

Regulation of endothelial cell plasticity by TGF- β

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Abstract Recent evidence has demonstrated that endothelial cells can have a remarkable plasticity. By a process called Endothelial-to-Mesenchymal Transition (EndMT) endothelial cells convert to a more mesenchymal cell type that can give rise to cells such as fibroblasts, but also bone cells. EndMT is essential during embryonic development and tissue regeneration. Interestingly, it also plays a role in pathological conditions like fibrosis of organs such as the heart and kidney. In addition, EndMT contributes to the generation of cancer associated fibroblasts that are known to influence the tumor-microenvironment favorable for the tumor cells. EndMT is a form of the more widely known and studied Epithelial-to-Mesenchymal Transition (EMT). Like EMT, EndMT can be induced by transforming growth factor (TGF)- β . Indeed many studies have pointed to the important role of TGF- β receptor/Smad signaling and downstream targets, such as Snail transcriptional repressor in EndMT. By selective targeting of TGF- β receptor signaling pathological EndMT may be inhibited for the therapeutic benefit of patients with cancer and fibrosis.

Keywords Angiogenesis · Cancer associated fibroblasts · Endothelial cells · EndMT · Fibrosis · Epithelial-to-mesenchymal transition · Fibrodysplasia ossificans progressiva · TGF- β

Abbreviations

| | |
|---------------|---|
| ActR | Activin receptor |
| ALK | Activin receptor-like kinase |
| BMP | Bone Morphogenetic Protein |
| CAF | Cancer-Associated Fibroblast |
| E-Cadherin | Epithelial-cadherin |
| EMT | Epithelial-to-Mesenchymal Transition |
| EndMT | Endothelial to Mesenchymal Transition |
| FOP | Fibrodysplasia Ossificans Progressiva |
| Id | Inhibitor of differentiation/DNA binding |
| IPF | Idiopathic Pulmonary Fibrosis |
| MI | Myocardial infarction |
| N-Cadherin | Neural-Cadherin |
| PAI-1 | Plasminogen Activator Inhibitor-1 |
| SMA | Smooth Muscle cell Actin |
| Smad | Small phenotype and Mothers Against Decapentaplegic related protein |
| TGF- β | Transforming Growth Factor- β |
| TGF β R | Transforming Growth Factor- β receptor |
| VE-Cadherin | Vascular-endothelial cadherin |

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Introduction

Complex body architecture is reliant on efficient supply of oxygen and nutrients to all tissues. The vascular system achieves this task efficiently via a highly branched network of blood vessels. The inner layer of blood vessels is lined with a thin layer of endothelial cells, stabilized by a basal lamina

around this endothelium (Adams and Alitalo 2007). Endothelial cells however do perform additional functions besides lining the vessel wall. One very interesting example that is emerging from recent studies is the transition of endothelial cells into mesenchymal cells (Goumans et al. 2008). This form of endothelial cells plasticity is called endothelial-to-mesenchymal transition (EndMT) and is the subject of this review.

During EndMT, endothelial cells delaminate from the organized layer of cells in the vessel-lining and migrate away possibly invading the underlying tissue. The mesenchymal phenotype is characterized by the acquisition of mesenchymal markers such as smooth muscle cell actin (SMA) and Neural (N)-Cadherin and the complementary loss of endothelial marker such as CD31/Pecam-1 and Vascular-Endothelial (VE)-Cadherin. In addition, the endothelial cells lose their cell-cell junctions and gain migratory and invasive capacity. Although discovered in the process of heart-development (Markwald et al. 1975), the mechanism of EndMT has now been implicated in a wide variety of pathological conditions like fibrosis of several organs as well as in cancer (Potenta et al. 2008).

EndMT is related to the more widely known mechanism of epithelial-to-mesenchymal transition (EMT). EMT allows a polarized epithelial cell, which normally interacts with basement membrane via its basal surface, to undergo multiple changes that enable it to assume a mesenchymal cell phenotype, which includes enhanced migratory capacity, invasiveness and elevated resistance to apoptosis. One of the molecular hallmarks of EMT is the loss of Epithelial (E)-cadherin expression that is frequently mediated by the upregulation of Snail transcription factor family members. EMT can occur in many epithelial cells and is, as EndMT, a critical process in embryonic development (Thiery and Sleeman 2006). In addition EMT is thought to be very important in cancer development since an epithelial cell needs to lose cell-cell junctions before it would be able to invade the surrounding tissue and migrate away ultimately leading to metastasis.

