RBCs were morphologically and functionally efficient. The scalability of production was assured in bioreactor culture expansion with indigenous novel biocompatible nano-fiber scaffold. Oxygen affinity and dissociation were efficient with five sets of experiments.

Conclusion:

This method of producing RBCs in large scale with a simple in-vitro reproducible protocol for making scalable and functional RBCs which is devoid of WBCs would be advantageous for frequently transfused patients, who are suffering from blood dyscrasias and exsanguination due to severe injury.
Further in-vivo stability evaluation with various animal experiments and clinical trials would make the possibility of clinically applicable, off the shelf packed RBCs which do not require screening for viral diseases as the cell source and media are pre-tested.

1Frontier lifeline pvt ltd, Stem cell and Tissue engineering, Chennai, India, 2Frontier lifeline pvt ltd, Cardiac surgery, Chennai, India, 3Frontier lifeline pvt ltd, Chennai, India

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research: Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700055

Hematopoietic-mesenchymal stem cell interactions in a three-dimensional culture system of the bone marrow niche

I. Leisten[1], M.V. Ferreira[1], W. Wagner[2], R. Knüchel[1], S. Neuss[1], R.K. Schneider[1]

Abstract

Mesenchymal stem cells (MSC) control hematopoietic stem cell (HSC) differentiation and proliferation by production of growth factors/cytokines, extracellular matrix (ECM) as well as cell-cell-interactions. It was shown that the co-culture of bone marrow-derived MSC (BM-MSC) with umbilical cord HSC (UC-HSC) can be utilized for cord blood expansion purposes as they preserve the necessary hematopoietic microenvironment. For clinical transplantation, it may be preferred to obtain HSC and MSC from the same donor, thereby eliminating complications resulting from a HSC and MSC genetic mismatch. In a previous study, we established a three-dimensional bone microenvironment using MSC and a collagen matrix (Schneider et al., Biomaterials 2010). In the current study we analyzed the potential of UC-MSC to act as a stromal support for UC-HSC compared to BM-MSC in our established three-dimensional bone marrow niche.

As previously described, we generated 3D-collagen gels with and without embedded UC- and BM-MSC. Enrichment for CD34+ cells from umbilical cord bloodwas performed by using immunomagnetic beads. We analyzed cell divisions of HSC by using cell division tracking with CFSE. Further, we analyzed HSC migration and immunophenotypic differentiation as well as remodelling of the extracellular matrix by MSC. We demonstrated a positive influence of the cell-free, three-dimensional collagen gel on HSC proliferation in comparison to monolayer culture.

The proliferation rate was even enhanced when HSC were co-cultured on collagen gels containing UC-MSC or BM-MSC.Nevertheless, the co-culture of HSC and UC-MSC also accelerated the differentiation of HSC. CD34 expression decreased after a cultivation period of up to 14 days, whereas CD38 expression was up-regulated as well as the expression of the differentiation markers CD13, CD45 and CD56. In contrast, in co-culture with BM-MSC the more primitive CD34(+) CD38(-) fraction of HSC was maintained in longterm-culture. Histological analysis demonstrated that UC-MSC as well as BM-MSC might have a chemotactic influence on HSC as migration into the collagen gel was accelerated by MSC. Migrated HSC showed the typical cobblestone-like pattern of proliferating hematopoietic progenitor cells in the collagenous matrix. Besides, HSC maintained their differentiation potential in the collagenous matrix and showed signs of maturation. Interestingly, in particular UC-MSC caused intensive ECM remodelling (immunohistochemistry) and produced a dense matrix of fibronectin, osteopontin and collagen I, typical for the bone marrow niche.

Concluding, in our three-dimensional culture system BM-MSC support in particular the maintenance of the primitive CD34+ HSC subpopulation in long-term culture while UC-MSC accelerate proliferation, differentiation and matrix remodelling. Our culture system is an optimal system for studying HSC differentiation, proliferation and matrix remodelling in physiological and pathophysiological processes.

1University Hospital RWTH Aachen, Institute of Pathology, Aachen, Germany, 2University Hospital RWTH Aachen, Helmholtz-Institute for Biomedical Engineering, Aachen, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research; Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700056

Adenovirus-mediated expression of the NF-E2-related factor 2(Nrf2) protein within the MSCs alleviated cisplatin-induced acute kidney injury in rat

M. Habibi Roudkenar[1], M. Mohammadzadeh[1]

Abstract

Background and objectives:

Poor MSCs survival after transplantation is one of the major challenges in their therapeutic application. Therefore, it is necessary to augment MSCs in order to improve the efficacy of cell therapy. In this study, we manipulated MSCs with a cytoprotective factor i.e Nrf2 and evaluate its efficiency flowing MSCs transplantation to cisplatin-induced acute kidney injury in a rat model.

Materials and methods:

Mesenchymal stem cells were isolated from bone marrow. Nrf2 was isolated and TOPO cloned into the pENTR vector. The recombinant vector was transfered into pAD/CMV/V5-DEST vector by gateway technology. Recombinant adenovirus was produced in AD293 cells, followed by infecting into MSCs. The Nrf2 engineered MSCs were exposed to hypoxic, serum deprived and oxidative stress conditions followed by evaluation of the cells viability and apoptosis. Finally, Nrf2-MSCs were transplanted to cisplatin-induced acute kidney injury in rats and their therapeutic efficacy was assayed by blood urea nitrogen, creatinine determination and morphologic analysis.

Results:

Nrf2 was successfully expressed in MSCs. Nrf2-MSCs retained their multi-differentiation capacity. Comparing to the controls, following exposure of the Nrf2 transduced cells to hypoxia, serum deprivation and oxidative stress conditions, cell viability was found to be higher, while their apoptosis rate was lower. Nrf2 alleviates cisplatin-induced acute kidney injury.

Conclusions:

Our findings are good demonstration of how an understanding of the cellular stress response can be leveraged for practical application. Enhanced anti-apoptotic and anti oxidative capabilities of MSCs following Nrf2 infection via adenoviral vectors, could be a graft cell death prevention strategy in transplantation and may emerge an alternative plan for stem cell therapy.

Key words:

Mesenchymal stem cells, Nrf2, Adenoviral Vector, Oxidative stress, Cisplatin, Kidney injury

1Research Center, Iranian Blood Transfusion Organization, Tehran, Iran, Islamic Republic of

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research; Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700057

In vitro generation of pancreatic islet bodies from mouse embryonic stem cells is increased using Activin A and a 3D-Differentiation model

A. Hommel[1], D. Borg[2], A. Meinhardt[3], I. Burtscher[4], H. Lickert[4], E. Tanaka[3], E. Bonifacio[2]

Abstract

Transplantation of human islets can cure type 1 diabetes, but amongst other factors, the shortage of donor islets limits its wide-scale application. Differentiation of beta cells from pluripotent sources is an attractive alternative source, but the efficiency of in vitro differentiation protocols is limited. We established a robust in vitro process to direct mouse-derived pluripotent embryonic stem cell (mESC) differentiation into pancreatic islet bodies. Two factors proved effective in increasing differentiation efficiency. First, the addition of a high concentration of Activin A during embryoid body formation increased differentiation into definitive endoderm (DE). Using a transgenic mouse cell line carrying a Sox17-Cherry fusion (SCF) knock-in, we were able to analyze the amount of DE cells by fluorescence- activated cell sorting. Activin A increased the number of SOX17-positive cells approximately 2-fold compared to the control group without Activin A treatment. A second factor that markedly increased islet body differentiation was the use of 3D culture.
Embryoid bodies enriched for definitive endoderm were transferred into 3D culture using Matrigel. By day 28 of differentiation, these three-dimensional islet bodies expressed pancreatic lineage markers PDX1, NGN3 and C-Peptide, and a yield of seven islet bodies per EB were obtained. Whilst the generation of complex three-dimensional organ tissues in vitro remains a major challenge for translational studies, it is hoped that similar modifications may also improve differentiation of beta cells from human ES and iPS cells and eventually allow for transplantation of pluripotent derived islet bodies.

1DFG-Center for Regenerative Therapies Dresden, Preclinical Approaches to Stem Cell Therapy / Diabetes, Dresden, Germany, 2DFG-Center for Regenerative Therapies Dresden, Dresden, Germany, 3Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany, 4Institute of Stem Cell Research, Helmholtz Zentrum München, Munich, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research: Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700058

Human and rat tendons harbour a population of insulin producing cells expressing stem- and pancreatic ß- cell associated markers

C. Lehner[1], R. Gehwolf[2], C. Hirzinger[1], P. Augat[3], D. Stephan[3], A. Wagner[4], H. Resch[1], H.-C. Bauer[2,4], H. Tempfer[2]

Abstract

Diabetes and obesity are risk factors for tendinopathy, tendon injury and impaired tendon healing. So far, the mechanisms of tendon impairment in diabetic patients remain a matter of debate. Altered metabolic parameters as well as changes in the vasculature may play a role.

Along our search for stem cells suitable for tendon repair, we discovered a tendon-derived cell type readily differentiating towards insulin producing cells in vitro. Here we describe a population of tendon cells expressing the pancreatic ß-cell associated proteins Insulin and Glucagon in vivo.

Tissue samples from intact human biceps-, supraspinatus, and semitendinosus tendons were obtained with patients´ informed consents, tissue donors were aged from 23-63 (n=5) years.

By immunocytochemistry, Laser Capture Microdissection and quantitative PCR we show that these cells are mainly located in the perivascular area, but also in the dense, collagenous parts of tendon tissue. Single cell PCR analysis of isolated tendon perivascular cells reveals a coexpression of Insulin with the stem cell-associated markers Oct4 and Nanog.Both Insulin and Insulin-related mRNA are significantly reduced in Achilles tendons of rats treated with 60µg/kg of the ß-cell specific cytostatic agent Streptozotocin 5 days after the treatment. Biomechanical testing revealed that this treatment also results in a significant (p=0,018) reduction in tendon tensile strength of 39,9% (n=12) after 5 days.

In our hands, enzymatically released tendon cells neither show glucose-dependent insulin secretion nor staining with Dithizone, a zinc-chelating agent commonly used for the detection of pancreatic ß-cells.

These findings indicate that, despite several similarities with pancreatic ß-cells, these tendon- derived cells have different properties and functions.

Whether these cells are affected by changing metabolic parameters or diabetes, or whether these cells may be a potential source for tissue engineering or cell therapy in diabetes treatment will have to be elucidated by further experiments.

1University Hospital Salzburg, Department of Trauma Surgery and Sports Medicine, Salzburg, Austria, 2Paracelsus Medical University, Applied Cell Biology Unit, Salzburg, Austria, 3Trauma Center Murnau, Biomechanics Laboratory, Murnau, Germany, 4University of Salzburg, Department of Organismic Biology, Salzburg, Austria

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research: Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700059

Diabetic glucose impairs embryonic stem cell osteogenic differentiation through persistent activation of Akt

K.C. Keller[1], D.D. Ehnes[1], A. Dienelt[2], N.I. zur Nieden[1,2]

Abstract

Objectives:

The mammalian embryo is said to have a high amount of plasticity, as observed by the ability of the embryo to develop in a wide variety of conditions in vitro. Following fertilization, many changes occur to an early stage embryo in regards to metabolism and embryonic nutrition. The requirement for glucose (Glc) during development is thought to be for the biosynthesis of macromolecules such as phospholipids and nucleic acids, however studies have shown that diabetic Glc levels can be toxic to the developing embryo. For instance, newborns of diabetic mothers may exhibit skeletal malformations.
Embryonic stem cells (ESCs) are pluripotent cells that are capable of modeling osteogenesis and thus often serve researchers to study embryonic skeletal development. Against this background, we aimed to gain insight into the molecular mechanism whereby Glc may influence the process of osteogenesis.

Methods:

Murine ESCs were differentiated in media containing either physiological (1.0 g/l) or diabetic (4.5 g/l) concentrations of D-glucose while simultaneously being triggered into osteogenesis using 1alpha,25(OH)2 vitamin D3. The osteogenic differentiation ability of the cells was evaluated by histochemical stainings, quantitative bone marker gene expression and flow cytometric analyses at distinct time points of maturity. In addition, the calcification of the secreted extracellular matrix was quantified and signaling pathway activation studied.

Results:

mRNA expression and van Gieson staining suggested that more collagen was produced and secreted by cells differentiated in diabetic conditions, while matrix calcification was severely impaired, suggesting that cells were prevented from maturation. Instead, cells differentiated in physiological Glc conditions showed normal osteogenic progression, including stage specific activity of alkaline phosphatase. Follow-up studies then revealed that the Glc-mediated osteogenic impairment was already detectable at the level of an osteoprogenitor state. Western blotting then allowed us to correlate persisting Col I mRNA expression to elevated protein levels of AKT and activated AKT (phosphor-S473), which has been previously reported to control Col I mRNA expression in other cell types. Stage-specific blockage of AKT activation using the AKT inhibitor 124005 restored normal Col I mRNA levels and led to a calcification rescue in diabetic conditions.

Conclusions:

In conclusion, we were able to show that diabetic Glc inhibits the formation of matrix calcifying osteoblasts in embryonic stem cell cultures possibly through AKT mediated persistent expression of Col I mRNA.

1University of California Riverside, Department of Cell Biology and Neuroscience, Riverside, United States, 2Fraunhofer IZI, Department of Cell Therapy, Leipzig, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research; Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700060

Mesenchymal stem cells - Key players in vascular calcification of chronic kidney disease patients?

R.K. Schneider[1], R. Knüchel[1], W. Jahnen-Dechent[2], J. Floege[3], R. Kramann[3]

Abstract

Vascular calcification in chronic kidney disease (CKD) patients has emerged as a tightly regulated, coordinated and osteoblastic process resembling bone morphogenesis The current study is based on the hypothesis that mesenchymal stem cells (MSC) constitute critical cells for pro-calcific ECM remodeling in CKD patients.Human MSC were cultured in media supplemented with pooled sera from either healthy or uremic patients (20%). Exposure to uremic serum enhanced the proliferation of MSC (cell counting, BrdU incorporation) whereas apoptosis and necrosis were not affected (annexin V and 7-AAD staining). Uremic serum exposed MSC recapitulated osteogenesis by matrix calcification and expression of bone-related genes (BMP2-receptor, ALP, osteopontin, Runx2) within 35 days. The uremic serum-induced osteogenesis was shown to be BMP2/4 dependent and was completely blocked by a BMP2/4 neutralizing antibody or its natural antagonist .

NOGGIN. Calcification and matrix remodelling were further analysed in a collagen-embedded osteogenesis model recapitulating the vascular collagen I/III environment.

The uremic serum induced calcification was shown to occur along collagen fibres as shown by SEM, energy-dispersive X-ray spectroscopy and von Kossa staining and was accompanied by extensive matrix remodelling.

MSC acquired a myofibroblastic phenotype, contracted the collagenous matrix and extensively remodelled the collagenous matrix by producing components of the ECM.

These changes in the artificial vascular wall were comparable to remodelling process observed in arteries of CKD patients (n=12) compared to vessels of young children (n=10).

Concluding, uremic serum induced in a BMP2/4-dependent manner an osteoblast-like phenotype in MSC accompanied by matrix remodelling and calcification comparable to the remodelling and calcification in CKD patients' arteries.

1 RWTH Aachen University, Institute of Pathology, Aachen, Germany, 2 RWTH Aachen University, Institute for Biomedical Engineering, Biointerface Laboratory, Aachen, Germany, 3 RWTH Aachen University, Department of Nephrology and Clinical Immunology, Aachen, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700061

Efficient ZFN-based gene targeting in transgenic human iPS cells as a model for gene editing in patient-specific cells

S. Merkert[1], K. Kahn[2], K. Schwanke[1], J. Meyer[2], T. Cathomen[2], U. Martin[1]

Abstract

Gene targeting by homologous recombination via customized zinc-finger nucleases (ZFN) is a powerful method to manipulate the genome and correct genetic defects. Although efficiency of ZFN based homologous recombination has been shown to be significantly higher than by means of conventional gene targeting, the selection of suitable clones still requires cells that proliferate in culture. Clinically applicable ZFN-based gene correction in patient-specific cells was hardly possible so far, due to the inability to sufficiently expand most adult (stem and progenitor) cells *in vitro*. However, the availability of human induced pluripotent stem (hiPS) cells with their almost unlimited potential for proliferation and differentiation now offers novel opportunities for the development of patient-specific regenerative therapies. As a first step towards ZFN-based gene targeting, a non-viral gene-transfer in human iPS cells with transfection rates of up to 80% and high cell vitality was established. Aiming at the development of a general ZFN-based recombination approach, transgenic human iPS cell clones stably expressing eGFP under an ubiquitous promoter were generated to investigate the functionality of an eGFP specific ZFN. For proof of principle we initially knocked out the eGFP via non-homologous end joining and achieved up to 3% $eGFP^{neg}$ cells. For the establishment of a general applicable protocol for ZFN based site-specific recombination an appropriate eGFP targeting vector encoding for RedStar was generated. Successful gene targeting resulted in $eGFP^{neg}$ $RedStar^{pos}$ human iPS cells and could be achieved with an efficiency of up to 1%. Hence we generated $eGFP^{neg}$ $RedStar^{pos}$ human iPS cell clones. So far these clones show stable RedStar expression for up to 20 passages and could be verified for specific integration via PCR analysis. Ultimately, the development of a generally applicable protocol for ZFN based site-specific recombination and gene correction in patient-specific hiPS cells may enable the development of cellular therapies for various genetic diseases.

[1]*Hannover Medical School, LEBAO, Hannover, Germany,* [2]*Hannover Medical School, Experimental Hematology, Hannover, Germany*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700062

Induced pluripotent stem cells of the common marmoset monkey

A. Wiedemann[1], E.C. Bunk[2], I. Bernemann[3], C. Figueiredo[1], S. Honndorf[1], J.C. Schwamborn[2], A. Schambach[4], R. Blasczyk[1], T. Müller[1]

Abstract

Objective:

Regenerative medicine is in need of a solid animal model as a link between rodents and human to evaluate functionality, immunogenicity and clinical safety of embryonic stem cells (ESCs) or reprogrammed cell types. The common marmoset monkey (*Callithrix jacchus*) is an excellent large animal model, genetically closely related to the human and readily used worldwide in clinical research. Besides the studies on marmoset ESCs, our group is focusing on reprogramming somatic cells of the common marmoset to explore their potential for preclinical studies.

Material and methods:

For reprogramming bone-marrow derived mesenchymal cells of the common marmoset were used. In the presence of TAV, SB431542, PD0325901 and ascorbic acid, the cells were reprogrammed via a lentiviral SFFV driven quad-cistronic vector system in mTESR medium and transferred onto mouse embryonic feeder cells at day 21.

Results:

The cells obtained showed typical ESC-like morphology and were positive for alkaline phosphatase and the endogenous pluripotency markers Oct3/4, Nanog, Sox2, KLF4 and MYC while exogenous genes were downregulated. From passage 19 on cells were also differentiated successfully to embryoid bodies. Marker genes and morphology of early neuronal progenitors, adipocytes, chondrocytes, osteogenic cells and megakaryocytes could be observed so far.

Conclusions:

These cells appear to be fully reprogrammed iPS cells of the common marmoset which display pluripotency markers and can be differentiated into various cell types. They are a promising tool for animal models close to human for preclinical studies in the field of regenerative medicine.

[1]*Institute for Transfusion Medicine, Hannover Medical School, Hannover, Germany,* [2]*Institute of Cell Biology, Stem Cell Biology and Regeneration group, University Muenster, Muenster, Germany,* [3]*Institute for Multiphase Processes, Leibniz University Hannover, Hannover, Germany,* [4]*Institute for Experimental Hematology, Hannover Medical School, Hannover, Germany*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research: Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700063

Expansion of human pluripotent stem cells (hPSC) in controlled, stirred bioreactors

R. Olmer[1], U. Martin[1], R. Zweigerdt[1]

Abstract

Therapeutic application of pluripotent stem cells and their derivatives requires large quantities of cells generated in defined conditions which cannot be produced via adherent culture. Standardized fully controlled stirred tank reactors are widely used in biopharmaceutical industry for culture volumes of up to 10.000 L. However, standard cell lines used for the production or recombinant factors are usually homogenous and robust and therefore relative easy to expand. In contrast, cultures of hPSC (including hiPSC/hESC) are much more demanding, particularly regarding maintenance of pluripotency and translation to suspension. A direct transfer of these complex cultures to established stirred bioreactor systems is therefore challenging. In recent publications we have identified a defined culture medium that resulted in up to six-fold increase in cell numbers within 4-7 days [1-3] The culture system is based on initial single cell dissociation which is critical for standardized process inoculation of suspension cultures. Cells maintained a stable karyotype, expression of pluripotency markers and their potential to differentiate into derivatives of all three germ layers[1-3]. Here we will present unpublished results on successful process translation to stirred bioreactors. These data provide key steps to establish scalable mass expansion of hiPS/hES aiming at the translation of stem cell research to clinical practice. Experiments to optimize culture conditions regarding control of cell aggregation, feeding strategies and other parameters will also be presented.

[1]Hannover Medical School, Lebao, Hannover, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700064

Development of buffalo embryonic stem cell colonies on synthetic matrices in different culture medium

G. Puri[1], B. Das[2], S. Bag[2]

Abstract

The present experiment was carried out to study the role of different culture conditions on development of embryonic stem cell colony from early stage IVF derived embryonic embryos in buffalo. The 8-16 cell stage IVF embryos were made zona free and the clumped blastomere were cultured on matrigel and fibronectin coated tissue culture dish in culture condition viz. Control (C-I), M-I:C-I+ stem cell factor, SCF),M-II: C-I+SCF+bFGF and M-III:CI+SCF+bFGF+IGF1) and were cultured at 37°C, 5% CO2 and 90% relative humidity in CO_2 incubator. Once the clumped blastomere cells multiplied and made stem cell colony, they were passaged mechanically. A total of 16 number of zona free embryos were cultured in each medium on matrigel or fibronectin coated dish. It was also observed that the ES cell colony formation was better on matrigel than on fibronectin.

