Biomatter 3:3, e24647; July/August/September 2013; © 2013 Landes Bioscience

Vascular endothelium as a novel source of stem cells for bioengineering

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Keywords: vascular, endothelial, stem cells, endothelial-mesenchymal transition, EMT

Endothelial plasticity, the ability of endothelial cells to alter their lineage commitment to generate other cell types, is involved in many developmental and pathological processes. It was recently shown that vascular endothelial cells are converted to a mesenchymal stem cell phenotype through a process known as endothelial-mesenchymal transition (EndMT). EndMT is characterized as a morphological and phenotypical transformation of endothelial cells that has been implicated in cardiac development, cancer, fibrosis and heterotopic ossification. Here we describe the molecular and cellular basis for EndMT-dependent generation of endothelial-derived stem cells and their potential for tissue engineering and regenerative medicine.

Introduction

Researchers have identified and isolated mesenchymal stem cells from numerous different tissues, including (but not limited to) bone marrow, adipose tissue, skeletal muscle, synovium and dental pulp.[1-5] Although many of these cell types have exhibited promising results for tissue engineering and regeneration, there are still many limitations in harvesting tissues from some of these sources, such as donor site morbidity[6,7] and the necessity for in vitro expansion and/or purification prior to re-implantation.[8]

More recently, it was found that vascular endothelial cells transform into mesenchymal stem cells through the process of EndMT. It has been shown that these cells exhibit multipotency by their ability to differentiate into osteoblasts, chondrocytes, adipocytes, smooth muscle cells or fibroblasts in vitro and in vivo.[9-11] These cells may have the ability to overcome some of the limitations of mesenchymal stem cells derived from other tissues. Here we provide a brief overview of EndMT in generating endothelial-derived stem cells and their potential use for regenerative medicine.

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Submitted: 03/31/13; Accepted: 04/08/13
Citation: Susienka MJ, Medici D. Vascular endothelium as a novel source of stem cells for bioengineering. Biomatter 2013; 3:e24647; http://dx.doi.org/10.4161/biom.24647.

Endothelial-Mesenchymal Transition

Vascular endothelial cells comprise the inner lining of blood vessels and provide an interface between the circulating blood in the lumen and the rest of the vessel wall.[12] Endothelial cells express a unique set of biomarkers, such as VE-cadherin, CD31, TIE1, TIE2 and von Willebrand factor (vWF).[12] During EndMT, expression of these endothelial biomarkers is markedly reduced while expression of mesenchymal markers such as fibroblast-specific protein-1 (FSP-1), α-smooth muscle actin (α-SMA), vimentin and N-cadherin, is increased.[12] Endothelial cells normally exhibit a tightly-clustered, cobblestone-shaped morphology in culture. Changes in gene and protein expression during EndMT cause the endothelial cells to rearrange their cytoskeleton and exhibit an elongated, spindle-shaped morphology resembling mesenchymal cells.

EndMT can be induced in cultured endothelial cells by stimulation with transforming growth factor beta 2 (TGFβ2) or bone morphogenic protein 4 (BMP4).[9,13-15] These ligands activate both activin-like kinase 2 (ALK2) and ALK5, which promote phosphorylation of Smad1/5/8 and Smad2/3, respectively.[9] Smad-independent pathways are also necessary for EndMT, including MEK, PI3K and p38 MAPK.[13] These Smad-dependent and Smad-independent signals have been linked to increased expression and function of the EndMT-inducing transcription factor Snail.[13] EndMT can be inhibited by BMP7 and vascular endothelial growth factor (VEGF) signaling.[11,16]

EndMT in Development and Disease

It has been previously established that epithelial-mesenchymal transition (EMT), by which epithelial cells transform into mesenchymal cells, is involved in cancer metastasis, embryonic development and wound healing.[17-22] More recently, it was shown that endothelial cells are capable of a similar process (EndMT) implicated in heart development, organ fibrosis, cancer progression and heterotopic ossification.[23]

EndMT was originally discovered as an embryonic mechanism necessary for cardiac development.[24] The heart valves and septa are formed by endothelial cells from the atrioventricular canal that have undergone EndMT and invaded adjacent tissues.[25] This process is heavily mediated by TGFβ and BMP

