Retraction

The following article from *Journal of Cellular and Molecular Medicine, 'In vitro* analysis of integrin expression in stem cells from bone marrow and cord blood during chondrogenic differentiation' by Ulrich Reinhart Goessler, Peter Bugert, Karen Bieback, Jens Stern-Straeter, Gregor Bran, Haneen Sadick, Karl Hörmann and Frank Riedel, published online on 4th August 2008 in Wiley InterScience (www.interscience.wiley.com), has been retracted by agreement between the journal Editor in Chief, Professor LM Popescu, and Blackwell Publishing Ltd. The retraction has been agreed due to overlap between this article and the following article published in the *International Journal of Molecular Medicine*; 'Integrin expression in stem cells from bone marrow and adipose tissue during chondrogenic differentiation' by Ulrich Reinhart Goessler, Peter Bugert, Karen Bieback, Jens Stern-Straeter, Gregor Bran, Karl Hörmann and Frank Riedel, Volume 21 Issue 3, 2008, pages 271–279.

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In vitro analysis of integrin expression in stem cells from bone marrow and cord blood during chondrogenic differentiation

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Abstract

The use of adult mesenchymal stem cells (MSC) in cartilage tissue engineering has been implemented in the field of regenerative medicine and offers new perspectives in the generation of transplants for reconstructive surgery. The extracellular matrix (ECM) plays a key role in modulating function and phenotype of the embedded cells and contains the integrins as adhesion receptors mediating cell–cell and cell–matrix interactions. In our study, characteristic changes in integrin expression during the course of chondrogenic differentiation of MSC from bone marrow and foetal cord blood were compared. MSC were isolated from bone marrow biopsies and cord blood. During cell culture, chondrogenic differentiation was performed. The expression of integrins and their signalling components were analysed with microarray and immunohistochemistry in freshly isolated MSC and after chondrogenic differentiation. The fibronectin-receptor (integrin a5b1) was expressed by undifferentiated MSC, expression rose during chondrogenic differentiation in both types of MSC. The components of the vitronectin/osteopontin-receptors (avb5) were not expressed by freshly isolated MSC, expression rose with ongoing differentiation. Receptors for collagens (a1b1, a2b1, a3b1) were weakly expressed by undifferentiated MSC and were activated during differentiation. As intracellular signalling components integrin linked kinase (ILK) and CD47 showed increasing expression with ongoing differentiation. For all integrins, no significant differences could be found in the two types of MSC. Integrin-mediated signalling seems to play an important role in the generation and maintenance of the chondrocytic phenotype during chondrogenic differentiation. Especially the receptors for fibronectin, vitronectin, osteopontin and collagens might be involved in the generation of the ECM. Intracellularly, their signals might be transduced by ILK and CD47. To fully harness the potential of these cells, future studies should be directed to ascertain their cellular and molecular characteristics for optimal identification, isolation and expansion.

Keywords: integrin • cartilage • tissue engineering • differentiation • extracellular matrix • mesenchymal stem cells • chondrogenic differentiation

Introduction

Improved healthcare has resulted in dramatic demographic changes in developed countries, causing an increase in the prevalence of diseases associated with aging. Stem cell research and regenerative medicine offer unique opportunities for developing new therapeutic approaches to prevent and treat these debilitating and life-threatening diseases, and new ways to explore fundamental questions of biology.

Still, each year, millions suffer from organ failure or tissue loss due to injury, disease or congenital malformation [1, 2]. With a progressively aging population, there is an increasing demand for therapies to regenerate or replace musculoskeletal tissues. More than three million musculoskeletal procedures are performed annually. The existing shortage of donor tissue and organs available for transplantation has driven a multidisciplinary effort to develop therapeutic solutions.

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Table 1 Different integrins and their functions

Integrin	Receptor for
Integrin $\alpha 5\beta 1$	Fibronectin
Integrin α 4 β 1	VCAM, Fibronectin
Integrin α 6 β 1 and α 7 β 1	Laminin
Integrin α 1 β 1	Collagen, Laminin, Tenascin
Integrin α 2 β 1	Laminin, Collagen
Integrin α v β 5	Osteopontin

The emerging field of tissue engineering promises to deliver improvements in the technologies and therapies for musculoskeletal disorders through the development of biological substitutes for tissue replacement. The creation of functional tissue addresses very complex biological problems, comprising a wide range of engineering, science and clinical disciplines. The next generation of engineered musculoskeletal tissues will be more complex and structurally organized to better mimic normal tissue structure and function. Tissue engineering as an interdisciplinary approach utilizes specific combinations of cells, scaffolds and bioactive factors to create, influence and maintain cellular phenotype and function [3].

Cartilage as a unique avascular, aneural and alymphatic loadbearing live tissue is unique in that the extracellular matrix is composed of a complex combination of type II collagen fibrils which are specifically arranged and have bonded to them by very large water-retaining molecules called aggrecan molecules [4]. The promise of tissue engineering is perhaps most relevant to chondrogenic defects because cartilage has little self-healing potential.