However on both matrigel and fibronectin, the efficiency of embryonic blastomemere attachment, stem cell colony formation and propagation were significantly higher when ES cell medium was supplemented with only stem cell factor SCF(M-I) or supplemented with SCF+bFGF+IGF1 (M-III) followed by supplementation of SCF+bFGF (M-II) as compared to control media. Media-I and III were found to be significantly ($P< 0.01$) better as compared to Media-II, however, there was no significant difference between Media-II and control. The better ES cell colony formation on matrigel as well as on Medai-1 and 3 than fibronectin and Medium 2 and control may be due to the fact that the pluripotent gene expression was better in former conditions. It can be concluded that buffalo ES cell colonies can be established in ES cell medium supplemented with SCF alone or SCF+ bFGF4+IGF1 on matrigel and fibronectin synthetic matrices.

[1]College of Veterinary & Animal Scie, HPKV, Dept. of Vety Physiology, Palampur, India, [2]Indian Veterinary Research Institution, Division of Physiology & Climatology, Bareilly, India

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700065

Expression of stella positively correlates with the activation of imprinted genes of the Dlk-Dio3 imprinting cluster during reprogramming

X. Xu[1], L. Smorag[1], K. Pantakani[1], J. Nolte[1], W. Engel[1]

Abstract

Pluripotent stem cells are capable of differentiating into all three germ layers namely, endoderm, mesoderm, and ectoderm. Since the reprogramming of somatic cells into pluripotent state has been very well established, induced pluripotent stem (iPS) cells hold the great promise in custom-tailored regenerative medicine therapies. Molecular and functional characterization of iPS cells revealed a strong correlation between aberrant expression of genes from Dlk1-Dio3 imprinting cluster and the germ-line transmission ability. Moreover, defects in the imprinting status of Dlk1-Dio3 gene cluster was also observed in iPS cells generated by various independent research groups, making it as a common defect occurring during reprogramming. The activation of Gtl2 expression ($Gtl2^{on}$), one of the genes from Dlk1-Dio3 cluster, is currently being used as a bona fide marker of authentic pluripotent cells competent of germ-line transmission.

Recently, we have shown that during reprogramming germ-cell (GC) marker genes expression precede the expression of pluripotency-related genes. Hence, in the present study we aimed to analyze the role of GC marker genes in reprogramming and also in the activation of Gtl2 gene. Screening of several GC marker genes revealed that Stella, a maternal factor with a role in imprinting maintenance, as a potential candidate. We found that the expression of Stella along with the expression of known reprogramming factors (Oct4, Sox2, Klf4 and c-Myc) reduces the time needed for reprogramming and also increases the efficiency of iPS generation. Interestingly, addition of Stella to the reprogramming factor cock-tail leads to the generation of all $Gtl2^{on}$ iPS cells. Currently, we are investigating these cells for their pluripotent cell characteristics and germ-line transmission competency.

[1]*Goettingen University, Goettingen, Germany*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700066

Generation of induced pluripotent stem cells from cynomolgus monkey and the differentiation towards functional

S. Wunderlich[1], A. Haase[1], S. Merkert[1], J. Beier[1], A. Schambach[2], S. Glage[3], G. Göhring[4], E.C. Curnow[5], U. Martin[1]

Abstract

Induced pluripotent stem cells (iPSCs) may represent an ideal cell source for future regenerative therapies. Clearly, the development of iPSC-based therapeutic concepts requires suitable animal models. Nonhuman primates show the highest similarities to humans concerning the physiological, cellular and molecular level and macaques are frequently used in preclinical research and pharmacology. Considering the establishment of allogeneic / autologous cell transplantation models, we have generated iPSCs from cynomolgus monkeys (M. fascicularis, cyiPSC). Instead of the commonly human immunodeficiency virus (HIV) based reprogramming vectors that show poor transduction of simian cells, cynomolgus skin fibroblasts were reprogrammed using simian immunodeficiency virus (SIV) based vectors encoding OCT4, SOX2, KLF4, and c-myc. Resulting cyiPSCs show all typical characteristics of primate embryonic stem cells (ESCs). Culture characteristics and cell / colony morphology is almost identical to cynomolgus ESCs and cyiPSCs stain positive for typical pluripotency markers such as OCT4, NANOG, and TRA-1-60. CyiPSCs differentiate in vivo and in vitro into derivatives of all three germlayers. Furthermore, differentiation into functional cardiomyocytes could be demonstrated. Our data indicate that the generated cyiPSCs are highly similar to human iPSCs and should therefore represent an excellent cell source for allogeneic / autologous preclinical cell transplantation models.

[1]Hannover Medical School, LEBAO, Hannover, Germany, [2]Hannover Medical School, Experimental Hematology, Hannover, Germany, [3]Hannover Medical School, Institute for Laboratory Animal Science, Hannover, Germany, [4]Hannover Medical School, Cellular and Molecular Pathology, Hannover. Germany. [5]Washington National Primate Research Center. Seattle. United States

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700067

Differentiation of isolated porcine mesenchymal bone marrow stem cells into endothelial-like cells by application of

F. Schlegel[1], S. Dhein[1], F.W. Mohr[2], P.M. Dohmen[2]

Abstract

Question:

This study was induced to investigate the possibility of reprogramming mesenchymal bone marrow stem cells (BMSCs) into endothelial cells by application of growth factors into culture media.

Methods:

Isolation of BMSCs were performed by bone marrow aspiration of the mini-pig femur. Bone marrow was filtered with a 100µm cell strainer to eliminate bone remains and tissue fragments. After centrifugation pellet was dissolved in PBS-EDTA solution and the mononuclear cells were isolated by density gradient centrifugation with histopaque. Additionally, cells were washed with media and centrifuged further for 5min. The pellet was solved in medium containing endothelial cell growth factor supplement (10%). Medium was changed every second day. Confluent cells were analysed for the expression of eNOS by immunfluorescence staining and Western blot. Furthermore, the functionality of cells to release NO was examined by spectrophotometric investigation.

Results:

Isolated BMSCs cultured with endothelial cell growth factor exhibited a typical cobblestone-like endothelial cell phenotype. Immunfluorescence staining and Western blot analysis with eNOS antibody showed eNOS expression in the same manner than venous endothelial cells. Investigations of the NO release exhibited also the functionality of endothelial-like BMSCs to liberate NO, which is an essential feature for the functionality of endothelial cells.

Conclusion:

The reprogramming of isolated pig BMSCs into endothelial-like cells with endothelial characteristics was possible by adding of endothelial cell growth factor. In future studies BMSCs could be a source for production functional endothelial cells.

[1]*Herzzentrum Leipzig, Forschung und Lehre/Chirurgie, Leipzig, Germany,* [2]*Herzzentrum Leipzig, Chirurgie, Leipzig, Germany*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700068

Optimisation of serum-free culture media for human embryonic stem cells using factor screening design analysis

F. Knöspel[1], R.K. Schindler[2], S. Riekenberg[2], E.C. Wönne[1], K. Bräutigam[1], J.C. Gerlach[3], K. Zeilinger[1]

Abstract

This study aimed to explore relevant factors to optimise the composition of a serum-free defined culture medium intended to support the expansion of human embryonic stem cells (hESC) and induced pluripotent stem cells (iPSC) under feeder-free conditions *in vitro*. Ten out of 19 potentially important factors for hESC proliferation were selected in an initial screening and then further investigated using a Plackett-Burman design. The medium composition was based on mTeSR medium as proposed by Ludwig and Thomson (Current Protocols in Stem Cell Biology, 1C.2.1-1C.2.16., John Wiley & Sons). Resulting from previous experiments bovine serum albumin was replaced by polyvinyl alcohol in the basal medium due to its distinct negative influence on the proliferation. The potential influencing medium factors [ascorbic acid, human transferrin, γ-aminobutyric acid (GABA), pipecolic acid, basic fibroblast growth factor (bFGF), insulin, lipids, transforming growth factor β 1(TGF β1), platelet derived growth factor (PDGF-AB) and sphingosin-1-phosphate (S-1-P)] and in addition the oxygen tension were varied at two concentration levels in 12 different media compositions. The used hESC (H1) were cultivated on Matrigel-coated culture dishes over five days and the proliferation activity was evaluated on culture day 2 and 5 using the CellTiter-Blue assay as response parameter. In addition, the metabolic parameters glucose and lactate were measured in culture supernatants. The selection of the most effective factors was performed by means of Analysis of Variance (ANOVA) using the software Design Expert. The generated ranking list of the screening showed that PDGF-AB and S-1-P had the most positive effect on the proliferation rate of hESC, while high levels of ascorbic acid, GABA and insulin negatively influenced the cell growth. The undifferentiated state of the hESC was confirmed by immunofluorescence staining with the pluripotency markers Oct 3/4, Nanog and SSEA-4. In conclusion, the applied CellTiter-Blue assay enables repeated analysis of the hESC on different culture days and the collection of more than one set of data (e.g. different assays) from one culture sample. The DoE methodology allows an efficient identification of critical compounds for stem cell expansion media. In order to substantiate these findings a minimal-run-resolution IV design with a higher statistical resolution has to be applied, which also allows discriminating active factors from factor interaction effects.

[1]*Berlin Brandenburg Center for Regenerative Therapies (BCRT), Charité Universitätsmedizin Berlin, Campus Virchow-Klinikum, Bioreactor Group, Div. of Experimental Surgery, Berlin, Germany,* [2]*Biochrom AG, Berlin, Germany,* [3]*University of Pittsburgh, McGowan Institute for Regenerative Medicine, Pittsburgh, United States*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700069

Isolation and characterization of mouse mesenchymal stem cells

S. Raeth[1], T. Hauk[1], G. Siegel[2], K. Pfizenmaier[1], A. Hausser[1]

Abstract

Mesenchymal stem cells (MSC) are multipotent adult stem cells. Bone-marrow derived MSCs are able to differentiate at least into osteocytes, adipocytes and chondrocytes *in vitro*. Because of their multipotency and ability to migrate to sites of injury in animal models, MSC moved into the focus of regenerative medicine. The general properties of MSC are discussed controversially in literature. Therefore, a comprehensive understanding of signaling pathways involved in proliferation and differentiation is essential considering the potential use of MSC in therapy. Especially mouse MSC could be a versatile tool to investigate important factors and proteins involved in mesenchymal stem cell signaling as a variety of transgenic mouse models are available. In this study, mMSC were isolated according to Nadri and characterized with respect to proliferation and differentiation. Remarkably, cells isolated showed a long lag phase with no growth after the isolation. Preliminary experiments suggest that oxidative stress could be involved in this quiescent phase after isolation. By characterizing different isolates from C57BL/6 mice, general characteristics concerning proliferation and differentiation were analyzed. The cell populations displayed a heterogeneous marker profile, though all isolates did not show the expression of hematopoietic markers (CD34, CD45). Surprisingly, cell isolates displayed an increased proliferation in later passages. The cell populations consisted of unipotent, bipotent and tripotent cells with the tripotent isolates being considered as mMSC according to Pittenger. The osteogenic differentiation of the cells was analyzed in more detail by measuring the level of mineralization at different time points. Taken together, the isolation of mesenchymal stromal cells from C57BL/6 mice results in a heterogeneous cell population consisting of various precursor cells.

[1]*Institute of Cell Biology and Immunology/University of Stuttgart, Stuttgart, Germany,* [2]*Institute for Clinical and Experimental Transfusion Medicine/Eberhard Karls University Tübingen, Tübingen, Germany*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700070

Time dependent multilineage differentiation of human embryonic stem cells and its implications for developmental toxicity

K. Meganathan[1], S. Jagtap[1], V. Wagh[1], J.A. Gaspar[1], J. Hescheler[1], A. Sachinidis[1]

Abstract

The better understanding of early human embryonic development has been hampered with the availability of samples and eventually addressed with in vivo embryonic development (ED). As an initial step we have recapitulated the early human ED with multilineage differentiation of human embryonic stem cells (hESC) integrated with sensitive genomics approach in our prior publication. To further explore the human ED and its subsequent application in toxicogenomics we have assigned the time kinetic hESC ED assays. The global transcriptional profiling and the subsequent gene ontology analysis reveal that from day 3 the developmental biological processes were identified. Also, the gene signatures identified during the embryonic development were used for the assessment of early developmental toxicity. The known developmental toxicants and the negative compound were treated at sub lethal concentration during the Embryoid body development for 14 days. To demonstrate the toxicological application positive compounds methyl mercury, valproicacid and negative compounds mannitol were treated at IC10 and four times lower than IC10 concentrations. The principal component analysis showed, mannitol clustering close to control and the positive compounds showed significant number of differentially expressed transcripts. The further in depth analysis of gene expression data provided the proof of concept that this platform can demonstrate the developmental toxicity.

[1]*University of Cologne, Cologne, Germany*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700071

In vitro embryotoxicity testing with human embryonic stem cells reveals markers for all-trans-retinoic acid

S. Jagtap[1], K. Meganathan[1], V. Wagh[1], J.A. Gaspar[1], J. Hescheler[1], A. Sachinidis[1]

Abstract

Many characteristics of human embryonic stem cells (hESCs) including pluripotency are useful tools for studying the harmful effects of reference compounds during early development. The early embryogenesis is recapitulated by pluripotent embryonic stem cells when differentiated in vitro, which makes hESC an effective tool for developmental toxicology and embryotoxicology. All-*trans*-retinoic acid is known to be embryotoxic in laboratory animals and for humans when applied during critical stages of embryonic development. Characteristic malformation patterning of craniofacial structures and defects in cardiac development were observed in human offsprings.

In rat and mice, All-*trans*-retinoic acid treatment resulted in craniofacial malformations and limb anomalies associated with embryo fetal alterations. In the following study, All-*trans*-retinoic acid was treated on undifferentiated hESC in the presence and absence of bFGF for a period of 7 days and a toxicogenomics profile was studied. The results revealed that mesoderm markers such as MESP, TBX6 and DES, endoderm markers such as AFP, GATA4 and SERPINA1 were repressed. In contrast ectoderm markers such as NEUROD1, Shh and ENC1 were up regulated irrespective of the presence or absence of bGFG. The results are further used to identify embryotoxic markers and also to test other compounds for embryotoxic potential.

[1]*University of Cologne, Cologne, Germany*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700072

Electrophysiological maturation of fetal cardiomyocytes during electrical stimulation in vitro

S. Baumgartner[1], M. Halbach[1], G. Peinkofer[1], B. Krausgrill[1], J. Hescheler[2], J. Müller-Ehmsen[1]

Abstract

Objectives:

The aim of this study was to investigate whether continuous electrical stimulation affects electrophysiological properties and orientation of fetal cardiomyocytes (FCMs) in culture.

Methods:

FCMs at day 14.5 p.c. were harvested from murine hearts and electrically stimulated for six days in culture using a custom made stimulation chamber. A stimulation frequency of 10 Hz was applied continuously during cultivation. Subsequently, action potentials of FCMs were recorded with glass microelectrodes. Additionally, alpha-actinin immuno-stainings of the cultivated FCMs were performed and the angles between the electrical field lines and the longitudinal axis of the cells were measured. All data are presented as mean ± SEM.

Results:

Action potential duration to 50% repolarisation (APD50) of stimulated FCMs (37.34±1.43 ms, n=29) was significantly decreased as compared to controls (52.81±1.65 ms, n=21, p< 0.001). APD90 of stimulated cells (102.10±4.32 ms, n=29) was also significantly lower as compared to controls (148.30±11.21 ms, n=21, p< 0.001). The ratio of APD50/APD90 of stimulated FCMs (38.63±2.17 %, n=29) did not differ from controls (38.06±3.49 %, n=21, p=0.78). Spontaneous beating frequency of stimulated (1.86±0.17 Hz, n=29) and non-stimulated cells (2.17±0.17 Hz, n=21, p=0.22) was comparable. AP amplitude of electrically stimulated cells (67.42±1.47 mV, n=29) was equal to that of non-stimulated cells (71.28±1.35 mV, n=21, p=0.07).Maximal diastolic potential of stimulated cells (-56.87±0.96 mV, n=29) was not different from that of non stimulated cells (-58.55±1.02 mV, n=21, p=0.25). Maximal upstroke velocity (Vmax) of stimulated cells (25.78±3.97 V/s, n=29) and of non-stimulated cells (21.13±4.16 V/s, n=21) was comparable (p=0.43). The angles between the electrical field lines and the longitudinal axis of the stimulated cells (8.44±1.13°, n=31) were significantly lower as compared to the non-stimulated cells (49.43±3.33°, n=44, p< 0.001).

Conclusions:

The decrease of APD50 and APD90 observed in stimulated fetal cardiomyocytes in vitro corresponds to the electrophysiological maturation of fetal cardiomyocytes in vivo. To our knowledge, continuous electrical stimulation is the first condition leading to an electrophysiological maturation and parallel alignment of cultured murine cardiomyocytes.
This implies that the electrophysiological maturation of cardiomyocytes in vivo may at least partially depend on electrical activity.

[1]University Hospital of Cologne, Department III, Cardiology, Cologne, Germany, [2]University Hospital of Cologne, Institute of Neurophysiology, Cologne, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700073

Brain tumor stem cells and their diverse features

M. Loy[1], S. Roth[1], J. Leppert[1], I. Choschzick[1], A.M. Dorenberg[1], V. Tronnier[1], C. Zechel[1]

Abstract

Objective:

Malignant brain tumors, in particular the glioblastoma mutliforme (GBM), are amongst the most lethal solid human tumors. They grow invasively, develop resistance to radiation and chemotherapy, and frequently recur. Recently, GBM tissue was classified into four distinct molecular entities, the classical, mesenchymal, neural and proneural subtype based on a combined genome, transcriptome and proteome analysis. It remains unclear whether stem-cell like tumor cells (SCLC), which represent a small subfraction of the GBM, could be grouped into similar categories and whether GBMs harbor different types of SCLC.

Material and methods:

We screened 80 human brain tumor biopsies for the presence of SCLC by immunological techniques and isolated SCLC from different glioma entities, such as glioblastoma mutlifirme (GBM) and gliosarcoma (GSarc). SCLC were expanded in serum-free medium and transplanted into SCID mice. Expression of stemness factors and neural markers was investigated by immunocytochemistry, FACS, Western blot analysis and real time PCR. Single cells from SCLC cultures were seeded in 96 well plates, clonally expanded and subsequently subjected to phenotype analysis.

Results and conclusions:

We identified stem cell-like cells (SCLC) in all brain tumor biopsies analyzed so far. SCLCs that gave rise to proliferating cultures were transplanted into the brain of SCID mice to prove their tumorigenic potential. All SCLC expressed the intermediary filaments Nestin and the transcription factor Sox2. Co-expression of the intermediary filament GFAP or the marker CD133 (prominin-1), however, varied largely. The histostructural organization of Nestin+/GFAP- and Nestin+/GFAP+ cells differed between the various glioma tissues. This indicated that malignant gliomas show a high heterogeneity with respect to the presence and localization of SCLC. This also translated to SCLC cultures derived from GBM and GSarc biopsies. Several SCLC cultures exclusively contained Nestin+/GFAP+ cells, whereas others encompassed only Nestin+/GFAP- cells. Mixed forms were also observed. In these cultures the ratio of Nestin+/GFAP+ to Nestin+/GFAP- was remarkably stable over many passages. The same phenomenon was observed for the presence of CD133+ and CD133- cells. In addition, we found co-expression of Nestin and CD44 in almost all SCLC subtypes, which is in contrast to data published in the novel classification of molecular GBM entities. Clonal expansion of single SCLC cells from selected cultures revealed different degrees of heterogeneity, supporting the view that SCLC subtypes may co-exist in the same culture and presumably the same tumor.

First and second author = Contributed equally

[1]*University of Lübeck, Neurosurgery, Lübeck, Germany*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700074

Growth regulation and cellular crosstalk in human glioma

I. Choschzick[1], S. Willkomm[2], D. Hügging[1], S. Behling[1], E. Pawlak[1], A. Bernt[2], V. Tronnier[1], C. Zechel[1]

Abstract

Objective:

Malignant brain tumors are amongst the most lethal solid tumors. The standard treatment consists of surgical resection followed by adjuvant radio- and chemotherapy. It has been proposed that glioma cells with stem cell features, designated SCLC hereafter, contribute to initiation and progression of primary and recurrent glioma. Regulation of SCLC and non-SCLC growth and the crosstalk amongst themremains largely elusive.

Material and methods:

Up to now, we screened 80 human brain tumors for the presence and features of SCLC. Weexpanded brain tumor cells in vitro and analyzed the expression of receptor tyrosine kinases and peptide growth factorsin SCLC and non-SCLC by means of immunological staining as well as Western blot analysis and real time PCR. In addition, we investigated mitogen dependence (growth curves, proliferation and cell survival assays) and differentiation (immunocytochemistry) in the absence of exogenously provided growth factors.

Results and conclusions:

We identified stem cell-like cells (SCLC) in all brain tumor biopsies analyzed so far. These cellsexhibited a substantial heterogeneity with respect to morphology, proliferation rate, growth modus and the expression of neural markers and stemness factors.Therefore, we refer to these cells as SCLC subtypes. Both, SCLC and non-SCLCfrom the same tumor co-expressed several receptor tyrosine kinases (RTK), includingEGFR/HER1 and the SCF (stem cell factor) receptor cKIT. Co-expression of RTKs and their corresponding ligandswas observed in several cultures, indicating that SCLC and non-SCLC might stimulate themselves by autocrine mechanisms. Besides the full-length receptors, a truncated EGFR/HER1 and cKIT was observed in several cultures, indicating that these brain tumor cells may be activated in the absence of HER1 ligands and SCF, respectively. Secretion of the growth factors EGF and SCF was observed using cytokine ELISA techniques. In keeping with this, thedependence ongrowth factorssupplied with the medium was low and varied amongst the various SCLC and non-SCLC cultures. In addition, the RTK expression level of SCLC and non-SCLC from the same tumor differed. Together our data suggest that the human brain tumors cellsexertintracrine and autocrine growth control and cross-talkvia paracrine mechanisms.