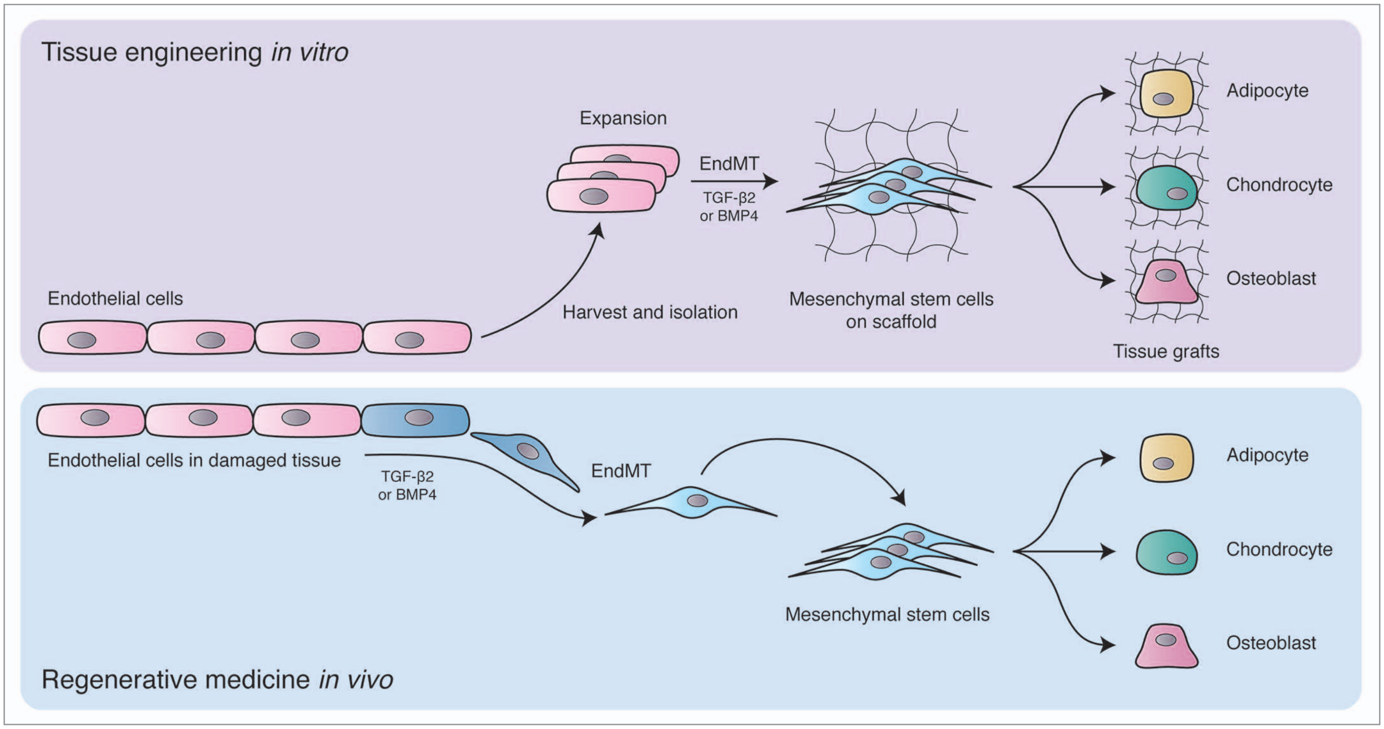

Figure 1. Tissue engineering and regeneration using endothelial-derived stem cells. Top panel: In the traditional in vitro tissue engineering approach, vascular endothelial cells could be harvested and isolated from capillary beds from a patient with degenerative disease, expanded in culture and stimulated with TGFβ2 or BMP4 to induce EndMT. These mesenchymal stem cells could then be seeded on a scaffold, differentiated into the desired cell type, then surgically implanted into the patient. Bottom panel: For the in vivo regeneration approach, endothelial cells in damaged or degenerating tissue could be stimulated with TGFβ2 or BMP4 to induce EndMT. The resulting mesenchymal stem cells could then be coaxed to differentiate into the desired cell types to regenerate the target tissue.

signaling, as suppression of EndMT via targeted inhibition of TGFβ2, BMP2, BMP4, ALK2 or ALK5 in mice results in defective cardiac development.[25]

Myocardial infarction and ischemia often result in cardiac fibrosis as a result of EndMT, which was confirmed by endothelial lineage tracing using Tie2-Cre reporter mice where it was found that the fibroblasts in the resulting scar tissue are of endothelial origin.[11] Similar results have been observed in systems of renal and pulmonary fibrosis.[26,27] Furthermore, it has been shown that cancer-associated fibroblasts (CAFs) in the microenvironment of malignant tumors co-express endothelial and mesenchymal markers and arise by EndMT, which was confirmed by endothelial-specific lineage tracing.[28]

Fibrodysplasia ossificans progressiva (FOP) is a rare bone disease characterized by inflammation-induced heterotopic ossification in soft tissues (e.g., muscles, tendons and ligaments), whereby they gradually turn into cartilage and bone.[27,28] FOP is caused by a heterozygous germline mutation (R206H) in the ALK2 receptor, involved in TGFβ and BMP signaling.[29] This mutation causes the ALK2 receptor to be constitutively active.[30] It was found that the chondrocytes and osteoblasts in ectopic lesions from patients with FOP stained positive for endothelial biomarkers, while normal bone and cartilage did not express such markers. Similar results were observed in a transgenic mutant ALK2 mouse model of FOP. The presence of these endothelial markers was shown to be a result of EndMT from vascular endothelial cells within inflamed soft tissues, followed by their differentiation into chondrocytes and osteoblasts.[9] This endothelial transition was confirmed by lineage tracing using Tie2-Cre reporter mice.[9] Although EndMT is detrimental in the context of debilitating diseases such as cancer and FOP, preliminary in vitro and in vivo studies have shown that this pathological process can be replicated and potentially used to generate mesenchymal stem cells for use in tissue engineering and regenerative medicine.