As shown for epithelial plasticity (Xu et al. 2009; Miyazono 2009) TGF- β signaling also plays an important role in regulating endothelial plasticity. Therefore we shall first discuss TGF- β receptor signaling in controlling endothelial cell function. Subsequently, we will focus on the role of endothelial cell plasticity in health and disease.

TGF- β signaling

TGF- β is one of the best known members of a large family of secreted pleiotrophic growth factors. Other members of the family include the bone morphogenetic proteins (BMPs) and activins. Many of these cytokines have vital roles in numerous processes during development, but also in maintenance of

tissue homeostasis and tissue repair in the adult (Massague 1998). Not unexpectedly, they also have roles in pathological conditions like cancer, vascular diseases and fibrosis (ten Dijke and Arthur 2007; Blobe et al. 2000).

Members of the TGF- β family mediate their effects by binding specific transmembrane receptors at the cell-membrane (Feng and Derynck 2005). They bind a complex of two type I and two type II serine/threonine kinase receptors. Upon ligand induced heteromeric complex formation, the type I receptor is phosphorylated by the type II receptor. There are seven different type I receptors (ALK1 to ALK7) (also known as activin receptor-like kinases (ALK)) and five type II receptors (activin receptor type IIA (ActRIIA), activin receptor type IIB (ActRIIB), BMP type II receptor (BMPRII), TGF- β type II receptor (TGF β RII) and AMH type II receptor (AMHRII)). Different ligands can bind different combinations of type I and type II receptor thereby creating specificity of signaling. TGF- β signals mostly via ALK5 and TGF β RII, activins via ALK4 with ActRIIA and ActRIIB, and BMPs signal via ALK1, 2, 3 and 6 together with BMPRII, ActRIIA and ActRIIB. For regulation of endothelial function by TGF- β , ALK1 and ALK5 signaling are most important (van Meeteren et al. 2011).

After binding of the ligand the type I receptor phosphorylates specific transcription factors called receptor regulated (R)-Smads. Upon activation by type I receptors, R-Smads form heteromeric complexes with the common mediator (Co)-Smad (Smad4) and these heteromeric complexes accumulate into the nucleus, where they regulate the transcription of specific target genes (Moustakas and Heldin 2009).

Inhibitory Smads (I-Smads) inhibit the activation of R-Smads by competing for type I receptor interaction and by recruiting phosphatases and ubiquitin ligases to the activated receptor complex leading to dephosphorylation or proteosomal degradation of the receptor complex (Itoh and ten Dijke 2007). R-Smads can be divided in 2 groups based on the type I receptor that is activating them. The first group consists of Smad1, 5 and 8 and these are activated by ALK1, 2, 3 and 6. The second group contains Smad2 and 3 and is activated by ALK4, 5 and 7. In addition to Smad signaling TGF- β and BMP signaling can result in activation of pathways where Smads are not involved. Non-Smad pathways include various branches of MAP kinase pathways, Rho-like GTPase signaling pathways, and PI3K/AKT pathways (Zhang 2009; Moustakas and Heldin 2005).

Co-receptors are receptors that do not signal by themselves since they lack intracellular enzymatic domains such as kinase-domains. For TGF- β signaling co-receptors endoglin and betaglycan (also called TGF- β receptor III) have been identified. Both receptors are structurally related and have a small intracellular tail and a large extracellular domain.

Endoglin is highly expressed in proliferating endothelial cells, hence its name endoglin (ten Dijke et al. 2008).

To regulate the activation state of endothelial cells TGF- β can differentially activate two type I receptors, ALK1 and ALK5 (Fig. 1) (Oh et al. 2000; Goumans et al. 2002). ALK5 is ubiquitously expressed in most tissues, but ALK1 expression is typically restricted to endothelial cells. TGF- β induced ALK5 signaling leads to Smad2 and Smad3 phosphorylation resulting in inhibition of angiogenesis by inhibiting endothelial cell proliferation, migration and organization (Goumans et al. 2002, 2003). The ALK5 kinase inhibitor SB-431542 facilitated proliferation and sheet formation of embryonic stem cell derived endothelial cells and in fetal mouse metatarsal assays (Watabe et al. 2003; Liu et al. 2009). In addition, SB-431542 upregulated the expression of claudin-5, an endothelial specific component of tight junctions, suggesting a role of ALK5 signaling in regulating vascular permeability (Watabe et al. 2003). Indeed, ALK5 has been reported before to be important for TGF- β induced permeability and cytoskeletal remodeling of endothelial cells (Birukova et al. 2005). In summary, ALK5 signaling results in keeping endothelial cells in a quiescent state.