Recently, the successful isolation of human stem cells from bone marrow (BM), periosteum and other newer sources was established by different groups [5–8]. These cells are highly proliferative and are capable of differentiating into different types of tissue such as bone, cartilage, tendon, muscle or fat. Human mesenchymal stem cells are characterized by a specific pattern of cell surface markers, growth factors, cytokine receptors, integrins and other adhesion molecules [9, 10].

Although BM has been the main source for the isolation of multipotent MSCs and BM-MSCs are well characterized and safe in handling, the harvest of BM is a highly invasive procedure and the number, differentiation potential, and maximal life span of MSCs from BM decline with increasing age [11–13]. Therefore, alternative sources from which to isolate MSCs are subject to intensive investigation.

As one alternative source umbilical cord blood (UCB) has proven to offer excellent potential for clinical scale allogenic transplantation. UCB can be obtained by a less invasive method, without harm for the mother or the infant [14, 15]. Essential preclinical studies proved a higher percentage of $CD34^{+}CD38^{-}$ cells in UCB compared to BM, suggesting that more primitive progenitors may be abundant in neonatal blood [16]. The same might apply for the presence of MSCs or progenitor cells. However, previous attempts to isolate MSCs from UCB either failed [17–19] or have demonstrated a low frequency of mesenchymal progenitors [20, 21]. Controversy still exists whether full-term UCB can serve as a fully acknowledged source for isolating multipotent MSCs: although some groups did not succeed in isolating MSCs [18, 19], we and other groups succeeded in isolating MSCs from full-term UCB [20, 22, 23].

As cellular function and phenotype are influenced by intrinsic and extrinsic stimuli, the cell–cell and cell–matrix interactions are of special interest in understanding factors crucial to generation of a distinct cellular phenotype. The integrin family of cell surface receptors appears to play a major role in the mediation of the cell–ECM interactions associated with structural and functional changes in surrounding tissues [24–27]. The integrins are heterodimeric glycoproteins that are composed of an α- and a β-subunit, each of which has extracellular and cytoplasmic domains. The extracellular domains bind to a number of ECM-proteins, including collagen types II and VI, fibronectin and matrix Gla-protein. Several recent studies have provided evidence that chondrocytes express integrins [28–33]. Salter *et al.* used immunohistochemical staining in normal adult articular cartilage, and noted that integrin $\alpha 5\beta 1$ was the most prominently expressed chondrocyte integrin [32]. A more recent study demonstrated that the chondrocyte expression of $\alpha 1\beta 1$, $\alpha 5\beta 1$ and $\alpha v\beta 5$ were accompanied by weak expression of integrin $\alpha 3\beta 1$ and $\alpha v\beta 3$ [31]. Other integrins are known to have distinct functions in binding components of the ECM (Table 1).

Integrin-mediated signalling is involved in a variety of cellular processes such as differentiation, adhesion and migration. Hannigan *et al.* found that integrin-linked kinase (ILK) coimmunoprecipitated with $\beta 1$ integrin from cell lysates, and that overexpression of ILK disrupted cell architecture and inhibited adhesion to integrin substrates, suggesting that ILK regulates integrin-mediated signal transduction [34]. In addition to ILK, integrin cytoplasmic domain-associated protein 1 (ICAP1) interacts with the cytoplasmic domain of $\beta 1$ integrin [35]. CD47 or integrin-associated protein (IAP) is a membrane protein that is involved in the increase in intracellular calcium concentration that occurs upon cell adhesion to extracellular matrix [36].

As the stem cell is responsible for modulating its environment and the chondrocyte phenotype is influenced by the diverse components of the extracellular matrix, the investigation of the molecular basis of distinct changes during developmental processes – for the generation of cartilage transplants especially the process of chondrogenic differentiation – might broaden the understanding of impediments in the field of tissue engineering. As BM-MSCs are best characterized, we asked whether MSCs derived from other sources share characteristic expression patterns of BM-MSCs. The aim of our study was to analyse MSCs isolated from BM and UCB under identical *in vitro* conditions and during chondrogenic differentiation with respect to integrin expression.

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Materials and methods

Collection and isolation of MSC from BM

BM was obtained from the femoral shaft of patients undergoing total hip replacement at the orthopaedic department of the University Hospital Mannheim. Cells were aspirated into a 5 ml syringe containing CPD anticoagulant. In total, six specimens from female patients were obtained, with the donor age ranging from 68 to 84 years.

To isolate mononuclear cells (MNC), the BM aspirates were diluted 1:5 with PBS/2mM EDTA (Nexell, Baxter, Unterschleißheim, Germany, and Merck, Darmstadt, Germany) and carefully loaded onto Ficoll-Hypaque solution (Amersham, Freiburg, Germany). After density gradient centrifugation at 435 $\times$ *g* for 30 min. at room temperature, MNC were removed from the interphase and washed two to three times with PBS/EDTA. Cell counts were performed using an automated cell analyzer (Cell-Dyn 3200, Abbott, Wiesbaden, Germany).