[1]*University of Lübeck, Department of Neurosurgery, Lübeck, Germany,* [2]*University of Lübeck, Lübeck, Germany*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700075

Does the tumescent liposuction affect the viability of Adipose-Derived Stem Cells?

N. Gurtowska[1], J. Olkowska[1], A. Bajek[1], T. Kloskowski[1], M. Gajdus[2], W. Bienkowski[3], T. Drewa[1,4]

Abstract

Objectives:

Adipose tissue is easily accessible and abundant source of stem cells and can be harvested during liposuction. Aspiration of liposuction alone does not significantly alter the viability of Adipose Derived Stem Cells (ADSCs). However, liposuction is usually performed under general anaesthetic with infiltration of local anaesthetic fluid. That is why the aim of this study was to assess the influence of anaesthetic fluid on ADSCs viability.

Methods:

Adipose tissue was collected during liposuction. Briefly, ADSCs were isolated by collagenase digestion (0.075%, 30 min in 37°C) and followed centrifugal separation. Cells were cultured in DMEM/HAM'S F-12 medium supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic. After 14 days, cells were seeded on 24-wells plate ($1x10^4$ cells/well) and cultured in complete medium for 24 h. Then cells were exposed on anaesthetic fluid (NaCl 0.9%, Adrenaline 0.1%, Articaine hydrochloride 4%,. Natrium bicarbonicum 8.4%) for 1 hour. Cells in control were cultured in complete medium. The viability of cells was assessed by MTT assay

Results:

ADSCs in control had a regular shape and size. Cells were elongated and spindle-shaped, with numerous cytoplasmatic lamellipodia. After incubation with anaesthetic fluid decrease in cell number, compared to control was observed. Cells began to detach from the well surface. The viability of examined cells was 57,6% and was much lower comparing to control.

Conclusions:

Incubation of ADSCs with anaesthetic fluid significantly decreased their viability. It can be a limitation of adipose tissue as a source of stem cells. There is a necessary to optimize the method of adipose tissue collection for tissue engineering and regenerative medicine purposes.

[1]*Nicolaus Copernicus University, Tissue Engineering Department, Bydgoszcz, Poland,* [2]*Nicolaus Copernicus University, Department of Pharmacology and Therapy, Bydgoszcz, Poland,* [3]*Bienkowski Medicel Center, Bydgoszcz, Poland,* [4]*Institute of Oncology, Department of Urology, Kielce. Poland*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700076

Studying changes in gene expression of breast cancer stem cell-like cells markers, at different stages

P. Apostolou[1], M. Toloudi[1], M. Chatziioannou[1], E. Ioannou[1], I. Papasotiriou[1]

Abstract

Objectives:

Recent literature and experimental data, have demonstrated the significance of cancer stem cell-like cells (CSC-like cells), as an entity of circulating tumor cells (CTCs). Nanog, Okt 3/4, SOX2, Nestin and CD34 constitute specific markers of CSC-like cells and pilot studies showed a possible relationship between these transcription factors and clinical assessment of patients. The present study aims to determine the change of the above markers in correlation with the stage of the disease.

Methods:

In the first panel of this study, CTCs from more than forty (40) patients with breast cancer in different stages, according to TNM classification system, have been isolated. The quantification of CSC-like cells in CTCs cultures followed and in the second panel, real-time qPCR has been used for molecular analysis. Gene-specific primers for each marker and for endogenous gene (18S rRNA) have been designed and evaluated in reactions with positive control samples. The analysis has been performed by using relative quantification, normalized to the reference gene.

Results:

A first evaluation of the data suggests that there is no linear relationship between the gene expression of transcription factors and the stage of the disease. In most markers, the higher value is noted in stages II-III. Nanog's expression varies, while Okt3/4 seems to be overexpressed in stage II. The lower value of Nestin has been observed in stage IV, whereas for CD34 transcription factor has been observed in stage I.

Conclusions:

CSC-like cells may generate tumors through the stem cell processes of self-renewal and differentiation into multiple cell types. There are also resistant to chemotherapy, thus making difficult the treatment. This study demonstrates the correlation between gene expression of transcriptional markers, which is expressed particular in those cells, and the stage of disease in breast cancer. Concerning the above data, the question of whether these factors might be the target of new drugs arises. Further studies to confirm the above in a larger scale of samples, need to be performed.

[1]*Research Genetic Cancer Centre Ltd (R.G.C.C. Ltd), Filotas, Florina, Greece*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700077

Elucidating the function of Zfp819 in mouse embryonic stem cells

X. Tan[1], J. Pyczek[1], K. Pantakani[1], J. Nolte[1], W. Engel[1]

Abstract

Zinc finger proteins are among the most abundant proteins in eukaryotic genomes. Their functions are extraordinarily diverse and include DNA recognition, RNA packaging, transcriptional regulation, apoptosis, protein folding and assembly, and lipid binding. Zfp819 was identified in our comparative transcriptome analysis of undifferentiated and differentiated ESCs as a novel gene which is expressed highly in pluripotent cells but not in their differentiated counterparts. The protein encoded by Zfp819 belongs to C2H2-zinc finger (C2H2-Znf) family of proteins and bears a functional KRAB (Krueppel-associated box) domain on N terminal region of the protein, yet the function is not known. Recently, Zfp819 was shown to be being highly expressed in partially and fully reprogrammed induced pluripotent cells (iPSCs), but not in parental somatic cells. Therefore, these results suggest the possible crucial role for Zfp819 in establishments and maintenance of pluripotency.

To study the role of Zfp819 in pluripotency network, we altered the expression of Zfp819 by either overexpression or down-regulation and found no overt changes in the expression of key pluripotency marker genes. Unexpectedly, the expression of retroviral control elements such as Line1 and IAP gag were significantly up regulated upon Zfp819 down regulation in mouse ES cells. Further, to identify the interaction partners of Zfp819 in pluripotent cells, we performed a yeast two-hybrid screen on ESCs cDNA library with the N-terminal part of Zfp819 as bait. The identification of several putative interaction proteins suggest that Zfp819 might function as a transcriptional and cell cycle/apoptosis regulator. The characterization of interaction between Zfp819 and its putative interaction proteins is currently in progress. Further studies aimed at identifying the DNA targets bound by Zfp819 are in progress to unveil the role of Zfp819 in transcriptional regulation.

[1]*Goettingen University, Goettingen, Germany*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700078

Migratory properties of breast stem cell/breast cancer cell hybrids

B. Berndt[1], S. Haverkampf[1], G. Reith[1], B. Niggemann[1], K.S. Zänker[1], T. Dittmar[1]

Abstract

Objectives:

The biological phenomenon of cell fusion plays a fundamental role in several physiological and pathophysiological processes. In cancer cell fusion has been linked to the origin of more malignant tumor hybrid cells exhibiting an increased metastatogenic behavior as well as an enhanced drug resistance. Thereby, tumor hybrid cells could originate from the fusion between two tumor cells or between a tumor cell and a normal cell, such as a macrophage or an adult stem cell.

Methods:

In the present work the migratory behavior and induction of signal transduction pathways of two hybrid cell lines (M13HS-3 and M13HS-8) in comparison to the parental cells (M13SV1-EGF-Neo breast stem cells and HS578T-Hyg breast cancer cells) towards EGF and CCL21 was investigated.

Results:

All cell lines were positive for EGFR and HER2 expression. However, detectable levels of HER3 were solely found on M13SV1-EGFP-Neo breast stem cells and hybrid clone 8. By contrast, M13SV1-EGFP-Neo were negative for CCR7, whereas M13HS-2, M13HS-8 and HS578T-Hyg cells do express the CCL21 receptor. Cell migration is a prerequisite for metastasis formation and both factors have been demonstrated to be involved in the metastatic spreading of disseminated breast cancer cells. Particular for CCL21 it is assumed that this chemokine plays a crucial in mediating the specific metastatic spreading of breast cancer cells into regional lymph nodes. All investigated cell lines responded to EGF stimulation with an increased locomotory activity, whereas solely the hybrid cells responded to CCL21 treatment with an increased migratory activity.

Co-treatment of cells with EGF and CCL21 revealed an additive effect on the migration of M13HS-2 and M13HS-8 hybrid cells. Analysis of PLC activity by flow cytometry-based calcium measurements showed that both parental cell lines and the M13HS-8 hybrid cell line exhibited increased cytosolic calcium levels upon EGF stimulation. By contrast, CCL21 did not lead to elevated intracellular calcium levels in any of the analyzed cell lines indicating that PLC was not activated. Western Blot analysis of AKT and MAPKp42/44 and their phosphorylated counterparts showed increased pAKT and pMAKPp42/44 levels in EGF and CCL21 treated HS578T-Hyg breast cancer cells and M13HS-8 hybrid cells, but not in M13HS-2 hybrids. By contrast, solely EGF treatment resulted in increased pAKT and pMAKPp42/44 levels in M13SV1-EGFP-Neo cells, whereas CCL21 had no effect.

Conclusion:

We conclude from our data that they clearly depict the random nature of cell fusion resulting in unique hybrid cells exhibiting unique properties. The finding that hybrid cells, but not the parental tumor cells, responded to CCL21, which has been associated with lymph node metastasis of breast cancer suggests that cell fusion is a mechanism how tumor (hybrid) cells could become metastatic.

[1]*Witten/ Herdecke University, Institute of Immunology, Witten, Germany*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700079

Modulation of epigenetic marks at the promoters of direct Notch1 target genes

K. Bernoth[1], U. Just[1], R. Schwanbeck[1]

Abstract

Objectives:

Notch proteins are transmembrane receptors that influence cell fate decisions, differentiation, proliferation and apoptosis in many developmental systems. After ligand binding and activation, the Notch intracellular domain (N^{IC}) is cleaved from the cytoplasmic membrane and translocates into the nucleus to act as a transcription factor. N^{IC} binds to DNA via the adapter protein RBP-J (also termed CBF-1) and converts the transcriptional repressor RBP-J into a transcriptional activator. Recently we have shown that Notch signaling regulates the expression of genes playing key roles in cell differentiation, cell cycle control and apoptosis in a highly context dependent manner. Epigenetic events like histone modification, DNA methylation and chromatin remodeling are tightly involved in the control of gene expression.

Methods/results:

To investigate the role of chromatin modifications in cell-context dependent Notch1 target gene activation, we determined the chromatin modifications present at the regulatory regions of genes that we identified to be regulated in embryonic stem cells (ESC) by activated Notch1 using public databases. We found that the promoter regions of Notch1 target genes are marked by an enrichment of H3K4me3, an activating modification, and bivalent domains, containing an activating (H3K4me3) and repressing (H3K27me3) modification. We further screened the regulatory regions of the Notch1 target genes Hes5, Sox9 and Pax6 for potential RBP-J binding sites and analyzed the chromatin marks and their changes upon Notch1 activation in ESC using ChIP. Upon activation of Notch1 signaling for 15 min we observed changes in H3K4me3 and H3K27me3 at the RBP-J binding sites of the Notch1 target genes Hes5, Sox9 and Pax6. These results suggest that Notch1 signaling may alter the epigenetic marks, in particular the bivalent domains at the regulatory regions of its target genes. To further determine which of the RBP-J binding sites are used for Notch1 signaling at certain stages of development, we performed luciferase experiments with Sox9 RBP-J binding site mutant promoter constructs. When the two RBP-J binding sites located +40bp and -325bp relative to the transcription start site of Sox9 were mutated, the expression upon Notch1 activation was reduced compared to the wildtype Sox9 promoter.

Conclusion:

Our data indicate that epigenetic regulation at Notch1 target genes involves changes in histone lysine methylation (H3K4me3, H3K27me3) upon Notch1 activation that may promote gene expression. Results from luciferase reporter constructs revealed an important role for the two RBP-J binding sites near the transcription start site for Notch1 mediated Sox9 upregulation with the possibility that they may act in a synergistic manner.

[1]*Christian-Albrechts-Universität zu Kiel, Institute of Biochemistry, Kiel,*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700080

The influence of acute and chronic wound fluid on ADSC function

P. Koenen[1,2], O.C. Thamm[2], A. Richard[1], E.A. Neugebauer[1], G. Spilker[2]

Abstract

Introduction:

Chronic wounds represent a major problem in medicine today as their incidence is continuously increasing due to an aging population. Physiological wound healing is a complex biological process proceeding from the stage of inflammation through proliferation to maturation. It requires a well-orchestrated interaction of mediators, resident and infiltrating cells. In this context, mesenchymal stem cells play a crucial role as they are attracted to the wound site and influence wound healing processes via direct cell-cell interaction as well as paracrine secretion and transdifferentiation. In chronic wounds these finely tuned mechanisms are disturbed by mediators of the wound environment.

Methods:

We analysed the effect of acute and chronic wound fluid on adipose-derived stem cells (ADSCs). Therefore we successfully developed protocols to harvest wound fluid from acute and chronic wounds. We investigated the proliferation and migration capacity of ADSCs under the influence of acute and chronic wound fluid (AWF; CWF) using MTT test and transwell migration assay.

Results:

AWF and CWF positively influence ADSC migration. However, AWF has a significantly stronger chemotactic impact on ADSCs than CWF. Proliferation of ADSCs is inversely influenced by AWF and CWF, respectively. Whereas proliferation is stimulated by AWF, CWF has a negative effect on ADSC proliferation over time.

Conclusion:

These results give an insight into impaired ADSC function in chronic wounds. The less stimulating effect of CWF on ADSC proliferation and migration compared to AWF might be one reason for an insufficient wound healing process in chronic ulcers.

[1]*University of Witten/Herdecke, Institute for Research in Operative Medicine, Cologne, Germany,* [2]*University of Witten/Herdecke, Clinic for Plastic Surgery, Cologne-Merheim Medical Center, Cologne, Germany*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700081

CD 133+ stem cells migrate towards extracellular ATP in vitro

M. Laupheimer[1], C.A. Lux[1], N. Ma[1], G. Steinhoff[1]

Abstract

Objectives:

Bone marrow-derived stem cells have been shown to play a role in cardiac tissue regeneration. Cardiac ischemia causes HIF-1-dependent overexpression of stem cell homing factor SDF-1, and thus, stem cells are recruited to the area of tissue injury. Direct intramyocardiac transplantation may support this physiological process. In animal models as well as phase I / II clinical trials, transplantation of human bone marrow stem cells selected for the surface marker CD 133 led to improved heart function.

Besides SDF-1, however, various other factors such as chemokines, cytokines and nonpeptide molecules are released during cardiac injury. Elevated levels of extracellular adenosine-triphosphat (ATP) have been detected in ischemic vs. normal myocard. In the present study, we investigated the influence of extracellular ATP on CD 133+ cell migration.

Methods:

Sternal bone marrow samples were obtained from cardiac surgery patients who had given their informed consent. CD133+ cells were isolated by density centrifugation and MACS isolation. Cell purity was evaluated by flow cytometry. In vitro migration of untreated or preconditioned CD133+ cells towards SDF-1 and ATP was evaluated in a Boyden chamber followed by microscopic analyses. The influence of ATP on CD133+ cell viability was examined by Annexin/7-AAD staining detected via flow cytometry. Cellular proliferation after preconditioning was verified by CFU assay.

Results:

ATP as well as SDF-1 induced significant CD 133+ cell migration. The effect of ATP was dose-dependent, 100µM ATP enhanced cellular migration 6,3 fold compared to control (medium). CD133+ cells contained small numbers of apoptotic (13,6 % ± 4,78) and necrotic (4,13 % ± 1,38) cells. Preconditioning with 100 µM ATP for 16h lowered apoptosis rate slightly, yet insignificantly (8 % ± 1,63 apoptosis, 4,2 % ± 2,61 necrosis). We detected no influence of 100 µM ATP on viability or proliferation as evaluated by CFU count. Moreover, we investigated a possible “priming” effect of ATP preconditioning on migration towards SDF-1. Incubation of CD133+ cells with 100 µM ATP for 16h resulted in slightly enhanced migration towards SDF-1 (5,73 % ± 1,11 ATP vs. 3,89 ± 0,44 control). The difference, however, was not significant.

Conclusions:

We demonstrated that besides the well-known stem cell homing factor, SDF 1, ATP induces the migration of CD 133+ cells in vitro. Extracellular ATP may contribute to CD133+ cell migration towards ischemic tissue in vivo. Our data do not provide any evidence for a negative impact of ATP on CD133+ cells. Further studies are necessary to clarify the mechanism of ATP-induced migration.

[1]*Reference- and Translation Center for Cardiac Stem Cell Therapy (RTC), Rostock, Germany*

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Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700082

G-CSF dependent stem cell mobilization does not influence the cardioprotective effects of PTH after myocardial infarction

T. Weinberger[1], S. Brunner[1], B.C. Huber[1], M.-M. Zaruba[1], H.D. Theiss[1], G. Assmann[2], N. Herbach[3], R. Wanke[3], J. Mueller-Hoecker[2], W.-M. Franz[1]

Abstract

Aims:

PTH administration after myocardial infarction (MI) is known to attenuate myocardial function, survival and cardiac remodeling. These effects mainly resulted from an increase of mobilization and homing of CD34+/CD45+ stem cells into the ischemic myocardium. PTH related stem cell mobilization was shown to be related to an endogenous G-CSF release. The aim of our study is to determine the role of G-CSF on the cardioprotective effects of PTH.

Methods:

G-CSF +/+ (C57BL/6) and G-CSF -/- mice were treated with PTH (80 µg/kg/d) for 6 days after inducing a MI by LAD ligation. The myocardial homing factor SDF-1 was analyzed on day 2 with ELISA. Stem cell populations in peripheral blood and heart were examined on day 6 by FACS. Cardiac function and immuhistochemistry were investigated on day 6 and day 30.

Results:

PTH treatment resulted in a significant increase of CD45+/CD34+ cells in peripheral blood in G-CSF +/+, but not in G-CSF -/- mice. However, a significant increase of SDF-1 and enhanced migration of CD45+/CD34+ cells into the ischemic myocardium was revealed after PTH administration in both, G- G-CSF +/+ and G-CSF -/- mice. Enhanced stem cell homing was associated with an ameliorated cardiac function and post-MI survival after PTH treatment. Furthermore, infarct size, wall thickness and neovascularisation showed a significant improvement in both groups 30 days after MI.

Conclusion:

The cardioprotective effects of PTH could be shown to be independent from endogenous G-CSF release and therefore from stem cell mobilization. This puts more emphasis on the role of stem cell homing into the ischemic myocardium

[1]Ludwig-Maximilians-University, Department of Internal Medicine 1, Campus Großhadern, Munich, Germany, [2]Ludwig-Maximilians-University, Institute of Pathology, Munich, Germany, [3]Ludwig-Maximilians-University, Institute of Veterinary Pathology, Munich, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700083

"Epithelial-Mesenchymal Transition of small cell lung carcinoma (SCLC)" „Mesenchymal-Epitheliale Transition bei Zelllinien des

A. Krohn[1], T. Plönes[2], M. Burger[1]

Abstract

Small cell lung carcinomas (SCLC) are highly aggressive, invasive and early metastasizing tumors. The process of metastasis is going along with phenotypical changes. Cells have to detach from the primary tumor, invade the surrounding stroma, enter the circulation and escape the detection of the immune system until reaching metastatic sites. Epithelial-mesenchymal transition (EMT) processes, initially known from embryonic development, have recently been described to play an important role for these processes. EMT is going along with phenotypical changes and disbanding cell-cell connections that result in a transition from a more epithelial phenotype to a more mesenchymal-like appearance. It is described that the reverse process mesenchymal-epithelial transition (MET) happens at the site of metastasis, and it is postulated that these processes are linked to radio- and chemoresistance. There is very little knowledge on EMT processes and its impact on metastasis in SCLC.

SCLC cell lines NCI-H69, NCI-H82, NCI-N592 are usually forming floating cell clusters when cultured in RPMI 10%FCS, only very few cells are growing adherent to tissue culture flask (3-7%). FACS analysis shows different subpopulations of size and density within a cell line.We started to analyze the phenotypical morphology of the cell lines NCI-H69, NCI-H82, NCI-N592 and NCI-H446. The addition of low concentrations of BrdU to the culture is inducing a phenotypical change to mainly adherent growing cells. These changes are accompanied by changes of typical EMT markers on gene and protein levels.

For the induction of phenotypical changes 10µM BrdU was added to the culture medium for 14-21 days. Medium was changed every second day by centrifuging und resuspension. Once the majority of cells appear adherent BrdU was not added anymore. The cells remain adherent and show normal growth patterns. We show that the phenotypical changes go along with changes in membrane structures like Tight Junctions, Desmosome, Gap Junctions, Vimentin and Cadherins. Mesenchymal markers are upregulated in adherent cells, whereas epithelial markers are downregulated. We are postulating that under the influence of BrdU SCLC cell lines undergoing a mesenchymal shift. Our in vitro model might be interesting to study in vivo incidences of EMT.