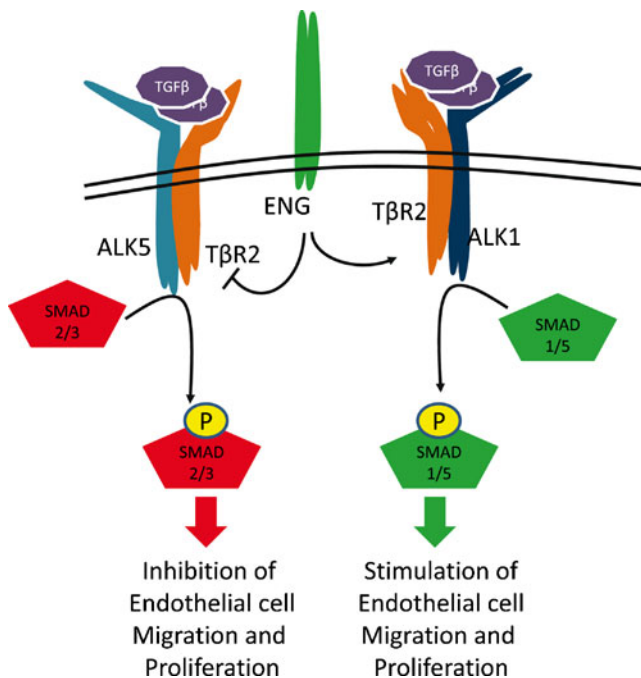


Fig. 1 TGF- β signaling in endothelial cells. TGF- β can bind to two distinct TGF- β type II/type I receptor complexes in which TGF- β type II receptor (TBR2) is a common component and two different type I receptors, i.e. activin receptor-like (ALK)5 and ALK1 determine signaling specificity. Whereas activation of ALK5 will inhibit endothelial cell proliferation, ALK1 will elicit opposite responses. Endoglin is an auxiliary receptor that modulates TGF- β signaling responses, i.e. it stimulates TGF- β /ALK1 but inhibits TGF- β /ALK5 signaling

In contrast, TGF- β induced ALK1 signaling activates Smad1 and Smad5 leading to endothelial cell proliferation, migration and organization (Goumans et al. 2003). An important intracellular effector of ALK1 is Inhibitor of Differentiation/DNA binding (Id)1; its upregulation was shown to be required for TGF- β /ALK1-induced endothelial cell migration and tube formation (Goumans et al. 2002). However, inhibitory effects of ALK1 signaling on endothelial cells have also been reported (David et al. 2007; Lamouille et al. 2002; Mallet et al. 2006). The effect of ALK1 is likely dependent on cellular context.

Although ALK1 and ALK5 have divergent effects on endothelial cells they do interact with each other physically. ALK5-deficient endothelial cells are not only defective in ALK5 signaling but also show impaired ALK1 responses; ALK5 was found to be essential for recruitment of ALK1 into a TGF- β receptor complex, and the kinase activity of ALK5 is essential for full ALK1 activation (Goumans et al. 2003). On the other hand, ALK1 can directly antagonize ALK5 signaling at the level of Smads (Oh et al. 2000; Goumans et al. 2002). In conclusion, the cross-talk between ALK1 and ALK5 signaling provides endothelial cells with a sophisticated mechanism to fine tune endothelial function (Fig. 1).

The TGF- β co-receptors endoglin and betaglycan can both be expressed by endothelial cells. Endoglin positive but betaglycan negative endothelial cells are only responsive to TGF- β isoforms 1 and 3 but not to isoform 2 (Cheifetz et al. 1990). In endothelial cells that express both co-receptors it was shown that endoglin can form a complex with betaglycan and the TGF- β receptor complex simultaneously (Wong et al. 2000).