BM-derived MNC were set in culture at a density of $1 \times 10^5/cm^2$ into 75 cm^2 tissue culture flasks (Nunc, Wiesbaden, Germany, www.nunc.de) in MSCGM medium (MSCGM BulletKit™, Cambrex, St. Katharinen, Germany).

After overnight incubation at 37°C in humidified atmosphere containing 5% CO_2, non-adherent cells were removed and fresh medium was added to the flasks. Cultures were maintained and remaining non-adherent cells were removed by complete exchange of culture medium every 3–4 days. The flasks were screened continuously to get hold of developing colonies of adherent cells. Fibroblastoid cells were recovered between day 7 and 10 after initial plating by using 0.04% Trypsin/0.03% EDTA (PromoCell, Heidelberg, Germany). Recovered cells were replated at a density of 4000–5000 cells/cm^2 as passage 1 (P1) cells and thereafter.

Collection of UCB

UCB units (n = 59) were collected as previously described from the unborn placenta of full-term deliveries in a multiple bag system containing 17 ml of citrate phosphate dextrose buffer (Cord Blood Collection System; Eltest, Bonn, Germany) [22, 37] and processed within 24 hrs of collection. The collection was performed in accordance with the ethical standards of the local ethical committee.

Isolation and culture of MNC from UCB

The isolation of MSCs was performed as described for BM with a few exceptions. Prior to the isolation of MNC, the anticoagulated cord blood was diluted 1:1 with 2 mM EDTA-PBS. The MNC fraction was initially seeded at a density of 1×10^6 MNC/cm^2 into foetal calf serum (FCS)-precoated culture plates (FCS batches S0113/1038E and S0113/892E; Biochrom, Berlin, Germany, http://www.biochrom.de) (Falcon, Becton, Dickinson and Company, Franklin Lakes, NJ, USA, http://www.bd.com) [22]. Nonadherent cells were removed 12–18 hrs after initial plating. The same culture conditions and media were applied as described for BM-FACs. Adherent fibroblastoid cells only appeared as CFU-F and were harvested at subconfluence using Trypsin (PromoCell). Cells at the second passage and thereafter were replated at a mean density of $3.5 \pm 4.8 \times 10^3/cm^2$.

Chondrogenic differentiation

To promote chondrogenic differentiation, 2.5×10^5 cells were gently centrifuged (150 $\times$ *g*, 5 min.) in a 15 ml polypropylene tube (Greiner) to form a pellet according to the protocol of Mackay *et al.* [38]. Without disturbing the pellet, the cells were cultured for 4 weeks in complete chondrogenic differentiation medium (Cambrex) including 10 ng/ml TGFβ3 (Strathmann Biotec AG, Hamburg, Germany) by feeding twice a week. After the culture period, cryosections were analysed by Safranin O staining. The sections were fixed with ice-cold acetone (Sigma) and stained with 0.1% aqueous Safranin O solution (Sigma). Cell nuclei were counterstained with Weigert's iron haematoxylin (Sigma).

For the RNA analysis we harvested and lysed the aggregates in RLT buffer (Qiagen, Hilden, Germany). The lysis was aggravated by freezing the pellet repeatedly in liquid nitrogen.

RNA extraction and microarray hybridization

Extraction of RNA was performed using RNA Mini Kit (Qiagen) according to the manufacturers' protocol and as published before [39]. The RNA concentration was estimated from the absorbance at 260 nm.

Approximately 1 μg total RNA was used in each microarray experiment and for amplification and labelling of mRNA the SMART technique (SMART Fluorescent Probe Amplification Kit; BD Clontech, Heidelberg, Germany) was applied according to the manufacturers' protocol. RNA samples from day 1 were labelled with Cy3 and day 6 or day 21 samples were labelled with Cy5 (Cy™3- and Cy™5-monoreactive dye; Amersham Pharmacia Biotech, Freiburg, Germany). Corresponding Cy3- and Cy5-labelled samples were mixed, vacuum dried and resuspended in 25 μl microarray hybridization buffer (MWG-Biotech; Ebersberg, Germany). Prior to hybridization the samples were heat denaturated at 95°C for 5 min. The human 10K (MWG-Biotech) oligo microarray systems on glass slides were used for mRNA profiling. Hybridization of Cy3/Cy5-cDNA was performed using cover slips and a hybridization chamber for 16 hrs at 42°C in a water bath. After stringent washing of the glass slides according to the manufacturers specifications the hybridization signals of the Cy3 and the Cy5 dyes were measured using a microarray laser scanner (GMS418; Affymetrix, MWG-Biotech).