[1]*Universitätsklinik Freiburg, Innere Medizin I, Hämatologie/Onkologie, Freiburg, Germany,* [2]*Universitätsklinik Freiburg, Abteilung Thoraxchirurgie, Freiburg, Germany*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700084

The landscape of cellular aging: long-term culture of mesenchymal stem cells is associated with specific changes in their DNA-

A. Schellenberg[1], Q. Lin[2], H. Schüler[3], C.M. Koch[1], S. Joussen[1], B. Denecke[4], G. Walenda[1], N. Pallua[5], C.V. Suschek[5], M. Zenke[2], W. Wagner[1]

Abstract

Mesenchymal stem cells (MSC) can only be culture expanded for a limited time until they reach a senescent state, the so called “Hayflick-limit”, which is accompanied by growth arrest, cell enlargement and reduced differentiation capacity. Therefore, culture associated changes in MSC may hamper their therapeutic potential. In this study, we have analyzed genetic and epigenetic changes upon long-term culture of MSC from human adipose tissue. The fibroblastoid colony-forming unit (CFU-f) frequency and the differentiation potential were already significantly impaired within the initial passages. Relevant chromosomal aberrations were not detected by karyotyping and SNP-microarrays and this supports the notion that human MSC possess relatively little genomic instability. Subsequently, we have compared DNA-methylation profiles with the Infinium HumanMethylation27 Bead Array and the profiles differed markedly in MSC derived from adipose tissue and bone marrow, indicating that the epigenetic makeup of MSC is highly dependent on the tissue of origin. Highly consistent senescence-associated modifications at specific CpG sites, especially in developmental genes, occurred already within the early expansion phase (between the passages 5 and 10). Remarkably, these DNA-methylation changes are highly enriched in regions with repressive histone modifications such as trimethylation of H3K9, H3K27 and EZH2 targets. These results indicate that cellular aging is not just a random accumulation of cellular defects, but that it is precisely regulated by epigenetic means in the course of culture expansion.

[1]Helmholtz-Institute for Biomedical Engineering, Medical Faculty, RWTH Aachen University, Aachen, Germany, [2]Department of Cell Biology, Medical Faculty, RWTH Aachen University, Aachen, Germany, [3]Institute of Human Genetics, Medical Faculty, RWTH Aachen University, Aachen, Germany, [4]Interdisciplinary Centre for Clinical Research (IZKF) Aachen, RWTH Aachen University, Aachen, Germany, [5]Department of Plastic and Reconstructive Surgery, Hand Surgery, Burn Center, Medical Faculty, RWTH Aachen University, Aachen, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700085

Delayed embryoid body formation and inhibition of cell adhesion induced by knock-down of p85α subunit of PI3 kinase

S.M.R. Gurney[1,2], R. Schwanbeck[2], U. Just[2], P. Forster[3]

Abstract

Objectives:

Embryoid bodies (EBs) are an accepted model of *in vitro* differentiation and embryogenesis. However, many factors can influence the direction of differentiation. Since the phosphatidylinositol-3-kinases (PI3Ks) exert a variety of signaling functions in eukaryotes regulating cellular survival, proliferation, differentiation, migration and trafficking we tested this pathway regarding the EB development. In eukaryotes there are three distinct classes of PI3Ks: class I (subdivided into IA and IB), class II and class III. The role of class IA PI3Ks, which we focus on, is to phosphorylate the second messenger lipid PIP2 to PIP3. Each of these complexes consists of one catalytic subunit (p110α, p110β, or p110δ, encoded by three different genes) and one regulatory subunit (p50α, p55α, p85α, p85β, or p55γ). These α, β and γ regulatory subunits are also encoded by three different genes: Pik3r1, Pik3r2 and Pik3r3, respectively. In the present work, we examined the role of the regulatory subunit p85α in undifferentiated ES cells and in early embryoid development.

Methods/results:

We suppressed the PI3K regulatory subunit p85α using a small interfering RNA (Pik3r1 siRNA) and examined the effects on EB development in hanging drop culture. We observed a 150% increase in the volume of the treated EBs within 24 h, compared to the negative controls. FACS assays showed that this increase in volume is not due to increased cellular proliferation. Instead, the increase in volume appears to be due to reduced cellular aggregation and adherence. This is further shown by our observation that 40% of treated EBs form twin instead of single EBs, and that they have a significantly reduced ability to adhere to culture dishes when plated. A time course over the first 96 h reveals that the impaired adherence is transient and explained by an initial 12-hour delay in EB development. Quantitative PCR expression analysis suggests that the adhesion molecule integrin-β1 (ITGB1) is transiently downregulated by the p85α suppression.

Conclusions:

Up to now, the function of the regulatory subunit p85α was rather poorly understood. Here, we provide first evidence that its gene Pik3r1 is involved in early embryoid development. We found that suppressing p85α leads to a delay in forming compact EBs, accompanied by a transient inability of the EBs to undergo normal cell-cell and cell-substrate adhesion.

[1]*University of Cambridge, Institute of Continuing Education, Madingley, United Kingdom,* [2]*University of Kiel, Institute of Biochemistry, Kiel, Germany,* [3]*University of Cambridge, Murray Edwards College, Cambridge, United Kingdom*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700086

Differentiation and selection of hepatocyte precursors in suspension spheroid culture of transgenic murine embryonic stem cells

Elke Gabriel[1], Eugen Kolossov[2], Stephanie Schievenbusch[3], Dirk Nierhoff[3], Tamara Rotshteyn[1], Heribert Bohlen[2], Jan G. Hengstler[4], Jürgen Hescheler[1], Irina Drobinskaya[1]

Abstract

Establishment of a hepatocyte-like in vitro system is a topical issue because of a need for a reproducible and accessible tool for early drug discovery and toxicological screenings. Also, such a system could prospectively serve as a source of hepatocytes feasible for clinical transplantations. In contrast to primary hepatocytes, which rapidly lose their functionality, embryonic stem (ES) cell-derived hepatocyte-like cells could represent a convenient material for both clinical trials and drug- and toxicology testing. Attempts to differentiate hepatocytes from ES cells are mostly based on application of defined-composed media containing growth factors and cytokines. However, the main associated problem is still an efficient differentiation and, in particular, selection of differentiated cells reliably imitating hepatic features. In this study we aimed to establish a system for generation of hepatocyte precursors capable of development to hepatocyte-like cells.

We used stable transgenic clones of murine ES cells possessing a bicistronic expression vector which contains the eGFP reporter gene and a puromycin resistance cassette under control of a common α-fetoprotein (AFP) gene promoter. Thus, in our experimental model, differentiating endoderm-derived hepatic progenitor- or hepatocyte-like cells with the activated AFP promoter coordinately developed eGFP fluorescence and puromycin resistance, allowing for their “live” monitoring and antibiotic selection. For high-yield generation of target cells, spinner flask cultivation mode was used.

Cell cultures were mainly investigated by real time qRT-PCR analysis, fluorescent immunostaining, and some functional assays.

We established an up-scaled embryoid body (EB) culture, in which eGFP-expressing cells developing in the outer rim of EBs could be preliminary selected by exposure to puromycin and then separated as aggregates by collagenase treatment. In the course of further cultivation and selection in spinner flasks using specifically optimized rotation parameters, they formed growing spheroids that comprised actively proliferating cells. RNA analysis of the spheroid culture revealed expression of hepatic precursor and hepatocyte marker genes, comparable to that in fetal liver. Once plated onto collagen I substrate, the spheroids outgrew as colonies expressing hepatic proteins albumin and α-1-antitrypsin, the main hepatic cytokeratin CK-18, cell membrane protein of periportal hepatocytes E-cadherin, and, at a lower level, liver specific organic anion transporter Lst-1. Glycogen-storing and indocyanine green up-taking cells were detected as well.

The data obtained suggest differentiation of hepatocyte precursor cells and likely also of a fraction of more advanced differentiated cells in the established spheroid culture. This confirms a feasibility to produce and to select cells, which are able to develop to hepatocytes, using high-yield dynamic suspension cultures facilitating early hepatic differentiation.

[1]*Institute of Neurophysiology, University of Cologne, Germany,* [2]*Axiogenesis AG, Cologne, Germany,* [3]*Clinic of Gastroenterology and Hepatology, University of Cologne, Germany,* [4]*Leibniz Research Centre for Working Environment and Human Factors, Dortmund, Germany*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700087

Key factors for the differentiation of murine embryonic stem cells and induced pluripotent stem cells into respiratory epithelial cells

S. Schmeckebier[1], K. Katsirntaki[1], C. Mauritz[1], M. Sgodda[2], V. Puppe[2], J. Duerr[3], A. Schmiedl[4], Q. Lin[5], M. Ochs[4], M. Zenke[5], T. Cantz[2], M. Mall[3], U. Martin[1]

Abstract

The ability to ex-vivo produce respiratory epithelial cell types would offer new therapeutic options to treat pulmonary injuries and diseases, including genetic surfactant deficiencies and cystic fibrosis. Pluripotent stem cells (PSCs) may represent a suitable cell source but the efficiency of existing differentiation protocols towards lung epithelial cells is still low. Therefore, we aimed at identifying key factors which drive the differentiation of murine embryonic (mESCs) as well as murine induced pluripotent stem cells (miPSCs) into airway epithelial Clara cells and alveolar epithelial type II (ATII) cells. We made use of miPSC clones established from two different strains of transgenic CCSP-rtTA2^{s}/GFP-tetO$_7$-lacZ mice which either confer transgene expression predominantly in Clara cells or ATII cells.

Keratinocyte growth factor (KGF, FGF-7), a member of the fibroblast growth factor family, is known to be a strong growth factor for primary airway and alveolar epithelial cells. Similar to KGF glucocorticoids also have a stimulating effect on the maturation of primary lung epithelial cells and this effect is even potentiated by the simultaneous addition of a cAMP analog and isobutylmethylxanthine. We thus hypothesized that the addition of KGF either alone or together with the three-factor combination dexamethasone, 8-bromoadenosine-cAMP and isobutylmethylxanthine (DCI) might support the pulmonary differentiation of mESCs and miPSCs. Indeed, we identified a synergistic effect of KGF and DCI on the differentiation into lung epithelial cell types with a surprising need for an early KGF supplementation already starting at day 0 or 5 of differentiation; suggesting a so far unknown effect of KGF on the generation of lung epithelial (progenitor) cells from PSCs per se during the early phase of differentiation when lung epithelial cells are not yet originated. This synergistic effect resulted in increased mRNA expression of the lung epithelial specific markers surfactant protein (SP-)B, SP-C and Clara cell secretory protein (CCSP). Affimetrix microarray analyses further confirmed the positive effect of KGF and DCI on pulmonary cell generation from PSCs. Finally, electron microscopy revealed the presence of ATII-like cells which contained composite body-like and more mature lamellar body-like structures, typical organelles of ATII cells. Those lamellar body-like structures had striking similarities with lamellar bodies of ATII cells of the adult rodent lung. In addition, cells were identified with ultrastructural features typical for Clara cells.

In conclusion, we were able to demonstrate a synergistic effect of KGF and DCI on the differentiation of PSCs resulting in increased expression of respiratory marker genes and cells with ultrastructural features typical for ATII and Clara cells. The successful generation of lung epithelial cells from iPSCs paves the way for an autologous cell therapy of lung diseases.

[1]Medical School Hannover, Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), Department for Cardiothoracic, Transplantation and Vascular Surgery, Hannover, Germany, [2]Medical School Hannover, Junior Research Group Stem Cell Biology, Hannover, Germany, [3]University of Heidelberg, Division of Pediatric Pulmonology and Cystic Fibrosis Center, Department of Pediatrics III, Heidelberg, Germany, [4]Medical School Hannover, Institute for Functional and Applied Anatomy, Hannover, Germany, [5]Aachen University Medical School, Institute for Biomedical Engineering-Cell Biology, Aachen, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700088

Rapid soft lithography for large-scale embryoid body formation of induced pluripotent stem cells on agarose microwells

J. Dahlmann[1], G. Kensah[1], D. Skvorc[1], A. Gawol[1], U. Martin[1], I. Gruh[1]

Abstract

Current research in the field of tissue engineering is focused on the production of large-scale amounts of pluripotent stem cell-derivatives (e.g. cardiomyocytes, hepatocytes, insulin-producing cells). To realize this, stem cell aggregation in microwells is a powerful approach yielding highly reproducible differentiation units in large quantities. We have developed a rapid and inexpensive soft lithography method to fabricate silicone masters, which can be used for agarose/DMEM patterning within a petri dish. Resulting non-adhesive "AgarOwell" plates containing800 microwells on one 10 cm dish were used for the aggregation of 1000 murine iPS cells / well into embryoid bodies (EBs). Comparison to the "gold standard" hanging drop method, led to similar results concerning harvesting efficiency (almost 100%), size distribution of EBs, cardiac differentiation efficiency (98-99% beating EBs on day 8) and marker expression. Thus, the presented method offers an inexpensive, fast and reproducible approach for large quantity/high quality embryoid body formation.

[1]Hannover Medical School, Lebao, Hannover, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700089

Differentiation of adipose tissue-derived mesenchymal stem cells into neuron-like cells on IKVAV-modified hydrogel scaffolds

L. Baranovicova[1,2], S. Kubinova[1], D. Marekova[1], E. Sykova[1, 2]

Abstract

The differentiation of adipose tissue-derived mesenchymal stem cells (AT-MSC) into neuron-like cells on an engineered biomaterial has been used in an effort to construct a cell-seeded scaffold that could be used as a permissive bridge that promotes axonal regeneration and the re-establishment of damaged connections after spinal cord injury. Modifications of poly(2-hydroxyethyl methacrylate) (PHEMA) with laminin derived peptide sequence IKVAV (Ile-Lys-Val-Ala-Val) have been developed to create highly superporous hydrogel scaffolds that promote cell-surface interactions.

AT-MSC were isolated from Wistar rats and seeded on IKVAV-PHEMA hydrogels as well as on control laminin-coated glass cover slips. The neuronal differentiation was induced by using Neurobasal media containing B27 and growth factors - EGF, bFGF and NGF during 11 days. The results were evaluated by qPCR and immunocytochemistry.

During neural differentiation, the increased expression of the neuronal markers ß-III-tubulin, MAP2 and nestin, GFAP, and the trophic factors BDNF, GDNF, EGF and FGF was observed in cells seeded on IKVAV-PHEMA hydrogels.

Immunocytochemisty revealed that during differentiation, the cells on IKVAV-PHEMA hydrogels became positive for MAP2, while they remained positive for nestin, ß-III-tubulin, NCAM, NG2 and p75. Neuronal differentiation on laminin-coated coverslips resulted in decreased immunostaining for nestin and ß-III-tubulin, while the cells remained positive for NCAM, NG2 and p75.

The results suggest that the IKVAV sequence has a supportive effect on neuronal differentiation and that IKVAV-modified hydrogels can be used as cell-seeded 3D scaffolds for neural tissue regeneration.

Supported by: GAČR: P304/11/0731, P304/11/0653, 108/10/1560, 309/08/H079; 1M0538 and IAA500390902.

[1]Institute of Experimental Medicine, Prague, Czech Republic, [2]2nd Medical Faculty of Charles University in Prague, Center for Cell Therapy and Tissue Repair and Department of Neuroscience, Prague, Czech Republic

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700090

Epithelial differentiation potential of adipose-derived stromal / stem cells

P.C. Baer[1]

Abstract

Adipose-derived stromal / stem cells (ASCs) possess a multilineage differentiation potential, can be used from an autologous origin and are, therefore, attractive candidates for clinical applications to repair or regenerate damaged tissues and organs. Adipose tissue as a stem cell source is ubiquitously available and has several advantages compared to other sources. It is easily accessible in large quantities with a minimal invasive harvesting procedure and the isolation of ASCs yields a high amount of stem cells, which is essential for stem cell-based therapies and tissue engineering. Differentiation of ASCs into cell types of mesodermal origin has been shown in a variety of studies. The plasticity of ASCs towards cells of the mesodermal lineage has been shown by their differentiation into chondrocytes, osteoblasts, adipocytes, and myocytes. Their potential to differentiate into lineages with non-mesodermal origin is even more exciting: ASCs are also able to differentiate into cells of ecto- and endodermal origin. Various *in vitro* and *in vivo* studies documented the induced differentiation into hepatocytes, pancreatic islet cells, endothelial cells, and other epithelial cells. Here, I summarize the current knowledge of the potential of ASCs to differentiate into the epithelial lineage. The differentiation of ASCs into different types of epithelial cells including hepatocytes, pancreatic cells and endothelial cells is highlighted. Furthermore, results from our lab demonstrating the induction of different epithelial markers by induced ASCs were shown.

[1]*Uniklinik Frankfurt, ZIM Nephrologie, Frankfurt, Germany*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700091

Chemical modification epigenetically 'renews' old human adipose derived mesenchymal stem cells and improves their differentiation into

X. Yan[1], C. Seeliger[1], P. De Sousa[2], L. Schyschka[1], U. Stöckle[3], S. Ehnert[3], A. Nüssler[3]

Abstract

Objective:

Adipose derived mesenchymal cell (Ad-MSCs) is a promising source for autologous cell therapy because of their safety and lower risk of tumorogenesis. But Ad-MSCs from old donors are less pluripotent and do not proliferate as good as the ones from young donors. We previously found this age-related loss of capacity had a link to the epigenetic and pluripotency status, and consequently lead to their lower hepatic and osteogenic differentiation capacity. The aim of the present study was to find out whether old Ad-MSCs could be modified epigenetically by 5-Azacytidine (AZA) or BIX 01294 (BIX) to improve their biological function after differentiation into hepatocyte or osteoblasts like cells.

Material and methods:

Ad-MSCs were isolated from abdominal adipose tissue of young (≤45yrs) and old (>45yrs) donors according to the ethical guidelines of TU Munich. Each group was pretreated with AZA (5, 20 µM) and BIX (0.1, 0.2 µM) over 24 and 48 h and then analysed immunocytochemically for genomic distribution of 5-methylcytosine and 5-hydroxymethylcytosine and expression of pluripotency genes ((Oct4, Nanog, Sox2, c-Myc) and TET oncogenes (1, 2, 3) responsible for hydroxymethylation. To verify differentiation potential into hepatic and osteogenic lineages, specific enzymatic and metabolic parameters were tested post in vitro differentiation. This included assessment of CYP activity, urea production and alkaline phosphatase activity for hepatic function and Alizarin Red and von Kossa staining for osteoblast-like structure.

Results:

Both AZA and BIX treatment significantly decreased the global DNA methylation of Ad-MSCs, most prominent in cells derived from old donors (AZA caused a decrease by 30%, BIX by over 60%). At 48h treatment of AZA and BIX led to an increase of TET1 expression and a higher 5-hmC level in AdMSCs, which was associated with changes in the expression of pluripotency related genes: BIX treatment gave rise to a significant ($p < 0.05$ vs. untreated) elevated Nanog, c-Myc and Oct4 expression in 'old` Ad-MSCs. Furthermore, pretreatment of 'old' Ad-MSCs with AZA or BIX and a subsequent differentiation into hepatocyte or osteoblast-like cells resulted in a significant improvement of function and activity of liver and bone-related markers: pretreated hepatocyte-like-cells demonstrated an elevated urea and glucose metabolic capacity as well as Phase I/II enzyme activity. In some cases AZA/BIX-treated cells even reached similar CYP450 activities and urea levels as seen in primary human hepatocytes. Similarly, AP activity, Alizarin Red and von Kossa staining in osteoblast-like-cells from old donors were found improved in comparison to non-AZA/BIX-treated cells.

Conclusion:

AZA and BIX treatment seems to be a promising approach to modify human Ad-MSCs from old donors up to the level of cells from young donors.This approach would be safer because it avoids viral transfection as used in iPS technology thus reducing the risk of tumorogenesis

[1]Department of Traumatology, Klinikum rechts der Isar, TU München, Germany, Munich, Germany, [2]Scottish Centre for Regenerative Medicine, University of Edinburgh, Edinburgh, United Kingdom, [3]BG Trauma Hospital Tübingen, Eberhard Karls University Tübingen, Tübingen, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700092

Germline-derived pluripotent stem cells for tissue engineering of skin

J. van de Kamp[1], R. Kramann[2], W. Jahnen-Dechent[3], M. Zenke[4], H.R. Schöler[5], R. Knüchel[1], S. Neuss[1,3], R.K. Schneider[1]

Abstract

A dedicated goal in the interdisciplinary area of biomedical engineering and stem cell research is the establishment of complex tissues that resemble their in vivo counterparts.

During embryonic development, the skin forms as a result of reciprocal interactions between mesoderm and ectoderm in the presence of bone morphogenetic protein-4 (BMP-4). Embryonic stem cells (ESC) are able to recapitulate developmental steps in vitro. Recently, germline-derived pluripotent stem cells (gPSC) were generated from explanted murine testis without introducing exogenous transcription factors with tumorigenic potential. Hence, gPSC represent a promising and novel cell type for tissue engineering.

In the current study, we combined mesenchymal-epithelial interactions by cultivating gPSC and ESC air-exposed and on three-dimensional organotypic skin equivalents. We analyzed the differentiation capacity as well as the epidermal cell fate of gPSC and ESC in the presence and absence of BMP-4. Interestingly, gPSC displayed a stronger and accelerated propensity to differentiate compared to ESC by qt-RT-PCR. Under organotypic culture conditions of skin, gPSC generated complex tubulocystic structures, lined by a multilayered, stratified ($CK5/6^+$, $CK8/18^-$) squamous epithelium in ≥50%. In contrast, ESC underwent apoptosis (as shown by staining against caspase 3) under the same epidermal differentiation pressure and only formed small tubulocystic structures lined by simple, $CK8/18^+$ epithelia. Furthermore, gPSC produced a dense eosinophilic basal membrane surrounding the epithelial structures and spontaneously differentiated into mesodermal and endodermal lineages. The addition of BMP-4 neither enhanced the epithelial nor epidermal differentiation of the stem cells. Given that monolayer cultures induced an epithelial but not an epidermal differentiation of both pluripotent cell types, the air-liquid interphase appears to be a strong and essential stimulus for epidermal differentiation of gPSC.