Signals controlling EndMT

Many endothelial cells can be induced to undergo EndMT in cell culture experiments. This has opened the opportunity to study the signaling mechanism in EndMT. To induce EndMT of endothelial cells, they are often stimulated with TGF- β or Notch ligands (Frid et al. 2002; Ishisaki et al. 2003; Timmerman et al. 2004; Nosedá et al. 2004; Zeisberg et al. 2007b; Zeisberg et al. 2007a).

The molecular mechanism behind TGF- β induced EndMT has been found to involve the Snail family of transcription repressors. In mouse embryonic stem cell derived endothelial cells TGF- β induced EndMT and expression of Snail. This upregulation of Snail by TGF- β was shown to be dependent on the activation of Smad, MEK, PI3K and p38 MAPK by TGF- β (Medici et al. 2011). Subsequent knockdown of Snail blocked the TGF- β induced EndMT (Kokudo et al. 2008). Although overexpression of Snail was sufficient to induce EMT (Cano et

al. 2000) for EndMT Snail expression alone is insufficient. The inhibitor of Snail, GSK-3 β , needs to be inhibited by phosphorylation by kinases such as AKT to induce EndMT (Medici et al. 2011).

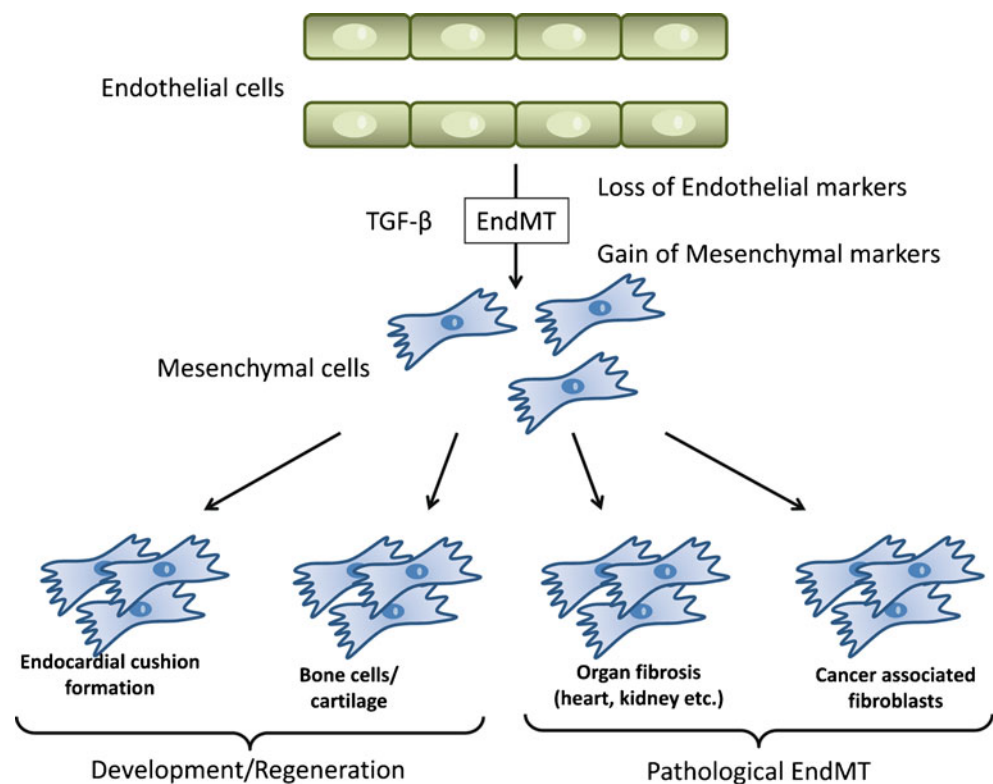
As mentioned above Notch can, as TGF- β , used in vitro to induce EndMT in endothelial cells in vitro. In this Notch induced EndMT the Snail family member Slug has been shown to be important (Leong et al. 2007). Snail and Slug are known to repress the expression of VE-cadherin (Lopez et al. 2009). Since VE-cadherin is essential for endothelial cell-cell junctions this obviously could provide a link to a mechanism by which EndMT occurs. A different factor involved in TGF- β induced EndMT was shown to be plasminogen activator inhibitor-1 (PAI-1). Although elevated levels of PAI-1 are implicated in tissue fibrosis (Ghosh and Vaughan 2011), lack of PAI-1 in the heart is associated with the development of cardiac fibrosis in aged mice (Ghosh et al. 2010). It was shown that in the PAI-deficient endothelial cells of these mice both Smad and non-Smad TGF- β signaling is spontaneously activated. This spontaneous activation leads to EndMT and subsequently the fibrosis observed in these animals (Ghosh et al. 2010). In addition, it was recently shown that c-Abelson tyrosine kinase (c-Abl) and Protein Kinase C (PKC)- δ are crucial for TGF- β -induced EndMT and therefore that imatinib mesylate and rottlerin (inhibitors of c-Abl and PKC- δ , respectively) might be effective therapeutic agents for fibroproliferative pathologies in which EndMT plays a role (Li and Jimenez 2011).