Microarray data analysis and statistics

The ArrayVision (Imaging Research, Inc., St. Catharines, ON, Canada) software has been used for evaluation and calculation of signal intensities from the raw data images in 16-bit tagged-image-file (TIF) format as described previously [39]. In brief, for evaluation of hybridization results we defined a negative (<3.000), a grey area (3.000–4.999) and a positive range (≥5.000) of hybridization signal intensities. Signal-to-background (S/B) values were calculated by dividing the signal intensity for each spot with the background signal intensities of the hybridized glass slide. Computer-assisted evaluation of the raw data provides the mean signal intensity and the signal to background ration for each individual gene spot. For statistical evaluation the mean signal intensity and standard deviation (S.D.) was calculated for each spot from the values obtained in the 10 individual experiments. Functional grouping of genes was performed on the basis of the database supplied by the array manufacturer.

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Immunohistochemistry

Immunohistochemistry for integrin αv, integrin β1, integrin β5, CD47 and the integrin-linked kinase (ILK) was performed by using a streptavidin-biotin complex procedure. Endogenous peroxidase was blocked with 0.3% hydrogen peroxide for 30 min. Sections were washed with phosphate-buffered saline (PBS) and incubated with normal rabbit serum in PBS for 30 min. at room temperature to block non-specific antibody reaction. The sections were then incubated over night at 4°C with the primary antibody (all from Santa Cruz Biotechnologies, Heidelberg, Germany). The slides were washed in several changes of PBS. The sections were then incubated with a peroxidase-conjugated secondary antibody (DAKO, Hamburg, Germany). After being washed twice in PBS, sections were then treated with a streptavidin-biotin-peroxidase complex and peroxidase reaction was performed using Diaminobenzidine DAB (DAKO, Hamburg, Germany) as chromogen. The different antibodies were diluted to the desired concentrations in PBS. Controls were carried out by omitting the primary antibody. Light microscopically investigation was performed using a Zeiss Axiophot microscope.

Results

Microarray analysis

In MSC from bone marrow (BM-MSC, Table 2, Fig. 1), for the components of the fibronectin-receptor (integrin α5/β1) a constant expression for integrin α5 (day0: 11790, day20: 9144, Ratio day20/day0: 0,78) could be found, integrin β1 was inactivated (day0: 32134, day20: 15557, Ratio day20/day0: 0,48). The components of the receptor for VCAM and Fibronectin (integrin α4/β1) were not expressed (integrin α4, day0: 811, day20: 2548, Ratio day20/day0: 3,19) and an inactivation of the gene for integrin β1 (day0: 32134, day20: 15557, Ratio day20/day0: 0,48). The components of the receptor for laminin (integrin α6/β1 and integrin α7/β1) showed a constant expression of integrin α7 (day0: 2872, day20: 4826, Ratio day20/day0: 1,68) and an inactivation of integrin β1 (day0: 32134, day 20: 15557, Ratio day20/day0: 0,64). The components of the receptor for collagen, laminin and tenascin (integrin α1/β1) were inactivated (integrin α1: day0: 7345, day20: 4639, Ratio day20/day0: 0,63; integrin β1: day0: 32134, day20: 15557, Ratio day20/day0: 0,48). The components of the receptor for laminin and collagen (integrin α2/β1) revealed a constant expression of integrin α2 (day0: 23892, day20: 30795, Ratio day20/day0: 1,29) and an inactivation of integrin β1 (day0: 32134, day20: 15557, Ratio day20/day0: 0,48). For the receptor for osteopontin (integrin αv/β5), an inactivation of integrin αv (day0: 4044, day20: 3306, Ratio day20/day0: 0,82) and an activation of integrin β5 (day0: 2958, day20: 15601, Ratio day20/day0: 5,27). The components of the intracellular signalling cascade revealed a constant expression of ILK (day0: 1931, day20: 2625, Ratio day20/day0: 1,36) and of CD47 (day0: 22758, day20: 23360, Ratio day20/day0: 1,03). ICAP-1 was not expressed (day0: 1041, day20: 2147, Ratio day20/day0: 2,06).