Stem Cell Phenotype Generated by EndMT

In addition to the endothelial-derived bone and cartilage in FOP, osteoblasts in calcifications observed in prostate carcinomas stain positive for endothelial marker CD31, suggesting that they may be of endothelial origin.[31] More recently, Tran et al.[32] showed that white and brown fat cells arise from vascular endothelium by lineage tracing in VE-cadherin-Cre reporter mice, providing additional evidence that endothelial cell differentiation is a natural process.

Endothelial cells in culture expressing a constitutively active mutant ALK2 gene have been shown to undergo EndMT and express the mesenchymal stem cell markers STRO-1, CD10, CD44, CD71, CD90 and CD117.[9] These cells were also shown to have multipotent differentiation capabilities by transforming into osteoblasts, chondrocytes or adipocytes in vitro using appropriate differentiation media. The long-term effects of using such endothelial cells overexpressing mutant ALK2 for tissue regeneration purposes is unclear. Perhaps a more efficient method for inducing EndMT is to use ligands such as TGFβ2 or BMP4 that activate the ALK2 receptor, an alternative which bypasses any potential risks associated with constitutively active ALK2. As observed with the mutant ALK2 gene expressed in endothelial cells, the mesenchymal stem cells generated from TGFβ2- or BMP4-dependent EndMT were successfully differentiated into osteoblasts, chondrocytes or adipocytes in culture.[9] Multipotency was confirmed by immunoblotting and staining for relevant osteoblast, chondrocyte or adipocyte markers.

Endothelial-Derived Stem Cells for Tissue Engineering and Regeneration

Endothelial-derived stem cells generated by EndMT have also been shown to exhibit multipotency in vivo.[9] Endothelial cells pre-treated with TGFβ2 or BMP4 were seeded on polylactic acid scaffolds, implanted subcutaneously into nude mice and locally injected with differentiation medium. Upon explant, the scaffolds were sectioned and tissues stained positively for bone, cartilage or fat.[9] The endothelial cells were labeled with fluorescent quantum dots prior to implantation, which confirmed the endothelial origin of the bone, cartilage and fat tissues that formed in these scaffolds. Endothelial-derived stem cells have also been shown to differentiate into vascular smooth muscle cells in collagen scaffolds. Krenning et al.[10] seeded endothelial progenitor cells (EPCs) on three-dimensional collagen sponges and induced them to undergo EndMT. Immunofluorescence and electron microscopy revealed that the differentiated EPCs exhibited f-actin bundling, cytoplasmic stress fibers and cell-matrix interactions characteristic of the vascular smooth muscle phenotype.[10] These findings suggest a promising use of endothelial-derived stem cells for both connective tissue and vascular tissue engineering.

Using endothelial cells as a source of stem cells is advantageous in that they can be harvested using minimally invasive techniques. For example, a single biopsy punch could be used to harvest a small portion of a capillary bed from a patch of skin, from which a population of dermal microvascular endothelial cells could be isolated.[33] This procedure would minimize the risk of complications associated with stem cell harvest from other tissue sources, such as bone marrow aspiration for isolation of bone marrow-derived stem cells. Furthermore, not only are most tissues highly vascularized, but also it is well known that both hypoxia and vascular injury stimulate angiogenesis,[34,35] which would ensure revascularization of the donor site. Another non-surgical option would be to isolate circulating endothelial progenitor cells (EPCs) from peripheral blood.[36,37] These cells could be expanded in vitro and then stimulated to undergo EndMT. Transformed cells could be seeded onto scaffolds that are currently being used for tissue engineering (e.g., collagen-, polymer- or hydrogel-based scaffolds) and differentiated into the appropriate tissue type for surgical implantation (**Fig. 1**).

Another possible application of endothelial-derived stem cells is for the repair of injured tissues or treatment of degenerative diseases such as osteoarthritis, osteoporosis, muscular dystrophy, etc. Since most tissues are highly vascularized, they could potentially be regenerated directly in vivo by targeted injection of TGFβ2 or BMP4 to induce EndMT of local vascular endothelial cells. These endothelial-derived stem cells could then be coaxed to differentiate into the appropriate cell type of need to regenerate the target tissue (**Fig. 1**). Other potential targets for tissue regeneration include cardiomyocytes, skeletal myocytes and neurons, which are known to arise from mesenchymal stem cells.[38] Loss of capillary blood vessels for regeneration should lead to hypoxia-induced angiogenesis to allow for revascularization of the target tissue.[34]

The use of EndMT to generate mesenchymal stem cells is a unique example of replicating a pathological mechanism such as in FOP, where endothelium is converted into cartilage and bone via EndMT, for use in tissue engineering or regeneration. This mechanism provides an innovative method for generating mesenchymal stem cells with many implications for the field of regenerative medicine.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

This work was supported by grant R01HL112860 (to DM) from the National Institutes of Health and a grant from the John Butler Mulliken Foundation (to DM).

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