It has also been reported that pathways other than TGF- β can lead to EndMT. In myocardial infarction (MI) for example, canonical (β -catenin-dependent) Wnt signaling is induced 4 days after experimental MI in the subepicardial endothelial cells and perivascular cells. Coincidentally with canonical Wnt activation EndMT was also triggered after the infarction. In addition, canonical Wnt signaling induced mesenchymal characteristics in cultured endothelial cells, suggesting a direct role of canonical Wnt signaling in EndMT (Aisagbonhi et al. 2011).

EndMT in development

EndMT was originally discovered in the embryonic heart during studies on heart development (Markwald et al. 1975). In heart development, endothelial cells lining the endocardial cushions undergo EndMT (Fig. 2). These cells subsequently invade the underlying tissue and participate in the formation of the septa and valves of the heart (Eisenberg and Markwald 1995). Studies from knockout mice have shown subsequently that signals directing EndMT in heart development include multiple TGF- β isoforms and receptors (Goumans and Mummery 2000). For example it was shown that endoglin, a TGF- β co-receptor, is required cell autonomously for EndMT during formation of the endocardial cushions (Nomura-Kitabayashi et al. 2009). In the embryonic heart the endothelial cells that undergo TGF-

Fig. 2 TGF- β in endothelial cell plasticity. TGF- β can stimulate the transition of endothelial into mesenchymal cells. EndMT is a complex process that involves disruption of polarized endothelial morphology into cells with spindle-shaped morphology with formation of actin stress fibers. Furthermore EndMT results in reduced cell–cell junctions through delocalization and downregulation of VE-cadherin and PECAM, increased cellular motility and expression of mesenchymal makers, such as α -SMC actin, N-Cadherin and FSP-1. EndMT plays an important role in development, regeneration and pathological processes, such as fibrosis and tumor stroma formation



β -induced EndMT are exposed to high blood flow and are devoid of primary cilia (Van der Heiden et al. 2008). Egorova et al recently showed that TGF- β /ALK5 signaling is activated by blood flow in these endothelial cells (Egorova et al. 2011b), and lack of primary cilia primes endothelial cells for EndMT, thereby linking primary cilia with flow-related endothelial differentiation (Egorova et al. 2011a).

Role of EndMT in cancer

Fibroblasts are one the most abundant cell type in the microenvironment of tumors, being particularly prominent in carcinomas of colon, breast, pancreas, and prostate. There is substantial evidence that cancer-associated fibroblasts (CAFs) contribute to tumor growth and metastasis. This is mediated by the release of classical growth factors such as TGF- β , epidermal growth factor, hepatocyte growth factor, as well as a range of chemokines that influence diverse aspects of tumor cell behavior (Allen and Louise Jones 2011).

CAFs form a heterogeneous population, most likely related to their diverse origin. Whereas activation of local stromal fibroblasts has traditionally been considered the major source of CAFs (Kalluri and Zeisberg 2006), recently it was shown that EndMT is another unique source of CAFs (Fig. 2) (Zeisberg et al. 2007a). In this study EndMT in tumors was reported in two different mouse models of cancer and demonstrated that a substantial proportion of CAFs in these models arise through EndMT. The CAFs were shown to co-express the endothelial marker CD31 along with one of the mesenchymal markers, fibroblast specific protein (FSP)1, or α -smooth muscle actin (α SMA). Approximately, 40% of FSP1-positive CAFs were also found to be CD31 positive. To study the origin of the CAFs in more detail, tumors were grown in *Tie2-Cre;R26R-lox-STOP-lox-lacZ* transgenic mice, a reporter strain in which all cells of endothelial origin can be irreversibly labeled with *lacZ*. In 30% of the CAFs in tumors of these transgenic mice *LacZ* was detected making the authors conclude that the CAFs originated from endothelial cells (Zeisberg et al. 2007a). Since *Tie2* is however also expressed in the hematopoietic lineage it is technically also possible that the *lacZ* positive CAFs originate from these cells (Tang et al. 2010; Gitler et al. 2004).