Concluding, we successfully differentiated gPSC into a multilayered, squamous epithelium on organotypic skin equivalents and demonstrated their extensive differentiation potential resembling to early embryogenesis. Challenges for future studies will be the derivation of pure populations of keratinocytes, as well as the elimination of undifferentiated, pluripotent cells to reduce the risk of tumor/teratoma formation.

[1]RWTH Aachen University, Institute of Pathology, Aachen, Germany, [2]RWTH Aachen University, Division of Nephrology and Clinical Immunology, Aachen, Germany, [3]RWTH Aachen University, Institute for Biomedical Engineering, Biointerface, Aachen, Germany, [4]RWTH Aachen University, Helmholtz Institute for Biomedical Engineering, Department of Cell Biology, Aachen, Germany, [5]Max Planck Institute for Molecular Biomedicine, Cell and Developmental Biology, Münster, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700093

Study of regulatory genes in retinal pigmented epithelium cell reprogramming and differentiation of retinal anlage during eye

Y. Markitantova[1], P. Avdonin[1], E. Grigoryan[1]

Abstract

Adult newts (Urodela) are able of eye regeneration even after complete removal of the original neural retina (NR). Because of this, visual system of the newt is a unique *in vivo* model for study of molecular mechanisms regulating retinal pigmented epithelium (RPE) cell proliferation and differentiation, from one side, and morphogenesis of newly forming NR, from another. At the basis of NR regeneration in the newt is RPE cell transdifferentiation, the process resembling a lot of the acquiring of “stemnessy” by iPSCs, what makes the model quite relevant in the present. After retina removal, RPE cells loose cell-type identity, stop to synthesize melanin, loose pigment granules and epithelial morphology, re-enter cell cycle with altered parameters, change cytoskeleton protein range and cell micro-surrounding. Then, dedifferentiated RPE cells form transitory, neuroblast-like cell population enable to produce all cell types of definitive NR. In parallel, in the newt, throughout their life, there is a slow growth of the cells at the periphery edge of the retina, in the ciliary marginal zone (CMZ) that consisting a small population of stem cells. Multipotent cells of the CMZ are also involved in NR regeneration in the newt. We study on the role of regulatory genes and signaling pathways which control RPE cell reprogramming to a stem cell-like state, subsequent differentiation to cell types of the NR and morphogenesis of the latter in the newt. Using PCR, real-time PCR and immunochemistry we analyzed the expression patterns of genes encode to signaling FGF 2-FGFR, transcriptional factors Pax6, Otx2, Prox1, Six3, Sox2, Pitx1, Pitx2, and nucleostemin, a multiplex regulator of cell cycle progression. Cell proliferation was studied also by means of several markers: [^{3}H]-TdR, PCNA and BrdU. Cell phenotypes of transdifferentiating RPE and regenerating NR were determined by means of cell-type specific markers: RPE65; b-II-tubulin, rhodopsin, and recoverin. We found the expression of FGFRs in the RPE and their activation by FGF2 signals in early and late time courses of NR regeneration. The co-expression of the proteins FGF2, FGFR2, Pax6, Otx2, Prox1, Six3, Sox2, Pitx2 and nucleostemin was detected at early stages of NR regeneration in proliferating multipotent stem-like neuroblasts from which the NR primordium was formed. The vivid expression of regulatory proteins we studied (FGF2, FGFR2, Pax6, Prox1, Six3, Rx, Sox2, Pitx2, nucleostemin and PCNA) were localized also in CMZ. In other words, the early NR regenerate and CMZ of the adult newt maintains regulative network described during embryonic eye development. Differences in the expression patterns of studied molecules may arise at the level of interactions of similar molecular components of the network, determining both, NR development and regeneration.

This work was supported by Russian Foundation for Fundamental Research (Grant № 11-04-00728; 11-04-00125).

[1]*Koltzov Institute of Developmental Biology RAS, Laboratoty of Regeneration Problems, Moscow, Russian Federation*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700094

Molecular and genetic mechanisms of rat skeletal muscle satellite cells transdifferentiation to cardiomyocyte-like ones

O.V. Balan[1], N.D. Ozernyuk[1]

Abstract

Satellite cells are myogenic stem cells responsible for growth, repair, and maintenance of skeletal muscles at all stages of ontogenesis. Isolated satellite cells *in vitro* represent a convenient model for an analysis of cellular and molecular mechanisms governing proliferation, differentiation, and apoptosis. It was shown that satellite cells under *in vitro* conditions are able to differentiate into osteogenic, adipogenic and chodrogenic cells. However it is not clear so far whether these cells can transform into cardiomyocytes. The answer for this question is important not only for understanding of fundamental aspects of satellite cell phenotype changes but also for the development of the approaches necessary for therapy of cardiac dystrophy. It is well known that Gata, Nkx, Tbx, Ptx, connexins and cadherins play an essential role in regulation of the initial stages of cardiomyogenesis. We studied on the expression of genes encoding transcriptional factors Nkx2.5, Gata4 and membrane proteins connexin-43 (Cx-43), N-cadherin (N-Ca) in the process of satellite cells transdifferentiation into cardiomyocytes *in vitro*. In rat development satellite cells have specificities of differentiation. Because of this we used both: fetal (E20-21) and adult (3 month) rats as a source of cells. In 7-day-old satellite cell culture obtained from fetal rat we revealed low *Gata4* expression. Then its level gradually raised throughout the process of transdifferentiation. Whereas in satellite cell culture obtained from adult skeletal muscle *Gata4* transcripts could be found only on the 14th day of cultivation. Expression of *Nkx2.5* was detected exclusively in fetal satellite cell culture and from the 14th day of transdifferentiation. As to a number of Gata4-possitive cells some of them appeared in 7-day-old satellite cell culture from fetal muscles and in 14-day-old satellite cell from adult ones. The majority of cells were Gata4-positive by the 28th day of transdifferentiation in both cases. Expression of *Cx-43* and *N-Ca* followed since the 7th (fetal satellite cell culture) and the 14th (adult satellite cell culture) days of transdifferentiation. Cx-43 and N-Ca-positive cells were identified in 14-, 21-, 28-days-old satellite cell cultures obtained from both, fetal and adult rat skeletal muscle. It is necessary to note also that expression of heart specific L-type Ca^{2*}-channel (*Cacna1c*) was revealed only in the course of fetal satellite cell conversion. In sum our observations suggest that in satellite cells isolated from fetal skeletal muscle the molecular program of cardiomyogenic differentiation is initiated *in vitro* leading to formation of cardiomyocyte specific cell contacts. This study was supported by the Presidium program of RAS «A biological variety. Gene pool dynamics».

[1]*Koltzov Institute of Developmental Biology RAS, Laboratory of Gevelopmental Biophysics, Moscow, Russian Federation*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700095

Reprogramming of mouse embryonic fibroblasts to cardiomyocytes

J. Vrbsky[1], M. Pesl[1], P. Dvorak[1], A.C. Meli[1]

Abstract

O3bjectives:

During the reprogramming of differentiated cells into induced pluripotent stem cell (iPS) by defined transcription factors, generation of other specific cell types was recently reported (Efe *et al.*, 2011). Our study is aimed to define optimal conditions during reprogramming of mouse embryonic fibroblasts (MEF) leading to cardiogenesis.

Methods:

Using viral transduction of mouse fibroblast derived from different parts of the body (i.e. tail tips, ears, abdominal pars) as well as from the whole body, we delivered transcription factors: Oct4, Sox2, Klf4, and c-Myc, the classical factors frequently used for the generation of iPS cells. We further tested other factors and conditions (BMP-4, Activin, DCA, FGF-2, Wnt inhibitors, VEGF, serum concentration, including different timing of treatment) that could potentially enhance cardiac differentiation. In order to confirm cardiogenesis, we performed immunostaining for specific cardiac markers. Functional characterization of derived cardiomyocytes is being done in order to control cardiac-specific functionality. Thus, development of action potentials, responses to the beta-adrenergic agonists as well as release of calcium from the sarcoplasmic reticulum is being investigated.

Results:

Our preliminary results indicate that within ~10 days from transgene induction and subsequent periodical stimulation by other factors, mouse embryonic fibroblasts (MEFs) are reprogrammed to spontaneously contracting clusters of cardiomyocytes. Process of newly emerging clusters in the fibroblast culture continues over period of several days. Before the detection of the first cardiomyocytes no intermediate iPS stage is observed. Our immuno-cytological experiments indicate positive signals of differentiated cardiomyocytes for the sarcomeric protein troponin I (TnI) as well as for the cardiac sarcoplasmic protein ryanodine receptor (RyR2) and others.

Conclusions:

Our first results indicate the possibility to activate early cardiac reprogramming from MEFs. In addition, our results could offer optimal conditions to enhance cardiogenesis and platform for derivation of specific and functional mouse cardiomyocytes. Transdifferentiation of MEF has the potential for being a reproducible method for generating cardiomyocytes in a quick and reliable way.

[1]*Faculty of Medicine Masaryk University, Department of Biology, Brno, Czech Republic*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700096

Pressure-controlled Intermittent Coronary Sinus Occlusion (PICSO) study on mechanical control of cardiac tissue morphogenesis

N. Khatami[1], V. Wagh[2], P. Wadowski[1], J. Hescheler[2], A. Sachinidis[2], W. Mohl[1]

Abstract

Background:

Despite improved heart failure therapy, there remains a lack of clinically relevant concepts for structural regeneration of the diseased heart muscle. We have previously reported that Pressure controlled Intermittent Sinus Coronary Occlusion (PICSO) - a coronary sinus intervention, reduces infarct size clinically and induces washout clearing the ischemic/reperfused microcirculation. There is experimental and clinical evidence that besides the hemodynamic “mechanistic” effect another molecular pathway may exist influencing myocardial regeneration thus improving clinical outcome. We hypothesize that PICSO is a clinically feasible form activating coronary venous endothelium and its underlying mode of action is similar to the common understanding of stem cell research.

Objective:

We analyzed the regenerative potential of hitherto unknown molecules released in the Coronary venous blood of heart failure patients after 20 minutes of PICSO to confirm our hypothesis of reactivating developmental processes ("embryonic recall") by inducing proliferation in cell cultures as surrogate for regenerative pathways.

Design: Serum from healthy volunteers and Cardiomyopathy patients enrolled during the surgical intervention of resynchronization therapy, was collected pre and post 20 minutes of PICSO and compared to controls (n>10). Serum levels of *interleukin-6* (IL-6) and *N-terminal pro-brain natriuretic peptide* (NT-ProBNP) levels were analyzed. In addition, *in vitro* cellular proliferative and migrative evaluations were also measured.

Results:

Levels of NT-proBNP and IL-6 were seen in serum samples from cardiomyopathy patients increased after PICSO therapy. Serum IL-6 levels were associated with NT-ProBNP levels. Interestingly, 3 of 5 test serum samples showed also a significant increase in the capability of proliferation of human fibroblast cells compared to both control groups. In addition, cell scratch test showed increase migration capability (P>0.05) for test serum samples.

Conclusions:

Levels of established cardiovascular biomarkers (IL-6 and NT-ProBNP) could indicate an induction of the SAFE-pathway, leading to mitochondrial integrity and explaining reduction in infarct size. The *in vitro* data on proliferation and cell migration indicate that, besides the hemodynamic mechanistic effect also molecular pathways recapitalizing developmental processes may exist and that these effects are responsible for the beneficial effects of PICSO clinically. Based on our results we have to scrutinize the hitherto unknown molecules in further experiments to elucidate the conundrum of clinical relevant structural regeneration.

[1]*Medical University of Vienna AKH, Department of Cardiothoracic Surgery, Vienna, Austria,* [2]*Medical University of Cologne, Institute of Neurophysiology, Cologne, Germany*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700097

Amniotic membrane: Chondrogenic differentiation in toto

A. Lindenmair[1,2], S. Wolbank[1,2], G. Stadler[1], C. Hackl[3], A. Meinl[1,2,4], A. Peterbauer-Scherb[2,3], C. Gabriel[2,3], J. Eibl[5], M. van Griensven[1,2], H. Redl[1,2]

Abstract

Human amniotic membrane has already been applied in a lot of clinical trials due to its beneficial properties and is still of great interest for a multitude of areas of research. Tissue engineering strategies usually require cell isolation and combination with a suitable carrier substrate. Alternatively, the cell sheet technology enables transplantation of expanded cells without the use of carrier materials. In contrast, our approach is to differentiate stem cells within intact amniotic membrane (AM), which constitutes a pre-formed sheet of stem cells, without prior cell isolation. We have previously demonstrated osteogenic differentiation of stem cells within intact human AM in vitro. Beside bone, regeneration of cartilage is an important scope in orthopedic trauma. For this reason, also the chondrogenic differentiation potential of human AM was investigated. In vitro chondrogenic differentiation of human AM biopsies was induced by culture in Mesenchymal Stem Cell Chondrocyte Differentiation Medium (C, Lonza) supplemented with transforming growth factor beta 3 (Lonza), optionally supplemented with fibroblast growth factor 2 (FGF-2, R&D Systems; CF), and the medium described by Tallheden (T) for 16 weeks. Membrane viability was quantified by EZ4U-Nonradioactive Cell Proliferation and Cytotoxicity Assay (Biomedica). To determine chondrogenesis, cartilage-specific collagen II and glycosaminoglycans (GAG) were demonstrated by alcian blue staining of paraffin embedded histological sections. Furthermore, the amount of GAG was quantified using BlyscanTM Sulfated Glycosaminoglycan Assay (Biocolor). The highest hAM-viability was sustained in the medium described by Tallheden [d56: control medium (Co): 15.12%±1.47%; C: 24.56±4.26%; CF: 21.71%±13.31%; T: 56.18%±19.14% compared to d0]. Regardless of the medium applied, Alcian blue stainings of all AM-samples revealed accumulation of GAGs in the membranes. Nevertheless, stainings of samples cultured in control medium were less intense, whereas CF appeared to show the most intense staining. Quantitative evaluation showed that in the control medium (Co) the amount of GAG was decreasing during culture (52.0%±14.5% on d56 compared to d0). When culturing in C, the GAG-amount was increased compared to Co at any timepoint and remained stable throughout the culture period. Noticeable, 3-fold increased GAG production compared to Co was obtained when adding FGF-2 to the medium. Chondrogenic differentiation of intact human amniotic membrane could be induced by chondrogenic medium (C) and further increased with supplemented FGF-2. Thus, these results are another promising step towards using intact human amnion with its residing stem cells for tissue engineering.

[1]Ludwig Boltzmann Institut für experimentelle und klinische Traumatologie, Vienna, Austria, [2]Austrian Cluster for Tissue Regeneration, Vienna, Austria, [3]Red Cross Blood Transfusion Service of Upper Austria, Linz, Austria, [4]Bernhard Gottlieb University School of Dentistry, Vienna, Austria, [5]Bio-Products & Bio-Engineering AG, Vienna, Austria

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700098

Establishment of a dissociated inferior colliculus culture

A. Warnecke[1], U. Reich[1], P. Aliuos[1], T. Lenarz[1], K. Wissel[1]

Abstract

The auditory midbrain implant (AMI) is a novel prosthesis directly activating the auditory neurons within the inferior colliculus for hearing restoration in patients with bilateral neural deafness. As a response to insertion trauma, the formation of a reactive fibrillary sheath around the electrode is observed. From cochlear implant-related research it is known that fibrosis or gliosis can impair electrode-tissue-interaction. Structuring of the surface of the electrode, though, can hinder unspecific growth of fibrotic tissue within the cochlea. In order to test the influence of surface structuring on retrocochlear neuronal tissue, however, an adequate in vitro model is missing. Thus, aim of the study was the development of a dissociated inferior colliculus culture to study interactions between neurons and structured surfaces. Colliculi inferiores were isolated from neonatal rats and were seeded after dissociation in laminin-coated 96-well-plates. After a cultivation period of 2-5 days, cells were fixed and stained immunocytochemically. Using glial- and neuron-specific antibodies such as against S100, MAP2, Tuj1 and GAP43 the established cultures were characterized morphologically. We could demonstrate adhesion and survival of dissociated neurons and glial cells isolated from the inferior colliculus. Using this in vitro model, interactions between cells of the inferior colliculus and surface modifications of electrodes to be implanted could be investigated in order to minimize reactive gliosis formation around the implantation site and potentially for a more effective hearing restoration.

[1]*Hannover Medical School, Otorhinolaryngology, Hannover, Germany*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700099

Cardiac regenerativel biology strategies in experimental models

A. Arti[1], A. Bader

Abstract

Regeneration is a promising and also complex phenomena in biology and medicine. All species have some kind of capacity for regenerating but the level for this capacity depends on species and tissues are varied.

Regenerative biology is the process of replacing or regenerating animal cells, tissues or organs to restore or establish normal function. Methods for that are different but it could be by stimulating the body to repair or by replacing damaged tissues or organs.

The main strategies for regenerative biology in general and especially in cardiac regenerative biology are based on the two main following approaches: 1- Cell therapy (e.g. stem cell therapy) and 2- Tissue therapy (e.g. tissue engineering).

Stem cells unlike other cell types in the body have two main important properties: 1- They are self- renewal and unspecialized cells. 2- They have unlimited potency.

There are two main types of stem cells: 1- Embryonic stem cells (ESCs) and 2- Adult stem cells (ASCs). In this presentation a brief history and the recent findings of administration of ASCs on the treatment of the heart will be reviewed.

In some vertebrates such as Zebra fish cardiac regeneration was reported following severe injury of heart. The mechanism for this cardiac regeneration has not fully understood but it has suggested that it could be based on divisions of cardiomyocytes.

In animals the idea of regenerating damaged heart tissue after myocardium infarction (MI) using stem cell transplantation is very promising.

Adult stem cells are multipotent and can substitute to repair damaged tissues. Due to lack of ethical concerns they widely are used in the therapy of patients across the world in clinical trials for treatment of different diseases like cardiovascular disease (CVDs), however, adult stem cell therapy is not still a routine approach for treatment of diseases.

As before any clinical studies to undertake experimental studies are necessary, therefore to review the results of such experiments are very important too.

In this oral presentation I explain recent findings on regenerative biology strategies with emphasize on the treatment of heart diseases (especially MI) with stem cells in experimental models.

[1]*University of Leipzig, Cell Techniques and Applied Stem Cell Biology, Leipzig, Germany*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700100

Platelet lysate for cell therapy and regenerative medicine

K. Plöderl[1], A. Lindenmair[2], F. Hildner[1], A. Peterbauer-Scherb[1], H. Redl[2], S. Hennerbichler-Lugscheider[1], C. Gabriel[1]

Abstract

Cell cultures for cell therapy and regenerative medicine products are mainly performed in the presence of fetal calf serum (FCS). Due to its animal origin, the use of FCS bears the risk of prion disease transmission or immunogenicity of xenogeneic proteins. Several studies are already published that replaced FCS by human serum or use of defined media where recombinant growth factors were added. Due to its high concentrations in growth factors (GF), human platelet lysate (hPL) also turned out to be a potential substitute for FCS.

Our aim was to develop a production process according to GMP requirements for hPL to be used in cell culture. At the beginning, hPL was made out of outdated thrombocyte pools. The concentrates were stored at -80°C and thawed in a water bath at +37°C to cause platelet lysis. hPL was compared to FCS in several proliferation studies including adipose tissue derived stem cells (ASC), amniotic mesenchymal stroma cells, fibroblasts and chondrocytes. Furthermore, differentiation of ASC in presence of hPL as well as redifferentiation of chondrocytes cultured with hPL was achieved. In all these studies lower amounts of hPL were needed to obtain equal or superior results compared to FCS.

However, there is only a low amount of thrombocyte pools which need to be discarded and heparin needs to be added to the medium to avoid gel formation. Therefore, process adaptation was performed. Buffy coats with too low plasma levels that would be discarded are used. The plasma is replaced by 0.9% sodium chloride solution and volume reduction as well as increase in platelet concentration was achieved by centrifugation. Finally, several freeze thaw cycles were performed to achieve the highest yield in GF.

Preliminary cell culture results with fibroblasts and ASC showed that the modified hPL is also better than FCS but the ideal final concentration in the media needs to be determined. For process control, sterility testing is implemented. Measurement of pH-value, platelet concentration, and total protein content are defined as quality control parameters. Additionally, ELISA for platelet derived GF BB, transforming GF β1, insulin-like GF 1, epidermal GF and basic fibroblast GF were performed. We were able to define a GMP compliant production process for hPL that obtains better results in cell culture than FCS. Thus, hPL can be used in cell culture to avoid the use of non-human products in cell therapy and regenerative medicine.

[1]*Red Cross Blood Transfusion Service of Upper Austria, Linz, Austria,* [2]*Ludwig Boltzmann Institut für experimentelle und klinische Traumatologie, Linz, Austria*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700101

A new bioreactor for the development of beating cardiac patches

F. Herrmann[1], A. Lehner[1,2], T. Hollweck[1], U. Haas[1], F. Koenig[1,3], R. Kozlik-Feldmann[2], G. Eissner[1], E. Wintermantel[3], C. Schmitz[1], B. Akra[1]

Abstract

Objectives:

The development of functional myocardial patches requires several different tools, the most complex currently being the bioreactor. An ideal bioreactor for this task imitates the conditions inside the beating human heart so that the conditioned cells begin to beat, mimicking cardiomyocytes.

Methods:

The bioreactor engineered in our laboratory addresses two important variables in tissuegenesis of cardiac tissue. Firstly, the cell-seeded patches are stimulated using an electrical circuit. The electrical current imitates the ECG of physiological heart tissue in-vivo. Secondly the flow, created by a piston inside the bioreactor, is programmed to mimic the flow in the living heart. To achieve this, an ideal biomimetic flow curve is produced by programming the piston movement. The bioreactor consists of a Plexiglas shell with a custom built piston in the medium chamber. At the top of this chamber there is a ring for patch fixation.