In MSC from cord blood (Table 3, Fig. 2), the components of the fibronectin-receptor (integrin α5/β1) showed an inactivation for integrin α5 (day0: 7391, day20: 3358, Ratio day20/ day0: 0,45), and constant expression for integrin β1 (day0: 3474, day20: 3193, Ratio day20/ day0: 0,92). The components of the receptor for VCAM and fibronectin (integrin α4/β1) were not expressed (integrin α4: day0: 1018, day20: 2584, Ratio day20/ day0: 2,54) and constantly expressed (integrin β1: day0: 3474, day20: 3193, Ratio day20/ day0: 0,92). The components of the Receptor for Laminin (integrin α6/β1 and integrin α7/β1) revealed no expression of integrin α7 (day0: 1518, day20: 1936, Ratio day20/day0: 1,28) and constant expression of integrin β1 (day0: 681, day20: 1104, Ratio day20/ day0: 1,62). Integrin α6 (day0: 681, day20: 1104, Ratio day20/day0: 1,62) was not expressed. As for the receptor for collagen, laminin and tenascin (integrin α1/β1), a strong activation of integrin α1 (day0: 845, day20: 6783, Ratio day20/day0: 8,0) and constant expression of integrin β1 (day0: 3474, day20: 3193, Ratio day20/ day0: 0,92). The receptor for laminin and collagen (integrin α2/β1) revealed constant expression of integrin α2 (day0: 23892, day20: 30795, Ratio day20/day0: 1,29) and integrin β1 (day0: 3474, day20: 3193, Ratio day20/ day0: 0,92). For the receptor for osteopontin (integrin αv/β5), a constant expression of integrin αv (day0: 4259, day20: 5257, Ratio day20/day0: 1,23) and an inactivation of integrin β5 (day0: 7684, day20: 4344, Ratio day20/day0: 0,57) was found. The components of the intracellular signalling cascade showed a constant expression of ILK (day0: 6147, day20: 5222, Ratio day20/day0: 0,85) and inactivation of CD47 (day0: 21171, day20: 11173, Ratio day20/day0: 0,53), ICAP-1 was not expressed (day0: 1616, day20: 661, Ratio day20/day0: 0,41).

Immunohistochemistry

The analysis of integrin-expression on protein-level was analysed with monoclonal antibodys against integrin αv, integrin β1, integrin β5, CD47 and the integrin-linked kinase (ILK). For all markers, during the whole process of chondrogenic differentiation, a constant expression for all markers could be found (Fig. 3, Table 4).

Discussion

The field of regenerative medicine encompasses various areas of technology, such as tissue engineering, stem cells and cloning. Tissue engineering, one of the major components of regenerative medicine, follows the principles of cell transplantation, materials science and engineering towards the development of biological substitutes that can restore and maintain normal function.

BM-derived stem cells have been studied for decades, are well characterized and safe in even clinical settings. For clinical applications of stem cell transplantation therapy, direct manipulation of

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Table 2 Signal intensities of hybridization signals as measured using the microarray laser scanner and calculated by The ArrayVision software in MSC from bone marrow

Receptor for	Day 0	Day 20	S.D. Day 0	S.D. Day 20	Ratio	d20/d0
Fibronectin						
Integrin a5	11790	9144	4269	4093		0,78
Integrin b1	32134	15557	3040	3698		0,48
VCAM, Fibronectin						
Integrin a4	811	2584	307	821		3,19
Integrin b1	32134	15557	3040	3698		0,48
Laminin						
Integrin a6	1206	773	241	132		0,64
Integrin a7	2872	4826	1412	2412		1,68
Integrin b1	32134	15557	3040	3698		0,48
Collagen, Laminin, Tenascin						
Integrin a1	7345	4639	6079	4269		0,63
Integrin b1	32134	15557	3040	3698		0,48
Laminin, Collagen						
Integrin a2	23892	30795	9674	3988		1,29
Integrin b1	32134	15557	3040	3698		0,48
Osteopontin						
Integrin av	4044	3306	1112	1036		0,82
Integrin b5	2958	15601	804	8541		5,27
Signalling cascade						
CD47	1931	2625	1224	1921		1,36
ILK	22758	23360	11452	11496		1,03
ICAP-1	1041	2147	117	594		2,06

Signals were measured on day 1 and after chondrogenic differentiation on day 20. Signals are shown with standard deviation and the ratio day 20/day 1.

cells and their interactions would be desirable. Hitherto, establishing distinct protocols for precisely inducing and maintaining cellular differentiation with a defined phenotype and function has been extremely challenging. In addition, establishing knowledge about cell–cell and cell–matrix interactions would be desirable and the integrins as a family of adhesion receptors mediating these stimuli are a promising target for research.

As mentioned above, the harvest of BM is a highly invasive procedure and the number, differentiation potential and maximal life span of MSCs from BM decline with increasing age [11, 12]. Therefore, alternative sources from which to isolate MSCs are subject to intensive investigation. UCB is an alternative source that can be obtained by a less invasive method and in larger quantities than BM.

Expression of integrins was analysed in MSC from BM and cord blood during chondrogenic differentiation. The components of the fibronectin-receptor (integrin $\alpha5/\beta1$) showed in both types of MSC with ongoing differentiation a diminished expression of integrin $\alpha5$ in both MSC-types and inactivation of integrin $\beta1$ in BM-MSC and constant expression in CB-MSC, on protein-level, integrin $\beta1$ showed constant expression.