These studies suggest that EndMT is an important mechanism for CAF recruitment to the tumour stroma. Since TGF- β signaling is a known mediator of EndMT and TGF- β is abundantly expressed in many different tumors (Zeisberg et al. 2007a), EndMT may be mediated by TGF- β produced in the tumor. Yet, the molecular mechanism of EndMT in tumors has not yet been specifically studied, but is to be expected to involve similar pathways as in fibrosis.

EndMT in tissue fibrosis

Fibrosis is an essential process of proper wound healing. In many pathological conditions however deregulated or excessive fibrosis occurs. TGF- β signaling is involved in physiological fibrosis in wound healing. Therefore it is not surprising that TGF- β signaling also plays an important role in pathological fibrosis.

The predominant cellular mediators of fibrosis are assumed to be (myo)fibroblasts, not only in heart fibrosis but also in fibrosis of organs such as lung, kidney, and the liver. Fibrosis of all these organs share similar pathways. The origin of these (myo)fibroblasts may, besides resident interstitial fibroblast, be cells derived from the bone marrow as well as fibroblastic cells that have transdifferentiated from cells of epithelial origin. More interestingly, these cells can also be derived from endothelial cells that have undergone EndMT (Fig. 2).

In the kidney for example it was shown that EndMT can generate myofibroblasts in early diabetic renal fibrosis. Using endothelial-lineage tracing with *Tie2-cre; LoxP-eGFP* transgenic mice a significant number of interstitial α -smooth muscle actin-positive cells (myofibroblasts) were shown to be of endothelial origin in fibrotic kidneys from mice with streptozotocin-induced diabetic nephropathy. This indicated that EndMT can contribute to the early progression of diabetic nephropathy (Li et al. 2009; Kizu et al. 2009). Earlier it was already shown that fibroblasts expressed the endothelial marker CD31 in three different mouse models of renal disease: streptozotocin-induced diabetic nephropathy, unilateral ureteral obstructive nephropathy, and a mouse model of Alport syndrome (Zeisberg et al. 2008). Approximately 30% to 50% of fibroblasts formed in the kidneys of these models co-expressed the endothelial marker CD31 and the fibroblast/myofibroblast markers FSP1 and/or α SMA (Zeisberg et al. 2008). Since TGF- β is stimulating EndMT it is not surprising that interference with TGF- β signaling has been reported to inhibit EndMT and the subsequent kidney fibrosis. First it was reported that Smad3 conditional knockout mice are resistant to streptozotocin-induced renal fibrosis and tubulointerstitial fibrosis in unilateral urethral obstruction models (Fujimoto et al. 2003; Wang et al. 2007; Sato et al. 2003). Subsequently it was shown that a Smad3 inhibitor delays the early development of streptozotocin-induced diabetic nephropathy due to a blockade of EndMT (Li et al. 2010).

Likewise in the lung, fibrosis can cause serious pathological conditions such as idiopathic pulmonary fibrosis (IPF). IPF is characterized by progressive obliteration of normal alveolar lung architecture and replacement by fibrotic tissue. The result is declining lung function, progressive dyspnea, and ultimately death within 3 to 5 years of diagnosis (Nataraj et al. 2010). Hashimoto *et al* have demonstrated that pulmonary capillary endothelial

cells, through EndMT, can serve as a source of fibroblasts in pulmonary fibrosis (Hashimoto et al. 2010). They showed this by bleomycin-induced lung injury in mice where they found, using lineage tracing methods that the fibroblasts originated from the endothelial cells. Interestingly they also found a dependence on Ras for completion of EndMT. Only treatment with TGF- β in combination with activated Ras induced a persistent morphological change and suppression of endothelial markers consistent with EndMT (Hashimoto et al. 2010).