Results:

The architecture of the system allowed easy visualization of the medium flow as well as inspection of the ring for patch fixation. Seven day function testing showed that the bioreactor permits effective and sterile conditioning. Microbiological analysis of the used medium revealed no bacterial contamination. During this trial run various amplitudes and cardiac outflow rates were successfully tested.

Conclusion:

Unlike any other current bioreactor in myocardial tissue engineering our bioreactor achieves simultaneous electrical and mechanical stimulation. Both these stimuli as well as chemical signaling have been shown to be vital to cardiac tissue development. Combining chemical differentiation and electromechanical conditioning of stem cells in this bioreactor we hope to create a beating myocardial patch.

[1]*Ludwig Maximilian University, Department of Cardiac Surgery, Laboratory for Tissue Engineering, Grosshadern Medical Center, Munich, Germany,*
[2]*Ludwig Maximilian University, Department of Pediatric Cardiology and Pediatric Intensive Care, Grosshadern Medical Center, Munich, Germany,*
[3]*Technical University Munich, Chair of Medical Engineering, Garching, Germany*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700102

Beneficial modulation from a high-purity caviar-derived homogenate on chronological skin aging

F. Marotta[1]

Abstract

Collagen abnormalities have been shown to be a cause of the cutaneous changes taking place during physiological aging with reduction of thickness and alterations in its biomechanical properties. Concomitantly, increased elevated MMP expression with a related reduction in collagen synthesis are common associated mechanisms. Thus, although the exact mechanism of cutaneous aging is unclear, it is known that intrinsic biological skin aging results in a loss of collagen and an increase in MMP-1 expression. Moreover, very recently it has been found that a significant decrease in mitochondrial membrane potential in samples from aged donors, accompanied by a significant increase in ROS levels.his study tested the activity of LD-1227 (containing caviar-derived homogenate added with. CoQ_{10}-selenium component, CaviarLieri®, Lab-Dom, Switzerland) in aged human skin and its role on skin mitochondria function Human dermal fibroblasts were obtained from healthy donors over 70y old and treated with LD-1227 for 72 h. As compared to baseline, LD-1227 caused a robust (>67%) collagen type I synthesis ($p< 0.001$) and decreased fibronectin ($p< 0.05$) with significant fibronectin mRNA downregulation ($p< 0.05$, $r=0.78$). A significant collagen mRNA overexpression occurred with LD-1227 treatment ($p< 0.05$). Mitochondria cytosolic ATP level decreased in aged skin samples ($p< 0.05$ vs young control) but this phenomenon was reverted by LD-1227 (< p 0.01). These data show that LD-1227 modify the extracellular-matrix milieu in aged skin and beneficially affect mitochondrial function.

[1]*Reference and Translation Center for Cardiac Stem ReGenera Res Group for Aging Intervention, Milano, Italy*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700103

Is it feasible to implant transcatheter a tissue engineered pulmonary valve without tissue distortion?

F. Schlegel[1], A. Salameh[2], K. Oelmann[1], P. Kiefer[3], S. Dhein[1], F.W. Mohr[3], P.M. Dohmen[3]

Abstract

Objective:

Today glutaraldehyd fixed pulmonary valves are implanted in clinical trails, however limited by absence of regeneration, remodelling and growth potential. This feasibility study was performed to evaluate deliver-related tissue distortion of tissue engineered (TE) heart valves during implantation.

Methodes:

The use injectable TE heart valves was mounted on a self-expanding nitinol stent (n=7) and delivered into the pulmonary position of seven pigs (26-31 kg), performing a stenotomy or limited lateral thoracotomy. Prior to implantation, the injectable TE heart valve was crimped by using an applicator. The positioning of the implant was guided by fluoroscopy. Hemodynamic measurements were performed by epicardial echocardiography, angiography and invasive pressure measurements. Finally, the animals were sacrificed and the injectable TE heart valves were inspected by gross examination and histology.

Results:

Orthotropic delivery of the implanted TE heart valves (diameter 19mm) were successfully performed in all, expect in one due to valve migrated become of size discrepancy. Angiographically all other valves (n=6) showed normal valve function, supported by epicardial echochardiography in which no increase flow velocity was measured, neither valve regurgitation. Invasive pressure measurements showed a mean pressure gradient of 5mmHg. Histological evaluation demonstrated no integrity changes of extracellular matrix and absence of collagen and elastin distortion.

Conclusion:

Transcatheter implantation of an injectable TE heart valve seems to be possible without tissue distortion due to the delivery system.

[1]Herzzentrum Leipzig, FuL/ Chirurgie, Leipzig, Germany, [2]Herzzentrum Leipzig, Kinderkardiologie, Leipzig, Germany, [3]Herzzentrum Leipzig, Chirurgie, Leipzig, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700104

Case of malignant transformation of mesenchymal stem cell engineered tissue in a rat heart in vivo

C. Spath[1], F. Schlegel[1], S. Leontyev[2], M. Nichtitz[1], R. Schmiedel[1], F.W. Mohr[2], S. Dhein[1]

Abstract

Aim:

To find an alternative treatment option for heart failure we produced engineered tissue from mesenchymal stem cells (MSC) to enhance the immunological compatibility after implantation in rat heart.

Methods:

We used a rat bone marrow derived mesenchymal stem cell line (MSC, Gibco) to produce engineered tissues (MSC-ET). Low-passage (P6) MSCs were analyzed for phenotype characteristics by flow cytometry and adipogenic differentiation.

Cultured MSCs were mixed with matrigel, collagen and serum containing media and were casted into a circular structure for creating MSC-ETs. After 5 days of consolidation time the MSC-ETs were electrically stimulated (1Hz, 1mV and 0,1mA) for further 6 days. Subsequently MSC-ETs (n=3) were implanted around the beating heart of immunosupressed adult rats. After 1 month the rat hearts were surgically excised.

In vitro and in vivo MSC-ETs were histologically examined for collagen and elastic-fibers. By immunohistochemical investigation we measured expression of cardiac connexins (Cx40, Cx43 and Cx45), vessel associated von Willebrandt factor and mesenchymal stem cell-marker CD90.

Results:

At day 14 in vitro MSC-ETs (n=4) did not exhibit any contractions. Histological analysis showed rings containing 64.57±2.04% collagen tissue, but no elastic fibres. Furthermore we exhibited 19.7±8.1 micro vessels / 1mm².

In vivo 2 of 3 MSC-ETs were clearly distinguishable from the native heart. Our investigation showed that MSC-ETs in vivo consisted of less collagen (27.43±9.56%) than in vitro, but developed elastic fibers (0.93±0.18%). The number of micro vessels showed an increase up to 39.3± vessels /1mm².We also found CD90 and Cx43 expressing cells in vivo and in vitro.

Surprisingly, in 1 of 3 MSC-ET-transplanted rats the heart was completely surrounded by a huge, undifferentiated, pleomorphic sarcoma. Histological investigations of the growth and location of the tumour indicated that the tumour was part of the MSC-ET. Analysing the grade of the sarcoma by using the FNCLCC-grading system revealed, that the neoplasm was a high grade malignant sarcoma (total score= 7/8).

Dedifferentiated sarcoma cells lost the CD90 expression utterly, which certainly might indicate the malignant transformation.

Additionally, the majority of sarcoma cells were positive for the cardiac connexins (Cx40, Cx43, Cx45), but at the tumour-heart border their expression was left. Therefore, we hypothesize that the tumour was not able to communicate with the heart, which gives the sarcoma cells the opportunity to infiltrate healthy tissue.

Conclusion:

Low passage MSCs of a MSC-ET can dedifferentiate into a high grade malignant sarcoma in vivo. However, it remains unclear whether the transformation might be inborn to these cells or whether it might be caused by the treatment of these cells during the process of ET formation.

[1]Herzzentrum Leipzig, Forschung und Lehre/Chirurgie, Leipzig, Germany, [2]Herzzentrum Leipzig, Chirurgie, Leipzig, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700105

Adjustable mechanical properties of bioartificial cardiac tissue based on defined extracellular matrix blends

J. Dahlmann[1,2], G. Kensah[1,2], A. Krause[2,3], L. Möller[2,3], G. Dräger[2,3], U. Martin[1,2], I. Gruh[1,2]

Abstract

Carbohydrate hydrogels are attractive scaffold materials for myocardial tissue engineering since they provide high biocompatibility and chemical versatility adjustable to specific tissue demands. *In situ* cross-linking hydrogel-precursors based on chemically modified alginate (alg) and hyaluronic acid (hya) were developed, leading to gelation of the respective polymers upon mixing in presence of cells (hydrazone reaction). Entrapment of neonatal rat cardiomyocytes into this hydrogel system together with human collagen I (huCI) led to elongation and functional coupling of cardiomyocytes within resulting bioartificial cardiac tissue (BCT) constructs. Force measurements in our customized bioreactor revealed that different combinations of hydrogels lead to moderate differences in maximal systolic force (L_{max}: Hya/huCI: 1.9±0.28 mN, Hya/Alg/huCI: 1.5±0.12 mN and Alg/huCI: 1.5±0.33 mN), but dramatically distinct diastolic forces (at L_{max}: Hya/huCI: 2.1±1.6 mN, Hya/Alg/huCI: 6.7±1.9 mN and Alg/huCI: 15.0±3.2 mN). Morphologically all constructs resembled native myocardium with organized alignment of cardiomyocytes and well-developed sarcomeric structures. Our study demonstrates that choice and combination of matrix substances can be used to modify mechanical properties of bioartificial cardiac tissue. This strategy might be a valuable tool for the adjustment of stiffness parameters according to the conditions at the target site, ultimately enabling optimized heart muscle reconstruction.

[1]*Hannover Medical School, Lebao, Hannover, Germany,* [2]*REBIRTH, Cluster of Excellence, Hannover, Germany,* [3]*Institute of Organic Chemistry, Leibniz University Hannover, Hannover, Germany*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700106

Does the amniotic fluid is a good source of stem cells for tissue regeneration?

J. Olkowska[1], N. Gurtowska[1], A. Bajek[1], M. Walentowicz-Sadlecka[2], P. Sadlecki[2], T. Kloskowski[1], J. Tworkiewicz[1], I. Glaza[3], M. Grabiec[2], T. Drewa[1]

Abstract

Aims:

Human amniotic fluid stem cells (hAFSCs) have become an attractive stem cells source for medical therapy. The aims of this study were the isolation and characterization of human amniotic fluid stem cells.

Material and methods:

The human amniotic fluid was obtained from 23 patients in age from 19 to 45 years, during planned amniopunctions. Patients were divided into the following age groups: 1th - patients in age from 19 to 29, 2nd - from 30 to 39 and 3rd - from 40 to 45. The average of age in the following groups was respectively: 26 years (SD - Standard Deviation = 3,7), 35 years (SD = 2,7) and 43 (SD = 2,2). The samples were centrifuged at 350g for 10 min. After washing with PBS, cells were gently suspended and plated. When reached confluence the number of isolated cells and their phenotype were assessed. The phenotype was evaluated using: proliferation rate, differentiation potential and clonogenicity.

Results:

Human amniotic fluid-derived stem cells were allowed to adhere to plastic culture dishes and the proliferation rate ranged between $5x10^3$ to $15x10^3$ cells during 7 days of culture. Cells cultured in medium supplemented with differentiation factors toward osteogenic lineage showed change of morphology, from fibroblast-like to polygonal, after 5 days. hAFSCs formed an average of 8,2 ± 1,7 colonies. The clonogenicity that correlates with number of stem cells was 0,16%.
The average of cells number was respectively: in the 1st group - $20x10^4$ (SD = $11x10^4$), 2nd - $46x10^3$ (SD = $15x10^3$) and in the 3rd - $97x10^3$ (SD = $38x10^3$). The average of amniotic fluid amount was respectively: in the 1st group - 1ml (SD = 0,6), 2nd - 1,2ml (SD = 0,5) and 3rd - 2ml (SD = 0). The following results give the 30% of success rate.

Conclusions:

In conclusion, even if further investigations are required, the results obtained in this study support the finding that amniotic fluid contains rapidly growing cells with high differentiation potential. On the other hand, from such an amount of amniotic fluid the number of isolated cells is small, so the effectiveness of culture establishment is to little and limits their application in *in vitro* and *in vivo* studies.

[1]Collegium Medicum in Bydgoszcz Nicolaus Copernicus University in Torun, Tissue Engineering, Bydgoszcz, Poland, [2]Collegium Medicum in Bydgoszcz Nicolaus Copernicus University in Torun, Obstetrics, Gynecology and Gynecologic Oncology, Bydgoszcz, Poland, [3]Collegium Medicum in Bydgoszcz Nicolaus Copernicus University in Torun, Pharmacology and Therapy, Bydgoszcz, Poland

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700107

Cytotoxic and genotoxic effects of arsenic and lead on human adipose derived mesenchymal stem cells (AMSCs)

A.A. Chattha[1], A.R. Shakoori[1]

Abstract

Arsenic and lead are ubiquitously distributed in environment. Human exposure to arsenic and lead is increased due to rapid industrialization and their use in formulation of many products. Presence of arsenic in drinking water is serious health problem in many countries. Arsenic and lead are known to have genotoxic and mutagenic effects and cause different cancers. Liposuction material is rich source of stem cells. In the present study cytotoxic and genotoxic effects of arsenic and lead were tested on Adipose derived mesenchymal stem cells (AMSCs). Cells were exposed to 1-10 µg/ml and 10-100 µg/ml concentration of arsenic and lead respectively for 6, 12, 24 and 48 h and cytotoxic effects were measured by neutral red uptake assay. Genotoxic effects of arsenic and lead were tested by comet assay. There was gradual decrease in growth of cells with increase in time and concentration of arsenic. There was also change in morphology of cells and cell became round at higher arsenic concentrations (10 µg /ml). Decrease in growth of AMSCs was observed on lead exposure but lead proved to be less cytotoxic when cells were exposed for longer duration. No change in morphology of lead exposed cells was observed. DNA damage was observed in metal treated cells. Different parameters of comet assay were investigated for control and treated cells which indicated more DNA damage in arsenic treated cells as compared to lead. Intact nucleus was observed in control cells. Present study clearly demonstrate that both arsenic and lead have cytotoxic and genotoxic effects on AMSCs while arsenic have more deleterious effects on AMSCs as compared to lead.

Keywords:

Anti-proliferative, Cytotoxic, AMSCs, Comet assay, heavy metals, Genotoxic.

[1]*School of Biological Sciences, University of the Punjab, Lahore, Pakistan*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700108

Neuronal networks from stem cell derived neurons as in vitro alternative assay for neurotoxicity evaluation and prediction

T. Meyer[1], E. de Franchi, A. Novellino

Abstract

Testing of compounds for potential neurotoxic properties is required for all drugs and chemicals in Europe. Due to the complexity of neurotoxic effects especially systemic repeated dose toxicology testing is frequently performed in animals. Here we present a high throughput and high content screening platform based on stem cell derived neuronal networks cultured on microelectrode arrays. this assays allow parallell recording of nine neuronal networks with 28 electrodes each for extended periods of time. thus e can demonstrate repeated dose chronic toxicity as well as acute toxic effects with one integrated assay.

[1]*Multi Channel Systems MCS Gmbh, Reutlingen, Germany,* [2]*Natural & Medical sciences Institute at the Tübingen University, Electrophysiology, Reutlingen, Germany*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700109

Recent findings of the effects of mesenchymal stem cells on the treatment of patients following mi (a clinical studies review)

A. Arti[1], A. Bader

Abstract

Cardiovascular diseases (CVDs) are the leading cause of death in the developed countries. CVDs have different types and among them myocardium infarction (MI) is the most frequent form. MI occurs when the blood flow in coronary artery blocked. It might happen due to different reasons but some parameters like age, gender, smoking, lack of physical activity can be involve on the frequency of that.

The main strategies for the treatment of MI are: Oxygen therapy and beta- blockers medication treatment. The main goal for those therapy methods is to restore blood flow in the blocked coronary as fast as possible. The other strategies are like injection of Heparin and to administration of some medication with anti platelet like Aspirin or Clopidogrel and finally in acute condition to undertake the heart surgery is necessary.

To undertake new approaches as supplementary approaches in addition to routine therapy methods seems promising. Among them stem cell therapy is a less invasive, less expensive seems promising approach to accelerate treatment of the patients following MI.

There are two main types of stem cells: 1- Embryonic stem cells (ESCs) and 2- Adult Stem Cells (ASCs).

To discuss ESCs is out of scope of this review but ASCs there are different types of that but the most important type of ASCs is bone marrow stem cells (BMSc). BMSc are delivered to two main sources of 1- hematopoietic stem cells (HSCs) and 2- Mesenchymal stem cells (MSCs).

In patients after MI heart function will reduce like left ventricular ejection fraction (LVEF) and some the other important parameters also affect too, therefore to undertake a new approach to help routine treatments following MI is an important strategy. The first time Strauer et al in 2002 reported successfully clinical administration of BMSc and since then many researches across the world have done more promising experimental and clinical on this topic.

The goal for this review poster is to determine the effects of administration of MSCs on the therapy of patients following MI according to recent published studies on this topic.

[1]Biotechnology and Biomedicine, Medical Faculty, University of Leipzig, Cell Techniques and Applied Stem Cell Biology, Leipzig, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700110

Recent findings of the effects of mesenchymal stem cells on the treatment of heart failure patients (a clinical studies review)

A. Arti[1], A. Bader

Abstract

Hear Failure (HF) is one of the leading causes of death worldwide and especially in developed countries. When heart muscle does not capable to supply blood flow then HF occurs. Changes in human life style (e.g. lack of physical exercise), health problems (e.g. diabetes, hypertension,), smoking, age, and gender are most important risk factors of HF. HF mostly occur in men but the overall prevalence in both sexes are the same because women after HF more survive.

As HF is a chronic cardiovascular disease (CVD) therefore the treatment of that needs a life time management. In other words a patient suffer from HF needs to be under treatment for long time.
As HF has different symptoms depends on them different therapy strategies will undertake by clinicians. A combination of some medications is most common but in severe HF cases to undertake heart surgery is a must.

The main medications are used for HF therapy are: 1- Angiotensin- Converting enzyme (ACE) inhibitors 2- Angiotensin II receptor blockers (ARBs) 3- Beta- blockers 4- Digoxin.

If the HF be more chronic then heart surgery like coronary bypass surgery or in the most severe case heart transplant is the final option save the life of patient. In addition to routine therapies for HF patients it seems that new approaches can be useful. Among them stem cell therapy is a promising method. Stem cells are a group of cells that have the renewal capabilities and they can repair damaged cells and tissues.

There are two main types of stem cells. 1- Embryonic stem cells (ESCs) and 2- Adult stem cells (ASCs). In this review poster we only discuss the effects of the administration of mesenchymal stem cells (MSCs) that are main type of ASCs on HF patients.

In animal models the administration of MSCs led to improvement on heart function in animals with HF condition. In clinical studies also the administration of MSCs on HF patients is promising.

The goal of this review poster is to present an up-to-date on the effects of administration of MSCs on the therapy of patients with HF.

[1]*Center for Biotechnology and Biomedicine, Medical Faculty, University of Leipzig, Cell Techniques and Applied Stem Cell Biology, Leipzig, Germany*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700111

Cell therapy for the cardiovascular repair after an induced acute myocardial infarct in a swine animal model

A. Crovace[1], G. Rossi[2], G. Alessandri[3], F. Staffieri[1], L. Lacitignola[1], E. Francioso[1], G. Ferlan[1]

Abstract

The Aim of this study was to evaluate the results obtained after one year follow up, with the use of Bone Marrow derived Stromal Cells (BMSCs) and of Human Omentum - derived Fat Stromal Cells (HOFSCs) in the repairing of myocardial infarct in pigs.

The BMSCs were harvested from bone marrow collected from ileal crest of pigs while the HOFSCs were obtained from human patients undergoing abdominal surgery. After the isolation the cells were cultured and characterized, were also detected their production of growth factors and cytokines and their angiogenic potential in vitro. After the approval of the Italian Ministery of Health, 30 pigs were enrolled in the study. The myocardial infarct was obtained by a permanent ligation of the inter ventricular artery (IVA). The pigs were divided in three groups: group 1 - treated with BMSCs, group 2 - treated with HOFSCs and group 3 - control group treated with placebo (Saline). In the group 1 and in group 2 after two hours from the ligation of IVA, the cells were injected into the proximal ischemic border zone. After surgery the animals were monitoring periodically by echocardiography, myocardial scintigraphy and cardiac MRI. The animals were euthanized at 3, 6 and 12 months and the hearts were harvested for histological and immunoistochemistry evaluations. In the infarcted area the microvessel density was evaluated using sections labelled for the endothelial markers,

In vitro conditions the cells showed the capacity to differentiate into osteogenic, adipogenic and cardiomyogenic cell lineages and were homogeneous for many markers and produce growth factors, cytokines and an high level of angiogenic factors.

The instrumental evaluations of the heart functionality (echocardiography, myocardial scintigraphy and cardiac MRI) showed an improvement of myocardial function at 3 months post infarct and a significant decrease of distress symptoms in all pigs treated respect to the control group, but at 6 and at 12 months post infarct they do not give indications of amelioration of the hearts condition and these aspects seemed to be the same to the ones of the control group.

The histological examinations at 3 months evidenced, in the treated groups, a reduction of a fibrotic and necrotic tissue and an increment of myogenic, cardyomyogenic and vascular markers that had suggested a better vascularitation and cardiomyogenesis respect to the control group. At 6 and 12 months after the surgery whereas it was possible to observe a major presence of necrotic tissue and an high reduction of cardiomyogenesis with a tickness of the infarcted area and with heart lesions similar to those observed in the control group.