With RT-PCR analysis it could be shown in previous studies that freshly isolated MSC express Collagen 2 and 10 [40]. These previous results suit the high expression of integrin $\beta1\alpha5$ in

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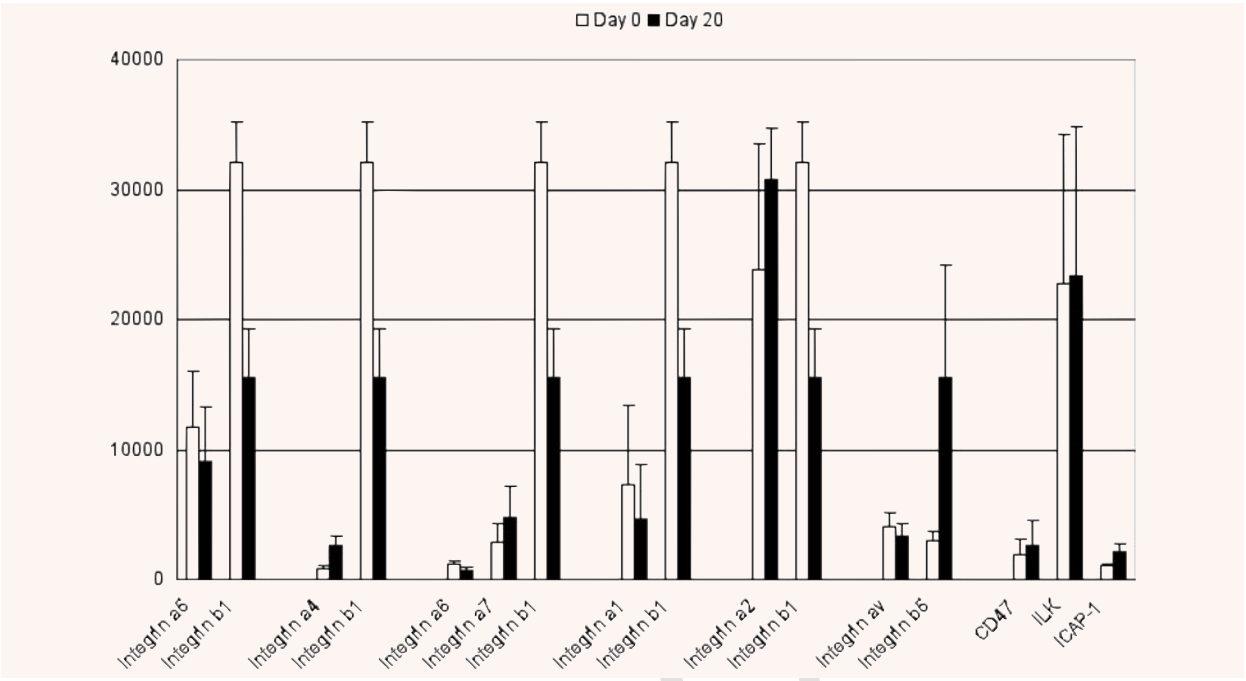

Fig. 1 Expression levels of genes for different integrins and integrin-associated proteins in MSC by microarray hybridization analysis. Results from mesenchymal stem cells from bone marrow (BM-MSC) during chondrogenic differentiation for the given genes in undifferentiated MSCs (white bars) and chondrocytes differentiated from the MSCs (black bars).

undifferentiated MSC, as the interaction of MSC and components of the ECM (*e.g.* Collagen 2) *via* this receptor is certain. So integrin β1α5 might exert an influence on cellular phenotype in undifferentiated MSC, with ongoing differentiation this receptor seems to become less important, in both types of MSCs at the same time.

The receptor for VCAM and fibronectin (integrin α4/β1) was not expressed by MSC from BM and CB in terms of integrin α4 and was inactivated during chondrogenic differentiation as for integrin β1, respectively. This receptor does not seem to be involved in the signalling during chondrogenic differentiation.

The components of the receptor for laminin (integrin α6/β1 und α7β1) showed no expression in the two types of MSC of integrin α6 and α7 as well as a diminished expression of integrin β1 during chondrogenic differentiation. So this receptor does not seem to play an important role during chondrogenic diferentiation.

The receptor for collagen, laminin and tenascin (integrin α1/β1) revealed adverse expression patterns in the two stem cell types: On RNA-level, inactivation of integrin α1 and β1 in BM MSC and activation of α1 with constant expression of integrin β1 in CB-MSC could be observed. However, on protein level, these diverging results could not be obtained, as integrin β1 showed constant expression in both types of MSC. These results do not allow a specific conclusion about the role of this receptor during chondrogenic differentiation.

The osteopontin receptor (integrin αv/β5) showed constant expression for integrin αv in both types of MSC. Integrin β5 was activated in BM-MSC and was inactivated in CB-MSC. Immunohistochemichal staining revealed constant expression of integrin β5 in both MSC-types. It has been established that integrin β5 plays a role in binding Vitronectin spielen könnte [41]. In addition, it has been concluded that the osteopontin receptor might be involved in processes of cellular migration and proliferation, especially in smooth muscle cells during vascular trauma [42]. Furthermore, this receptor might assist in the cellular differentiation *in vitro* [43]. The expression of this receptor during chondrogenic differentiation might reflect the influence of this receptor during the generation of a distinct ECM. The adhesion to surrounding ECM molecules might guide the process of differentiation.