Endothelial-to-mesenchymal transition in cardiac fibrosis

In cardiac fibrosis the heart valves abnormally thicken due to inappropriate proliferation of cardiac fibroblasts and of disruption of normal myocardial structure through excessive deposition of extracellular matrix (Krenning et al. 2010). Several studies have given evidence for the role of EndMT in cardiac fibrosis. For example Zeisberg et al. performed lineage analysis to trace the origin of the fibroblasts in cardiac fibrosis (Zeisberg et al. 2007b). Cardiac fibrosis was induced by exposing the heart to pressure overload for 5 days via aortic banding. Analysis of the fibrotic lesions revealed the presence of fibroblasts that originated from endothelial cells. This study elegantly shows that endothelial cells can undergo EndMT and contribute to the total pool of cardiac fibroblasts, similar as in formation of the atriocentric cushion in embryonic development.

TGF- β signaling stimulates the collagen-producing cardiac fibroblast and has therefore been implicated in the pathogenesis of cardiac fibrosis (Khan and Sheppard 2006). Progression of cardiac fibrosis is furthermore stimulated by a Smad3-dependent TGF- β signaling pathway inducing EndMT. In Smad3 heterozygous mice cardiac fibrosis was significantly reduced and associated with a decrease in the number of endothelial-derived fibroblasts (Dobaczewski et al. 2010; Bujak et al. 2007). In a different study BMP-7 inhibited EndMT and conserved the endothelial phenotype. The systemic administration of exogenous BMP-7 significantly inhibited EndMT and the progression of cardiac fibrosis in mouse models of chronic allograft rejection and pressure overload (Zeisberg et al. 2007b). Thus, although BMP cooperates with TGF- β in inducing EndMT during cushion formation, it is an antagonist in the TGF- β fibrotic pathway, preserving the endothelial phenotype and preventing fibrosis.

Targeting TGF- β in fibrotic diseases

With EndMT (and EMT) contributing to tissue fibrosis progression and TGF- β as pivotal mediator of this response

and also inducer of extracellular matrix deposition, this opens possibilities to intervene with fibrosis by targeting TGF- β . Several clinical trials on fibrosis with anti-TGF- β agents have been done or are currently running. For example in skin fibrosis a clinical trial is testing the effect of the synthetic peptide P144 (ClinicalTrials.gov Identifier: NCT00781053). P144 encodes a part of the ligand (TGF- β) binding domain of betaglycan and can block TGF- β activity. In animal models P144 inhibited carbon tetrachloride induced liver fibrosis and more importantly it inhibited bleomycin induced skin fibrosis (Ezquerro et al. 2003).

CAT-152 (Lerdelimumab) is a fully human TGF- β 2 neutralizing antibody with high affinity for TGF- β 2 and lower affinity for TGF- β 3 (Thompson et al. 1999). In rabbits it was capable of inhibiting scarring after glaucoma surgery (Mead et al. 2003). In the first clinical trials, CAT-152 showed possible effects in reducing scar formation in intractable glaucoma patients that received a trabeculectomy (Siriwardena et al. 2002). Disappointingly in larger phase III clinical trials these results could not be confirmed however (Khaw et al. 2007).

Systemic sclerosis is a disease in where the dermal layer of the skin undergoes fibrosis. In addition systemic sclerosis results in inflammatory responses, vascular changes and loss of function of internal organs due to scarring and extracellular matrix deposition. In a phase I/II trial CAT-192, a TGF- β 1 neutralizing antibody did not show effect on early stage diffuse cutaneous systemic sclerosis, together with more adverse events occurred in the CAT-192 treated groups (ClinicalTrials.gov Identifier: NCT00043706) (Denton et al. 2007). Nevertheless this antibody is now being tested in patients with myelofibrosis (ClinicalTrials.gov Identifier: NCT01291784).

GC2008 or Fresolimumab which is the humanized version of the pan-TGF- β neutralizing antibody 1D11 is also tested in clinical trials. These trials also treat patients with diffuse systemic sclerosis (ClinicalTrials.gov Identifier: NCT01284322).