The results obtained demonstrated that after a seeming amelioration at the initial stage (3 months) of the anatomical and clinical aspects, the cell therapy at the other interval of time (6 and 12 months) did not induce the expected improvement.

[1]University of Bari Aldo Moro, D.E.T.O., Valenzano, Italy, [2]University of Camerino, Department of Veterinary Science, Matelica, Italy, [3]Neurological Institute Carlo Besta, Milano, Italy

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700112

Embedment of highly purified murine iPS-derived cardiomyocytes in biodegradable macroporous microspheres as microcarriers facilitates intramyocardial injection of huge absolute cell numbers without altering low fractional cell engraftment

R.G. Sahito[1], B. Krausgrill[2], R. Hippler-Altenburg[1], M. Maaß[2], K. Urban[2], S.P. Potta[1], M. Halbach[2], D. Ladage[2], J. Müller-Ehmsen[2], A. Sachinidis[1], J. Hescheler[1]

Abstract

Objectives:

Heart failure is a major cause of morbidity and mortality in the world and cardiac cell replacement therapy is a promising strategy to restore cardiac function in heart failure. Since functional cardiomyocytes are the lacking cells in heart failure, iPS-derived cardiomyocytes (iPS-CM) are good candidates for transplantation. However, engraftment efficiency is very limited for purified iPS-CM and the small volumes suitable for intramyocardial injection in mice limits the absolute number of transplanted cells. Therefore, we tested embedment of purified murine iPS-CM in biodegradable macroporous microspheres as microcarriers for intramyocardial injection.

Methods:

Male murine Acta2-iPS expressing an antibiotic resistance and eGFP under the control of the Acta2-promotor were differentiated to Acta2-iPS-CM in hanging drops and highly purified with respective antibiotic treatment. Prepared single cell suspensions were either used directly for intramyocardial injections (iPS-CM alone) or they were cocultured with biodegradable macroporous microspheres and the loaded microcarriers were intramyocardially injected (iPS-CM in microspheres). Healthy syngeneic female mice served as recipients in an open chest surgery with 2 intramyocardial injections of 10µl each. Hearts were excised immediately after surgery (0h) or after 24h, genomic DNA was prepared and the number of persisting transplanted cells was determined using quantitative real-time PCR with y-chromosome specific primers. For every surgery day, 1 aliquot was mixed with an explanted heart ex-vivo and served as control and known dilutions of male in female DNA were included to derive a calibration curve.

Results:

After optimization, embedment of iPS-CM in microspheres was very efficient and loaded microspheres were not destroyed or lost when passed through the injection needle. Preliminary data suggest that immediately after intramyocardial injection of iPS-CM alone, only 14.6±5.9% of injected cells were detectable and this value further declined to 2.5±0.8% at 24h. Similarly, after intramyocardial injection of iPS-CM in microspheres numbers were 12.6±4.0% at 0h and 1.4±0.7% at 24h (both P=n.s. vs. iPS-CM alone). Absolute numbers were significantly higher in iPS-CM in microspheres than in iPS-CM alone in controls and at both time points studied, and the difference was 4-5-fold in controls and at 0h and declined to 2.5-fold at 24h.

Conclusions:

Embedment of highly purified murine iPS-derived cardiomyocytes in biodegradable macroporous microspheres as microcarriers is feasible and facilitates intramyocardial injection of huge absolute cell numbers without altering low fractional cell engraftment. Thus, this strategy could be useful to enable efficient cardiac cell replacement therapy.

[1]University Hospital of Cologne, Institute for Neurophysiology, Cologne, Germany, [2]University Hospital of Cologne, Department for Internal Medicine III, Cologne, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700113

Co-transplantation of mesenchymal bone-marrow cells increases persistence of murine iPS-derived cardiomyocytes at 24h after intramyocardial injection into healthy syngeneic hearts

M. Maaß[1], B. Krausgrill[1], C. Steigerwald[1], K. Urban[1], A. Fatima[2], M. Halbach[1], D. Ladage[1], J. Hescheler[2], T. Saric[2], J. Müller-Ehmsen[1]

Abstract

Objectives:

Cardiac cell replacement therapy is a promising therapy to improve cardiac function in heart failure, a major cause of morbidity and mortality in the world. In contrast to clinically intensively tested bone-marrow cells, embryonic or induced pluripotent stem cell can be differentiated into functional cardiomyocytes (ES-CM or iPS-CM) which after transplantation can integrate into host myocardium and thereby replace lost myocardium. Since engraftment and persistence of transplanted ES-CM and iPS-CM was found to be very limited, we tried to increase it in this study by co-transplantation of non-cardiomyocytes with iPS-CM.

Methods:

ES-CM and iPS-CM were derived and highly purifed from transgenic male murine embryonic or induced pluripotent stem cells using an antibiotic resistance under the control of a cardiac specific promoter. 300,000 ES-CM or iPS-CM with or without admixture of 300,000 000 syngeneic male murine mesenchymal stem cells (MSC) or syngeneic male murine embryonic fibroblasts (MEF) were intramyocardially injected into healthy hearts of syngeneic female mice. Hearts were explanted after 24h, DNA was isolated and the number of transplanted cells was determined using quantitative real-time PCR with transgene specific primers. For every surgery day, 1 additional aliquot was mixed with an explanted heart ex-vivo and served as control and known dilutions of transgene positive in transgene negative DNA were included to derive a calibration curve.

Results:

24h after intramyocardial injection of iPS-CM, we detected 0.62±0.44% of the transplanted cell number (approx. 1900 cells), which was significantly less than in the surgery day controls ($p < 0.001$) and similar to the intramyocardial injection of ES-CM with 0.93±0.28% (approx. 2800 cells). After co-transplantation of MSC numbers were 2.5-fold higher with 1.54±0.23% of transplanted transgenic cells ($p < 0.05$ vs. iPS-CM, approx. 4600 cells). Preliminary data suggest that co-transplantation of MEF showed similar results with 1.74±0.93% or approx. 5200 cells).

Conclusions:

Persistence of iPS-derived cardiomyocytes at 24 hours after intramyocardial injection is increased by co-transplantation of MSC, but it remains very limited. Nevertheless, this strategy could be useful to improve the efficiency of cardiac cell replacement therapy and should therefore be further investigated.

[1]*University Hospital of Cologne, Department for Internal Medicine III, Cologne, Germany,* [2]*University Hospital of Cologne, Institute for Neurophysiology, Cologne, Germany*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700114

Cardiac differentiation of human pluripotent stem cells by overexpression of transcription factors - a note of caution

S. Müller[1], K. Schwanke[1], S. Merkert[1], R. Zweigerdt[1], U. Martin[1]

Abstract

Cardiomyocytes (CMs) derived from human pluripotent stem cells (hPSC) possess a high potential for regenerative treatment of cardiovascular diseases as well as for drug screening and drug safety tests in pharmaceutical industry. However, one of the main obstacles to overcome is the low efficiency of *in vitro* cardiac differentiation of human induced pluripotent stem (hiPS) or human embryonic stem (hES) cells. Previous publications provided evidence that transcription factors (TFs) known to be essential for cardiac development could increase cardiomyogenesis upon constitutive (over)expression in mouse ES cells [1], preformed mouse mesoderm [2] and also hES cells [3]. After failure to establish transgenic clones stably expressing multiple TFs, we tested a set of TFs alone and in combination for induction of CMs from hPSCs in a transient approach.

Based on transfection by electroporation followed by a serum-free differentiation protocol, our monolayer-based system revealed Baf60c, Gata4 and Mesp1 (BGM) to be the most effective TF combination in hiPS derived from human cord blood endothelial cells (hcBEC-iPS) [4]. While there were no CMs in untransfected or control transfected cells, up to 60 cell clusters per well of BGM transfected cells stained positively for cardiac Troponin T (cTnT), alpha Myosin heavy chain (aMHC) and sarcomeric alpha-actinin although the clusters did not start beating. Removal of Baf60c from the transfection cocktail diminished cardiomyogenesis only marginally while removal of Gata4 or Mesp1 abolished CM formation completely. Each TF alone did not produce any CMs either.
Whilst these results are encouraging we would like to note that our experiments revealed a strong sensitivity of the system regarding the changeability of cell densities i.e. induced by variable cell survival post-transfection, subsequent proliferation rates during differentiation, as well as local cell assembly. Especially where cells accumulated at the rim of wells cardiomyogenesis was observed even in controls.

Dependence of CM formation on the starting cell number has also been reported in several differentiation systems based on embryoid bodies[5] and monolayers on matrigel [3] highlighting the importance of cell density for paracrine mesoderm and cardiac specification. Taken together, these results highlight the necessity for caution when assessing effects of TFs on directed CM generation *in vitro* in “monolayer protocols” as demonstrated by us and others.

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[1]*Medizinische Hochschule Hannover, LEBAO, Hannover, Germany*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700115

Electrophysiological integration and properties of transplanted fetal and induced pluripotent stem cell-derived cardiomyocytes

G. Peinkofer[1], M. Halbach[1], S. Baumgartner[1], B. Krausgrill[2], A. Fatima[1], T. Saric[1], J. Müller-Ehmsen[2], J. Hescheler[1]

Abstract

Purpose:

Cardiac cell replacement is a promising therapy for ischemic damaged heart tissue. A functional integration and maturation of transplanted cells prevents arrhythmias and enhances synergistic contractions of transplanted and host tissue. We compared the electrophysiological integration and properties of transplanted fetal cardiomyocytes (FCM) and induced pluripotent stem cell-derived cardiomyocytes (iPSCM), which are regarded as promising cell type for cardiac cell replacement therapy.

Methods:

FCM from transgenic mice expressing eGFP under control of the α-actin promoter were isolated at day 14.5. Genetically modified murine iPSCM, expressing eGFP and a puromycin resistance under control of the alpha-MHC promoter, were purified by antibiotic selection. FCM and purified iPSCM were injected into adult mouse hearts (2 injections, ~500,000 cells per site). At different times after transplantation (2-12 days), recipients were sacrificed and viable ventricular tissue slices (thickness: 150 µm) were prepared. Slices were focally stimulated by a unipolar electrode placed in host tissue. Recordings of action potentials were performed by glass microelectrodes in transplanted cells, which could be identified by their green fluorescence, and in host cardiomyocytes within the tissue slices.

Results:

Coupling of FCM and iPSCM to host tissue could be clearly demonstrated. Some transplanted FCM and iPSCM showed no conduction blocks even at high stimulation frequencies of up to 8 Hz, while others had blocks at lower stimulation frequencies or were not electrically integrated at all. Electrical integration was better in FCM than in iPSCM. The delay between stimulation artifact and Action Potential upstroke were significantly prolonged for FCM (19,84±3,77 ms at day 6 and 11,92±2,64 ms at day 12) and IPSCM (35,34±8,79 ms) in comparision to host tissue (6,96±1,0 ms). Electrophysiological properties of transplanted iPSCM differed significantly from those of host cardiomyocytes ($P < 0.05$ for all parameters). IPSCM had a lower maximum diastolic potential (-50.1±5.6 mV vs. -70.9±5.1mV), amplitude (49.7±3.4 mV vs. 75.0±11.5 mV) and maximum upstroke velocity (11.1±1.3 V/s vs. 102.2±31.7 V/s). APD50 was longer (21.7±10.1 ms vs. 13.8±10.0 ms), APD90 was shorter (37.2±15.4 ms vs. 95.7±20.2 ms). In FCM, action potentials were fetal-like at day 6 after transplantation, but were comparable to those of adult cells at day 12.

Conclusions:

Fetal cardiomyocytes and IPSCM are able to integrate electrically into host tissue, but conduction blocks occur and are more pronounced in iPSCM. Action potential properties of transplanted iPSCM differ considerably from those of recipient cardiomyocytes and are also more immature as compared to FCM.

[1]University Hospital of Cologne, Institute of Neurophysiology, Cologne, Germany, [2]University Hospital of Cologne, Department III of Internal Medicine, Cologne, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700116

Cardiac differentiation potential of bone marrow CD117+AT2R+ cell population

M. Ludwig[1], T. Noack[2], A. Skorska[1], A. Tölk[1], G. Steinhoff[1], J. Li[1]

Abstract

Background:

Ang II interferes with cardiac remodeling via its AT1 and AT2 receptor (R). It has been shown that the AT2R is up-regulated in fetus and following cardiovascular injury, and is involved in tissue regeneration and differentiation. We have recently identified the CD117+AT2R+ cell population in rat heart and bone marrow with the potential to enhance cardiac repair/regeneration upon AT2R stimulation. In the present study, we further characterize the cardiac differentiation potential of bone marrow CD117+AT2R+ cell population in response to myocardial infarction (MI) in mice.

Methods:

The CD117+AT2R+ cells were isolated from bone marrow samples of mice after acute MI, analyzed with FACS, Patch-Clamp technique, RT-PCR and immunostaining to assess their differentiation characteristics.

Results:

The isolated bone marrow CD117+AT2R+ cell population exhibited a distinct morphology in culture with a tendency to form cell aggregates. Whole-Cell-Patch-Clamp measurements detected an alteration of potassium channels in this cell population over a time span of 7 days. An increase in ion channel activation and potassium outward rectifier was also observed under defined *in vitro* conditions. In addition, the RT-PCR analysis revealed a changed gene expression profile in these cells upon AT2R stimulation. Moreover, immunostaining showed induced expression of cardiac differentiation markers including Connexin 43 and Mef-2.

Conclusion:

We demonstrate here that bone marrow CD117+AT2R+ cell population is characterized by AT2R-mediated cardiac differentiation potential, as shown by the development of ion channels and the expression of cardiac differentiation markers.

[1]*University Rostock, Reference and Translation Centre for Cardiac Stem Cell Therapy, Rostock, Germany,* [2]*University Rostock, Oscar Langendorff Institute of Physiology, Rostock, Germany*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700117

Recent findings of the effects of embryonic stem cells on the treatment of heart failure in animal models (a preclinical studies review)

A. Arti[1], A. Bader

Abstract

Heart failure (HF) is a serious health problem in developed countries and rest of the world. Typically in contrast with myocardium infarction (MI) that can cause suddenly, HF is a long term problem.

HF has two main types:
Systolic heart failure (SHF) that in this condition the problem arise as the heart pump function can not work sufficiently, therefore blood flow that leaves the heart (ejection fraction) decreases. This decreases depends on the severity of HF can be vary.

Diastolic heart failure (DHF) that in this condition the heart muscle can not fill with blood as diastolic blood pressure in this situation is low. The main reason for this situation is stiffness of the heart muscle.

HF is a common health problem across the world. It has estimated that in developed countries around 2% of adults suffer from it but with increasing the age this problem becomes severe and it rise to 6-10 % in patients over the age of 65. So, it shows that age and changing in life style (e.g. lack or reducing physical exercise) and in addition the other issues like hypertension, myocardium infarction (MI) can cause this main health problem. The researches on small animals especially on rats and mouse are developing rapidly to can answer to questions arise from different aspects of HF. In addition to routine treatments for HF new approaches seems necessary to can accelerate the treatment of this heart disease.
Stem cell therapy is a new promising approach. There are two main types of stem cells. 1- Adult stem cells (ASCs) 2- Embryonic stem cells (ESCs). In human to use ESCs is a challenging issue and it can in some countries use for therapy of patients under certain regulations, however, in animal models ESCs widely use in researches. ASCs is out of scope of this review.

The goal of this review is to evaluate the recent findings of the effects of ESCs on the treatment of HF in experimental models

[1]Biotechnology and Biomedicine, Medical Faculty, University of Leipzig, Cell Techniques and Applied Stem Cell Biology, Leipzig, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700118

Effect of embryonic stem cells on the treatment of myocardium infarction in animal models (a preclinical studies review)

A. Arti[1], A. Bader

Abstract

Myocardium infarction (MI) is the leading cause of mortality and morbidity in the developed countries. It is also one of the main cause of the mortality and morbidity in the developing countries and rest of the world. There are different risk factors for that and among them smoking, age, sex, health problems like diabetes, high blood pressure, and lack of physical exercise are more involved.

The consequence for MI is to reduce blood flow in heart tissue. There are different routine treatments for MI like oxygen therapy, injection of heparin and anti pallets medications like Aspirin or Clopidogrel to restore blood flow in the heart. There are new approaches for treatment of MI: These approaches can use as supplementary methods. Among them stem cell treatment is a promising approach. Animal models are good sources to do research for preclinical studies as with them we can mimic the clinical health problems like MI: The main advantage for stem cell administration is that it is a less expensive- less invasive promising treatment method. The supplementary treatment with stem cells in addition to the routine treatment methods is that it can improve the quality of treatment. In animals especially small animal's sudden death can arise of MI cause. There are different symptoms in animals after MI that among them myocardium scar formation, decreasing heart function, decreasing blood flow, increasing blood pressure are key parameters. MI in some animals like cats and dogs are rare but in small animals especially in rats and mouse causes after ligation when those animals are used as models to evaluate the effects of MI on them. These help researchers to have a better understanding of MI in experimental models to improve the quality of research and treatments in clinical studies. Studies shows that the administration of stem cells can improve the heart function following MI in animal models studies. There are two main types of stem cells but in this review we only review the embryonic stem cells (ESCs) and the other source (Adult stem cells) is out of scope of this review.

In this review poster we undertake a review on the recent findings on this topic in experimental studies.

[1]*Biotechnology and Biomedicine, Medical Faculty, University of Leipzig, Cell Techniques and Applied Stem Cell Biology, Leipzig, Germany*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700119

Analysis of transcripts with unknown function for cardiomyogenesis and function of cardiomyocytes in zebrafish (*Danio rerio*) and murine embryonic stem cells

R. Niemann[1], X. Doss[1], J. Winkler[2], J. Hescheler[1], M. Gajewski[2], A. Sachinidis[1]

Abstract

Heart failure due to the loss of functional cardiomyocytes is one of the most frequent cardiovascular diseases. Understanding the genetic network that leads to functional cardiomyocytes is the first step to develop future therapies. Two murine engineerd mesodermal ESC lines as well as a cardiac-specific ESC line were established in our group. A global transcriptome analysis yielded two genes up-regulated in cardiomyocytes, two genes up-regulated in cardiomyocytes and pure mesodermal cells and two genes up-regulated in pure mesodermal, but down-regulated in cardiomyocytes (Doss et al., 2007a+b*). We were interested in a fast screen for the functional role of transcripts with unknown function (TUFs) for an intact activity of the heart. Therefore we searched for homologues in the zebrafish genome and performed a morpholino-based *knockdown* approach. Morpholino-oligonucleotide injections caused highly specific cardiovascular defects in the majority of them such as altering of heart morphology and defects to a different extent and penetrance. For *knockdown* of the gene expression in murine ESCs to further analyze the potential for cellular differentiation, short hairpin RNA (shRNA) was used. PCR, Immunohistochemistry and microarray analysis indicated that the *knockdown* suppresses the expression of specific mesodermal and cardiac marker genes significantly. The present results demonstrate that, the in cardiomyocytes specifically up-regulated, genes play a crucial role in cardiomyogenesis.

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[1]*University of Cologne, Institute of Neurophysiology, and Center of Molecular Medicine (CMMC), Cologne, Germany,* [2]*University of Cologne, Institute for Genetics, Cologne, Germany*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700120

The neuronal co-repressor CoREST: An additional marker for immature neurons within the olfactory epithelium of the developing and adult mouse

C. Lehner[1,2], A. Wagner[2], H. Tempfer[3], R. Gehwolf[3], H. Bauer[2], H.-C. Bauer[3]

Abstract

In a previous study we have identified the mouse homologue of human Co-REST, a co-repressor to the zinc finger transcriptional repressor REST (repressor element-1 silencing transcription factor) and have determined its spatio-temporal expression pattern in the developing and newborn mouse brain (1). We have shown that CoREST is present in the developing neural tissue and persists in neurogenic areas throughout adulthood. Co-REST not only mediates the transcriptional repression of REST target genes, but also exerts REST-independent functions. CoREST target genes were found to be involved in pluripotency networks, neural stem cell differentiation, early neural fate decisions, and neuronal subtype specification (2).

In this project we have been focussing on the stage-specific expression of Co-REST in the mammalian olfactory epithelium (OE), which is known to harbour a pool of highly dynamic neurogenic cells (neural stem/precursor cells, NSCs/NPCs). Using immunohistochemistry we have determined the spatio-temporal expression pattern of Co-REST in the developing and adult mouse OE. Co-expression studies have been performed using antibodies against stem cell-related transcription factors, NPC-associated cytoplasmic proteins, and neuron-specific terminal differentiation markers.

Here we have shown that the stem cell-associated proteins Sox2 and Nestin are co-localized in the young OE, showing prominent staining in basal and apical regions. Interestingly, expression of Co-REST is detectable exclusively in Sox2/Nestin-free areas. From mid-gestation on, CoREST expression appears restricted to Doublecortin (Dcx) and GAP43-containing neuronal precursors. CoREST does not seem to colocalize with the olfactory marker protein (OMP), a marker being only expressed in mature olfactory receptor neurons (ORNs). Based on these results we conclude that CoREST is not responsible for stem cell-related gene repression, but is rather involved in a mechanism keeping ORNs in a precursor state.

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(2) Supported by grants from the Paracelsus Medical University (PMU, Salzburg, Project R-10/05/022-LEH) and by NEUROBID (EU 7th FP).