The analysis of the components of the receptors for laminin and collagen (integrin α2/β1) resulted in an inactivation of integrin α2 in BM-MSC, in CB-MSC a rising expression during chondrogenic differentiation. As mentioned above, integrin β1 showed diminishing expression during chondrogenic differentiation in BM-MSC and constant expression in CB-MSC.

For this receptor, a specific role during chondrogenic differentiation may not be established. A possible role might be in adhering to collagens to facilitate the synthesis of ECM. This process cannot be analysed in monolayer culture.

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Table 3 Signal intensities of hybridization signals as measured using the microarray laser scanner and calculated by The ArrayVision software in MSC from cord blood

Receptor for	Day 0	Day 20	S.D. Day 0	S.D. Day 20	Ratio	d20/d0
Fibronectin						
Integrin a5	7391	3358	5283	1542		0,45
Integrin b1	3474	3193	2845	1496		0,92
VCAM, Fibronectin						
Integrin a4	1018	2584	322	821		2,54
Integrin b1	3474	3193	2845	1496		0,92
Laminin						
Integrin a6	681	1104	373	397		1,62
Integrin a7	1518	1936	369	1233		1,28
Integrin b1	3474	3193	2845	1496		0,92
Collagen, Laminin, Tenascin						
Integrin a1	845	6783	442	2854		8,02
Integrin b1	3474	3193	2845	1496		0,92
Laminin, Collagen						
Integrin a2	5060	9458	2954	4956		1,87
Integrin b1	3474	3193	2845	1496		0,92
Osteopontin						
Integrin av	4259	5257	1788	1911		1,23
Integrin b5	7684	4344	4811	2937		0,57
Signalling cascade						
CD47	6147	5222	3134	2497		0,85
ILK	21171	11173	6070	887		0,53
ICAP-1	1616	661	315	104		0,41

Signals were measured on day 1 and after chondrogenic differentiation on day 20. Signals are shown with standard deviation and the ratio day 20/day 1.

In summary, different expression patterns were found only on RNA-level for 2 receptors if one compares the expression patterns in the two stem cell types. So one might conclude that there are no significant differences in chondrogenic differentiation capacity and the expression of integrins. Establishing new sources for MSCs might have a high impact on clinical usage these cells. Exploitation might be related to the abundance and expansion capacity of MSCs. Based on our results, both BM and CB are reliable sources for isolating and expanding MSCs in autologous settings. Advantages of CB-MSC include the less invasive harvesting. For the past decades, BM has been deployed as the main source for clinical application of MSCs, such as the treatment of osteogenesis imperfecta, graft *versus* host disease or acute myocardial infarction [44–46]. As an age-dependent decrease in number, frequency and differentiation capacity of BM-MSCs has been described, they could be clinically inefficient when derived from elderly patients. Taking into account all these factors, CB-MSC might provide a solid starting basis in reference to abundance, easy harvest and high MSC frequency.

Conclusion

In the present study, we analysed expression patterns of integrins and integrin-related signalling-proteins. One of the

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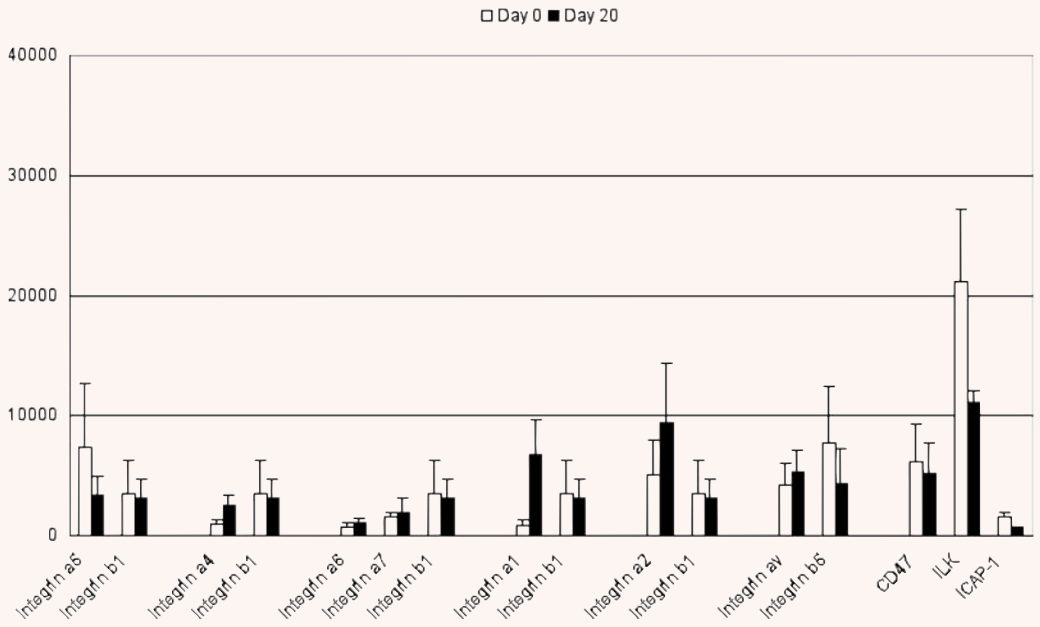

Fig. 2 Expression levels of genes for different integrins and integrin-associated proteins in MSC by microarray hybridization analysis. Results from mesenchymal stem cells from cord blood (CB-MSC) during chondrogenic differentiation for the given genes in undifferentiated MSCs (white bars) and chondrocytes differentiated from the MSCs (black bars).