EndMT leading to loss of endothelial function

As described above the result of EndMT in pathological conditions is fibrosis because of the generation of fibroblasts and excessive extracellular matrix. However EndMT also leads to the loss of endothelium. The loss of endothelial function can lead to badly perfused tissue and subsequent tissue damage. For example following traumatic spinal cord injury, significant vascular disruption occurs at the site(s) of injury. This interruption of vascular support is thought to be a key mediator of multiple secondary injury cascades, all of which contribute to loss of functional tissue. It was demonstrated that in response to spinal ischemia/

reperfusion injury TGF- β -responsive genes in the endothelial cell compartment were early and robustly transcriptionally activated (Benton et al. 2009). Given the known effects of TGF- β on endothelial cells it is attractive to speculate that the loss of endothelial functions, such as increased permeability of the endothelium in spinal cord injury is due to increased TGF- β signaling and conversion of endothelial cells to a more fibroblastic phenotype.

Endothelial transdifferentiation is not limited to fibrosis

Other than differentiation towards myofibroblasts endothelial cells can also differentiate to other cell types. Interestingly TGF- β signaling was shown to play an important role also in these transdifferentiation processes.

Fibrodysplasia ossificans progressiva (FOP) is a severely debilitating disorder caused by an activating mutation in the BMP type I receptor, ALK2 (Shore et al. 2006). In FOP-patients acute inflammation causes heterotopic ossification in soft tissues at nearly any site in the body (Kaplan et al. 2008). The source of the ossifying cells was previously unknown but Medici et al. showed that bone and cartilage cells from lesions of people with FOP and mice with mutant ALK2 expressed the endothelial markers Tie2 and von Willebrand factor. This suggests an endothelial origin of the ectopic mesenchymal cells that form the heterotopic tissues (Horwitz 2010; Medici et al. 2010). Furthermore, human endothelial cells treated with BMP4 or TGF- β 2, to activate endogenous ALK2, undergo EndMT resulting in mesenchymal stem cells. These mesenchymal stem cells may be of use in cell therapy to treat people in need of bone and cartilage regeneration (Medici et al. 2010).

Not only endothelial cells lining the vessel wall have been shown to be able to undergo EndMT. Also circulating endothelial progenitor cells have been shown to undergo EndMT. The conversion of these circulating endothelial progenitor cells to smooth muscle-like progeny was also shown to be stimulated by TGF- β (Moonen et al. 2010).

Endothelial plasticity also plays a role in the phenotype of the three main types of endothelial cells; arterial, venous and lymphatic. These 3 different phenotypes can be superimposed, or reverted to, by subtle alterations in the combination or in the expression levels of a few key regulators (e.g. Notch signaling, COUP-TFII and Prox1) (Johnson et al. 2008; You et al. 2005) (reviewed in (Oliver and Srinivasan 2010). Furthermore also during sprouting angiogenesis there are several different types of cells within the growing sprout. Tip cells and stalk cells are fundamentally different although they can revert to one and another quickly (Jakobsson et al. 2010). These changes in endothelial cell fates highlight again the once unappreciated plasticity of endothelial cells.

Concluding remarks

Recent studies have revealed the remarkable plasticity of endothelial cells. While initially met with skepticism (Tarin et al. 2005), EMT (and therefore also EndMT) was gaining acceptance. However, the concept of EMT/EndMT is currently under intense discussion (Quaggin and Kapus 2011; Zeisberg and Duffield 2010), in particular as it relates to kidney and liver fibrosis. Whereas early lineage tracing studies demonstrated EMT/EndMT, new lineage tracing experiments failed to do so (Humphreys et al. 2010). What is clear is that epithelial/endothelial cells can undergo morphological changes, i.e. demonstrate plastic behavior, and that TGF- β drives this process. In pathological situations like cancer and fibrosis, targeting of the TGF- β /Smad signaling pathway may have therapeutic benefit and should be further explored in experimental animal models and clinical trials. Recent advances of (intravital) imaging techniques should be applied in vivo studying diseases with possible links to EMT/EndMT to see whether further evidence of these processes can be obtained (Yoshino et al. 2011). As mentioned above several clinical trials are ongoing to test if anti-TGF- β therapy can inhibit pathological conditions, like fibrosis, in which endothelial (and epithelial) plasticity plays an important role. If successful not only many fibrotic pathological conditions but also the generation of cancer associated fibroblasts might be inhibited. This would lead to a less favorable micro-environment for tumor growth and ultimately would help to fight cancer in the clinic.

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