[1]*University Hospital Salzburg, Department of Trauma Surgery and Sports Medicine, Salzburg, Austria,* [2]*University of Salzburg, Department of Organismic Biology, Salzburg, Austria,* [3]*Paracelsus Medical University, Applied Cell Biology Unit, Salzburg, Austria*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700121

Cell transplantation of fetal stem/progenitor cells into the Mdr2 -/- mouse model

S. Schievenbusch[1], T. Schrammel[1], T. Goeser[1], D. Nierhoff[1]

Abstract

Backround:

Liver transplantation is the only established therapy for end-stage liver disease. However, due to the limited availability of donor organs, cell transplantation has been suggested as an alternative. Though the majority of patients waiting for liver transplantation has severe fibrosis or cirrhosis, most preclinical settings for the evaluation of cell transplantation are based on non-fibrotic liver injury. In the present study, we analyze the regenerative capacity of fetal stem/progenitor cells by transplantation into a mouse model of progressive liver fibrosis (Mdr2 -/- mice) at early and late stages of liver fibrosis.

Methods:

Fetal liver stem/progenitor cells were isolated from wild-type Balb/c mice. Cells were injected intrasplenically into Mdr2 -/- mice of different ages (sixth weeks vs sixth months) after 1/3 partial hepatectomy. Mice were sacrificed at 2 and 4 months after cell transplantation and liver tissues were taken for total RNA isolation. Engraftment of transplanted fetal liver stem/progenitor cells was investigated by quantitative expression analysis of hepatocyte-specific Mdr2 using RT-PCR.

Results:

Six months old Mdr2 -/- mice reveal only faint (< 0.5%) or rather no Mdr2 expression at any time point after cell transplantation. However, Mdr2 -/- mice of a younger age display considerably higher expression levels of Mdr2 after cell transplantation, rising from maximal 1 % at 2 months after transplantation up to 4 % at 4 months after transplantation.

Conclusion:

Repopulation of Mdr2-/- mice with fetal stem/progenitor cells demonstrated to be successful with mice of a younger age, i.e. less fibrosis. In contrast, Mdr2 -/- mice at a late stage of progressive liver fibrosis seem to be unaffected by fetal liver stem/progenitor cell transplantation. This is possibly due to a lower engraftment efficiency, as the transplanted cells fail to traverse the endothelial barrier in mice with significant matrix.

[1]*University Hospital of Cologne, Gastroenterology, Cologne, Germany*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700122

Neighbor of Punc E11 in the Mdr2 -/- mouse model: Novel marker of stem/progenitor cells in regenerating adult liver

S. Schievenbusch[1], T. Schrammel[1], T. Goeser[1], D. Nierhoff[1]

Abstract

Background:

The isolation of hepatic stem cells is demanding due to the lack of specific surface markers. Previously, we identified Neighbor of Punc E 11 (Nope) as a novel oncofetal marker of stem/progenitor cells in the murine liver. In the current study we focused on the expression pattern of Nope as a potential marker for stem/progenitor cells in normal adult liver as well as after acute (partial hepatectomy) and/or chronic liver injury (Mdr2 -/- mice).

Methods:

Liver tissues were obtained from adult Balb/c mice (10 weeks) and Mdr2 -/- mice of different age and stage of fibrosis. In subgroups with partial hepatectomy, livers were obtained 24 hours up to 7 days postoperatively. In selected mice, liver regeneration was modified by injection of DNA alkylating reagents 4 and 2 weeks before analysis. Expression levels of Nope were quantified using quantitative RT-PCR in homogenized liver tissue and after microdissection of bile ducts. Immunohistochemistry on cryosections was performed combining stainings for Nope with the biliary marker protein CK 19, an epithelial-specific Pancytokeratin (PanCK) and the canalicular membrane marker dipeptidylpeptidase (DPP) IV.

Results:

While normal adult liver shows only negligible expression of Nope, chronic liver injury in Mdr2 -/- mice leads to a considerably increased expression level of Nope. Co-stainings with CK19 demonstrated a bile-duct-specific expression of Nope in these mice. While acute injury in normal adult liver has no effect, an additional partial hepatectomy in the Mdr2 -/- mouse model results in sparse detection of Nope positive cell clusters if hepatocyte proliferation is blocked by DNA alkylating reagents. These Nope positive clusters are negative for CK19 but positive for PanCK and DPPIV.

Conclusion:

Here we report the expression of the oncofetal stem/progenitor cell marker Nope in the Mdr2 -/- mouse model of progressive liver fibrosis. While Nope is restricted to bile ducts in the chronic injury model, a rare population of regenerating stem/progenitor cells arises in case of an additional acute injury if physiological regeneration is blocked. We conclude that Nope is a potential marker for stem/progenitor cells in the regenerating adult liver.

[1]*University Hospital of Cologne, Gastroenterology, Cologne, Germany*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700123

Phenotipic characterization of normal human colon stem cells

A. Pastò[1], M. Marchesi[1], A. Amadori[1]

Abstract

Objective:

Aim of this study is to characterize the phenotypic profile of normal colon stem cells in order to compare this pattern to that found in colon cancer stem cells taking advantage of their different proliferative rate compared to non stem cells.

Materials:

Normal human colon biopsies were digested, cultured in serum-free medium and stained with PKH26. Cells were stained for the stemness marker Musashi-1 (Msi-1), Cytokeratin (CK) 20, CK18, Muc-1 and Ki67 and analyzed through confocal microscopy and cytofluorimetry.

Results:

PKH26 staining allowed to identify two subsets: PKH^{pos} e PKH^{neg} cells. PKH^{pos} cells showed high PKH intensity, reflecting a slow proliferation rate, whereas PKH^{neg} lost the PKH dye, due to high proliferation rate. Most PKH^{pos} cells lacked the expression of the differentiation marker CK20, and 5±0.9% of these cells were Msi-1^{+}. PKH^{neg} were >99.9% Msi-1^{-} but Muc-1and CK20 positive. In serum-free conditions, sorted PKH^{neg} cells died rapidly, whereas PKH^{pos} cells persisted for up to 35 days forming spheroid-like structures, morphologically comparable to those obtained from tumor colon tissues. In the presence of serum, PKH^{pos} cells differentiated into epithelial-like cells, and acquired CK20 and Muc-1 expression, while completely loosing Msi-1 expression. Furthermore, thanks to the different proliferative rate and markers expression, we could identify 4 different cell populations: $PKH^{high}/Msi^{+}/CK20^{-}$ dormant stem-like cells with low Ki67 expression, a subset of $PKH^{pos}/Msi^{+}/CK20^{+}$ stem-like cells in active proliferation that had acquired differentiation markers and expresses high levels of Ki67, $PKH^{low}/CK20^{+}/Msi^{-}$ actively proliferating cells that progressively lost PKH staining, eventually differentiating into terminal $PKH^{neg}/CK20^{+}/Msi^{-}$ cells.

Conclusion:

So far, the ability to form spheroids is reported for tumor colon samples but not for normal colon tissues. Here we demonstrate that stem-like cells derived from normal colon mucosa present this ability and show some specific features of stem cells, such as long lifespan in the absence of serum, expression of stemness markers, lack of differentiation markers and ability to differentiate into different cell types. Moreover, thanks to the PKH26 assay, we identified two stem cell populations: quiescent one and active and proliferating one.

[1]*University of Padua, Italy, Dept. of Oncology and Surgical Science, IOV-IRCSS, Padova, Italy*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700124

Spine fracture analysis with a finite element method and experimental data

S. Behforootan [1], M. Kasra [2], M. Moghimi [3]

Abstract

Osteoprosis is a disease characterized by low bone mass and micro-architectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture. Some studies in found vertebral strength could be better assessed with bone mineral density (BMD) while a strong correlation between failure force and BMD was observed in recent studies.

A motion segment consists of two discs and the vertebrae in between was modeled. Five distinct structural regions were simulated in the current model, namely discs annuluses, discs nucleuses, end plates, cortical and trabecular bone. The motion segment had being loaded until fracture happened. Load-displacement curve of upper disc was attained. In lower load the curve has the maximum gradient and when the stress become more than the yield stress of cortical and trabecular bone, the gradient of the curve become less.

Experimental data which were attained from this motion segment were applicable with the curve and errors were lower than 10%.

[1]*Dept of Biomedical Engineering, Amir Kabir University of Technology, Tehran, islamic republic of Iran,* [2]*Physics Unit, Biomedical Engineering, Amir Kabir University of Technology, Tehran,* islamic republic of Iran, [3]*Dept of mechanical Engineering, Khaje Nasir University of Technology, Tehran,* islamic republic of Iran

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700125

Comparison of HMGB1 levels between bone marrow and peripheral blood stem cell donation

F. Wenzel[1], V. Börger[1], J.C. Fischer[1], R. Sorg[1]

Abstract

Introduction:

High Mobility Group Box chromosomal protein 1 (HMGB1) is a nuclear DNA-binding protein acting as a proinflammatory cytokine. It is released from necrotic cells, activated macrophages dendritic cells and platelets. HMGB1 can induce prolonged inflammation and organ failure. Due to the preparation technique haematopoietic stem cell products consist not only of CD34+ cells but also of a mixture of different cell types, e.g. after GM-GCSF stimulation of a high addition of granulocytes, showing storage induced alterations. Therefore we evaluated HMGB1 levels in apheresis- and bone marrow (BM)-derived stem cell products.

Material and Methods:

In healthy BM donors (n = 3) and peripheral stem cell (PBSC) donors (n = 3), HMGB1 levels were determined in plasma samples (anticoagulated by EDTA (1.8 mg/mL)) of peripheral blood (PB) as well as in the respective stem cell product immediately after preparation and 24h after storage. HGMB1 was measured by an commercially available ELISA-Kit.

Results:

In the peripheral blood, HMGB1 levels of BM donors were in a lower range (5.6 ng/mL ± 1 ng/mL) than the levels of PBSC donors (12.4 ng/mL ± 6.3 ng/mL). In contrast, BM-derived stem cell products showed immediately after preparation higher HMGB1 levels (212 ng/mL ± 93 ng/mL) in comparison to the respective PBSC products (74 ng/mL ± 26 ng/mL). After 24h storage an accumulation of HMGB1 could be observed in the stem cell products, independent on the preparation technique and leading to comparable HMGB1 concentrations (228 ng/mL ± 80 ng/mL (BM) vs. 247 ng/mL ± 74 ng/mL (PBSC)).

Conclusions:

During haematopoietic stem cell preparation clear alterations of HMGB1 levels could be observed. In the peripheral blood of GM-GCSF-stimulated PBSC donors HMGB1 levels were found within a higher range in comparison to BM donors. However, after storage of the stem cell products an accumulation of HMGB1 occured independently on the preparation technique.

[1]*Universitätsklinik Düsseldorf, Institut für Transplantationsdiagnostik und Zelltherapeutika, Düsseldorf, Germany*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700126

Serum isolated after autologous transplantation stimulates proliferation and *in vitro* expansion of human CD34$^+$ hematopoietic stem- and progenitor cells

T. Walenda[1], G. Walenda[1], E. Jost[2], O. Galm[2], A. Schellenberg[1], C.M. Koch[1], D.M. Piroth[3], W. Drescher[4], T.H. Brümmendorf[2], W. Wagner[1]

Abstract

After hematopoietic stem cell transplantation (HSCT), regeneration of the hematopoietic system requires activation of the stem cell pool. So far, the mechanisms that recruit these cells into proliferation and self-renewal are scarcely understood. Here, we have addressed the question if activation of hematopoietic stem and progenitor cells (HPC) after autologous HSCT is mediated by systemically released cytokines and growth factors. Serum was taken from patients before chemotherapy, during neutropenia and after hematopoietic recovery. Subsequently, it was used as supplement for *in vitro* culture of CD34$^+$ cord blood HPC. Serum samples that were isolated during hematopoietic stress between 4 and 11 days after HSCT significantly enhanced HPC-proliferation and maintained primitive immunophenotype (CD34$^+$, CD133$^+$, CD38$^-$, CD45$^-$) over more cell divisions. The frequency of colony forming units (CFU) as well as the number of cobblestone area forming cells (CAFC) was also increased. More than 2 weeks after HSCT when hematopoietic recovery was almost completed, this stimulating effect declines to normal levels as observed with samples from before chemotherapy. Chemokine profiling revealed down-regulation of several growth factors after HSCT including platelet-derived growth factors PDGF-AA, PDGF-AB and PDGF-BB, whereas expression of monocyte chemotactic protein-1 (MCP-1) increased. Metabolomic profiling was used for identification of 46 metabolites that are currently tested for their functional relevance in HPC expansion. Taken together, these results demonstrate that systemically released factors stimulate hematopoiesis after autologous HSCT. This feedback mechanism opens new perspectives for *in vivo* stimulation of the stem cell pool.

[1]*RWTH Aachen University, Helmholtz Institute for Biomedical Engineering, Aachen, Germany,* [2]*RWTH Aachen University, Department for Hematology and Oncology, Aachen, Germany,* [3]*RWTH Aachen University, Department for Gynecology, Aachen, Germany,* [4]*RWTH Aachen University, Department for Orthopedics, Aachen, Germany*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700127

Mobilization mechanisms of human primary precursor-B-ALL cells in an i*n vivo* model system by the CXCR4-Antagonist AMD3100 and by Catecholamines

E.C. Buss[1,2], S. Kalinkovich[2], O. Kollet[2], A. Dar[2], M. Tesio[2,3], S. Fruehauf[4], M. Hotfilder[5], L.D. Shultz[6], A.D. Ho[1], T. Lapidot[2]

Abstract

Introduction:

Leukemia stem cells (LSC), similar to their normal counterparts (HSC), are well protected by adhesion to their niche in the bone marrow. Mobilization of LSC to the circulation might render them vulnerable to anti-leukemia therapy. The aim of this study was to explore mechanisms of leukemia mobilization from the BM with mobilizing agents like AMD3100 (AMD) in a pre-clinical immune deficient mouse model.

Methods:

Immunodeficient mice were engrafted with the childhood pre-B-ALL leukemic cell line G2 and with primary childhood precursor-B-ALL cells from 4 patients with up to 100% of transplanted mice being engrafted. Engraftment was without prior irradiation, thereby leading to a more physiological model of human leukemias.

Results:

Treatment with AMD lead to a significant mobilization of all transplanted leukemias with a mobilization level of between 3 - 8 times above baseline. Next, we examined the role of SDF-1 release by AMD. It could already be shown, that AMD3100 releases SDF-1 in healthy mice from the bone marrow to the peripheral blood, resulting in progenitor cell mobilization (Dar et al. Leukemia 2011). In the experiments reported here, inhibition of SDF-1 action with neutralizing CXCR4 antibodies abrogated AMD-induced leukemia mobilization. Recently we also demonstrated catecholamine receptor expression on hematopoietic stem and progenitor cells and of mobilization of these cells by catecholamines (Spiegel et al. Nat. Immunol. 2007). We showed now that the G2 cell line and all 4 examined precursor-B-ALL samples express the catecholamine receptors D3, D5 and beta-2. Treatment with high doses of epinephrine alone led to leukemia mobilization *in vivo* similar to AMD treatment. Lower doses of norepinephrine in combination with AMD increased leukemia mobilization up to 20 times above baseline.

Conclusions:

We could demonstrate the applicability of an *in vivo* xenotranplantation system of primary human precursor-B-ALL cells for research into leukemia cell mobilization. These leukemic cells can be mobilized efficiently by the CXCR4 antagonist AMD3100 and synergistically by cytecholamines. The AMD-induced mobilization mechanism is most likely via secretion of SDF-1. This mobilization approach could be potentially used for future mobilization protocols of leukemia in combination with established chemotherapy to improve eradication of minimal residual disease of leukemia.

[1]*University Hospital Heidelberg, Internal Medicine 5, Hematology/Oncology, Heidelberg, Germany,* [2]*Weizmann Institute of Science, Department of Immunology, Rehovot, Israel,* [3]*DKFZ, Division of Stem Cells and Cancer,, Heidelberg, Germany,* [4]*Paracelsus-Klinik, Zentrum für Tumordiagnostik und Therapie, Osnabrück, Germany,* [5]*University Children's Hospital, Department of Pediatric Hematology and Oncology, Münster, Germany,* [6]*The Jackson Laboratory, Bar Harbor, United States*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700128

Stem cell implantation in treatment of peripheral vascular disease

N.A. Doudar[1], S.S. Abdelshafy[2], M.M. EL Ansary[3]

Abstract

There is recent evidence from clinical trials that implantation of stem/progenitor cells improve limb ischemia. In the present study implantation of autologous peripheral blood mononuclear cells (PBMNCs) mobilized by granulocyte-colony stimulating factor (G-CSF) - investigated in patients with chronic limb ischemia. Twenty-four patients with chronic lower limb ischemia were enrolled and randomized (1:1) to either the implanted group or the control group. In the implanted group, the patients received subcutaneous injections of recombinant human G-CSF (300µg/day) for 5 days to mobilize stem/progenitor cells, and their PBMNCs were collected and implanted by multiple intramuscular injections into ischemic limbs while control group injected by sterile saline and receive medical treatment. All of the patients were followed up after at 12 week. At the end of the follow-up period, the main manifestations were significantly improved in the patients of the implanted group compared to the control group. Mean of rest pain decreased from 6.42±2.15 to 1.67±3.89 (P< 0.001). Mean of pain free walking distance increased from 25±29 to 409±204 (P< 0.001). Mean ankle-brachial pressure index increased from 0.45± 0.32 to 0.79± 0.38 (P = 0.005). A total of 7 of 9 limb ulcers and wounds (77.8%) of implanted patients healed after cell implantation. Two lower limb amputations occurred in the implanted patients. In contrast, eight control patients had to receive a lower limb amputation.

Key Words:

G-CSF -chronic limb ischemia-PBMNC- stem/progenitor cells.

[1]*Banisuef University, Clinical Pathology, Elwasta, Egypt,* [2]*Banisuef University, Clinical Pathology, Sheik zaid, Egypt,* [3]*Cairo University, Clinical Pathology, Cairo, Egypt*

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700129

Inducible overexpression in stably transfected hES cells

V. Markusova[1], E. Vaczy[1], K. Dofkova[1], A. Salykin[1], P. Dvorak[1], S. Kyrylenko[1]

Abstract

Objectives:

Overexpression of various regulatory proteins is a valuable tool to study molecular mechanisms of homeostasis in human embryonic stem cell (hESC). Transient overexpression can give answers to many questions, but as the overexpression rarely reaches 100 % of cells in the population the stable overexpression is preferred. However, hES cells are known to silence the transgenes and to adopt to the overexpressed proteins leading to artificial in-vitro phenomena. Therefore, inducible overexpression could be a method of choice because latent non-induced transgenes have better chances to remain in potentially active state.

Methods:

We set up to find conditions for establishing inducible hESC clones. First, we screened several available transfection methods including nucleofection (Lonza) and chemical transfection methods with Fugene 6, Fugene HD (Roche), NanoJuice (Merck), Lipofectamine 2000 (Invitrogen) transfection reagents in order to find the most suitable method for experiments with hES cells. Then, we screened several available inducible systems in order to find those which provide satisfactory inducibility and low basal expression in human ES cells. We then constructed a series of vectors overexpressing 3 different isoforms of FGF2, in particular isoform 1 (31 kDa), isoform 2 (22,3 kDa) and isoform 3 (18 kDa) as stand-alone proteins and fused to GFP.

Results:

We confirmed that the constructed vectors overexpress the target proteins upon induction with doxycycline. For that we derived subclones of hESC stably and constitutively overexpressing tetracycline-responsive transcriptional transactivator rtTA. In addition, we developed a PCR-based approach for transfection of hESC cells with combined linear DNA fragments containing both a gene of interest and a gene of a selection marker.

Conclusions:

We found that chemical transfection with Fugene HD and vectors of pTet-On Advanced system (Clontech) are the methods of choice for derivation of inducibly overexpressing clones of human ES cells. The work is now going on in order to derive stable hESC clones inducibly overexpressing various isoforms of FGF2 using both plasmid based and linear fragment based transfection.

[1]Masaryk University, Department of Biology, Brno, Czech Republic

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012

Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

Proceedings of the 6th Annual Meeting of the Germany Society for Stem Cell Research

JSRM Code: 008020700130

Setting standards: European and international standardization landscape in the field of medical devices utilizing tissues

K. Wenzelewski[1]

Abstract

Standards generate economic benefits which have been estimated at 16 billion euros a year for Germany alone. They facilitate trade, spread knowledge, disseminate innovative advances in technology, and share good management and conformity assessment practices. The poster provides an overview on the portfolio of European and International Standards available from the work of Technical Committee CEN/TC 316 „Medical devices utilizing tissues" of the European Standardization Organization (CEN) and its sister Technical Committees, ISO/TC 194/SC 1 „Biological evaluation of medicaldevices"/„Tissue product safety" and ISO/TC 150/SC 7 „Surgical implants"/„Tissue engineered medical products" of the International Organization for Standardization (ISO). Standardization provides excellent opportunities for shaping the business environment of current and future markets for tissue engineered medical devices and advanced therapy medicinal products both on the European scale and worldwide. Current projects and potential areas of future activities of the relevant CEN and ISO Technical Committees will be outlined and information given on how to get involved, be it by joining in current activities or by submitting proposals for new projects.The standardization toolkit comprises a variety of publication types. The range of publication types available within the European and International Standardization systems with their particularities will be described and an introduction to the mechanisms of consensus-based standardization given. Standardization is a strategic instrument for economic success. Learn more about how standards are made and how to get involved in CEN/TC 316 and/or its international counterparts to gain a competitive lead through timely access to information and knowledge. Join in to prepare the grounds which form an integral part of the business environment for the market success of your innovative products for application in regenerative medicine. For interested parties based in Germany, the German Standards Institute DIN Deutsches Institut für Normunge. V. can offer financial support for participation in standardization activities, within the framework of a BMBF funded project.

[1]DIN Deutsches Institut für Normung e.V., Aussenstelle Pforzheim, Pforzheim, Germany

Proceedings of 6th Annual Meeting of the Germany Society for Stem Cell Research;
Published online 10 Jun 2012