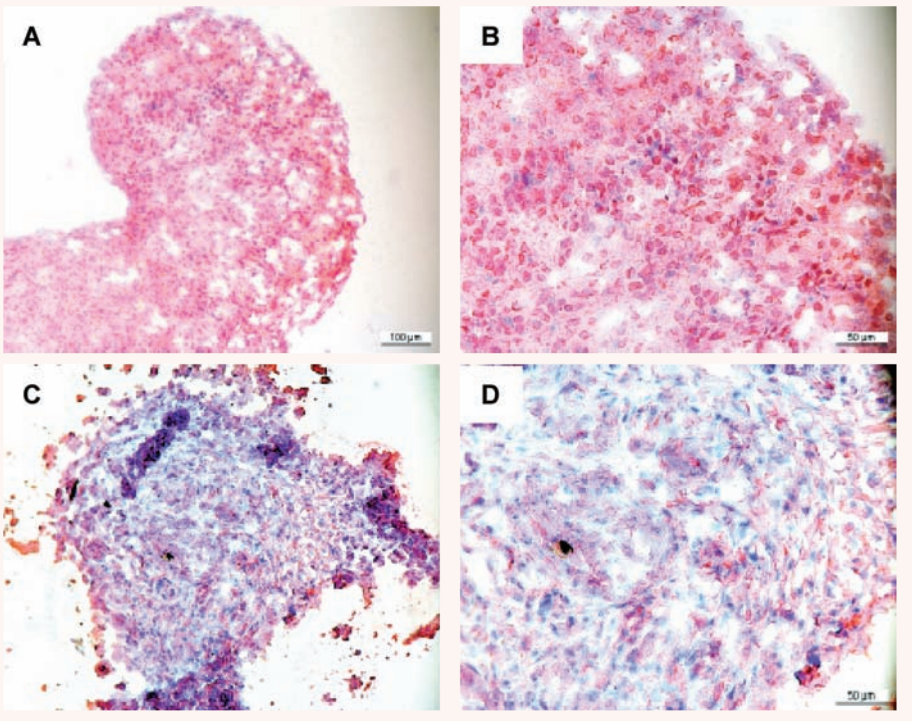

Fig. 3 Immunohistochemical staining against different integrins in MSC during chondrogenic differentiation. Day 1 (left) and day 20 (right) of cell culture. A Integrin β1 (day 1) in CB MSC; B Integrin β1 (day 20) in CB MSC; C ILK (day 1) in CB MSC; D ILK (day 20) in CB MSC.

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Table 4 Immunohistochemical detection of integrins and integrin-associated proteins in freshly isolated MSC (day 1) and during chondrogenic differentiation (days 1, 10, 20 and 30)

A					
Antibody specific for	**Staining pattern***				
BM MSC	**Day 0**	**Day 1**	**Day 10**	**Day 20**	**Day 30**
Integrin av	+++	+	++	+++	++
Integrin b5	++	+	+++	+++	++
Integrin b1	++	+	++	++	+
CD47	+++	++	+++	+++	++
ILK	+++	+++	+++	++	++
B					
Antibody specific for	**Staining pattern***				
CB MSC	**Day 0**	**Day 1**	**Day 10**	**Day 20**	**Day 30**
Integrin av	++	++	+++	+++	+++
Integrin b5	+++	++	++	++	+++
Integrin b1	++	++	+++	++	++
CD47	+++	+++	+++	+++	++
ILK	+++	+++	+++	+++	+++

*Amount of cells stained by the monoclonal antibodies is symbolized by ++++ (80−-100%), +++ (60–70%), ++ – +++ (50–60%), ++ (40–50%), + – ++ (30–40%), + (20–30%), ± (<20%) and − for no staining.

candidates for signal-transmission is the fibronectin receptor which might play a role in freshly isolated cells. Other receptors, *e.g.* for collagen, laminin and tenascin do not seem to be involved in signal transduction. The receptor for osteopontin seems to play a role during chondrogenic differentiation, in addition the receptor for laminin and collagen might assist the beginning chondrogenic differentiation. Intracellularly, ILK and CD47, but not ICAP1, might be involved in transduction of the integrin-dependent signals.

Integrin-mediated signalling seems to play an important role in the generation and maintenance of the chondrocytic phenotype during chondrogenic differentiation. To fully harness the potential of these cells, ascertaining their cellular and molecular characteristics for optimal identification, isolation and expansion belongs to future studies.

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