Endothelial-to-mesenchymal transition in diabetic retinopathy

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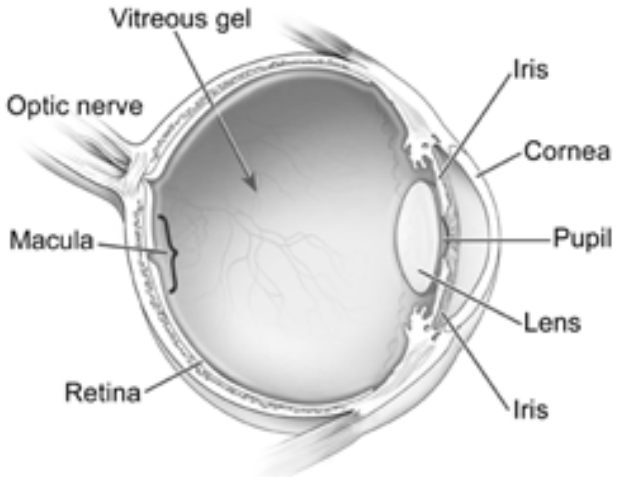

Original figure from National Eye Institute.
http://www.nei.nih.gov/health/diabetic/retinopathy.asp

Abstract

Diabetes is predicted to affect 380 million people by the year 2025.Diabetic retinopathy (DR) is a serious condition which is the leading cause of acquired blindness and which 90% of the patients with diabetes develop after 20 years. Recently, some studies have found that endothelial cells can differentiate into mesenchymal cells, a process called endothelial to mesenchymal transition (EndMT). EndMT has been linked to pathological fibrosis in the cornea and diabetic diseases, including diabetic nephropathy and diabetes mellitus–induced cardiac fibrosis. However, the occurrence of EndMT in the retina and the possible contribution to the pathogenesis of DR has not been investigated thoroughly. This thesis has discussed the indications that EndMT might also play a role in DR.

As well as in the cornea, diabetes mellitus–induced cardiac fibrosis and diabetic nephropathy, TGF-β induces the endothelial cells to undergo EndMT, leading to irreversible fibrosis. Oxidative stress may be an important factor in this, as it increases the TGF-β concentration. Although this thesis has mostly focused on TGF-β, as it seems to be the main initiator of EndMT, the identification of other factors, like ET-1 and FGF, is also of great value to provide targets for possible therapeutic interventions.

Further research is needed to understand the molecular mechanisms and pathways involved in EndMT. Moreover, human cell line studies and in vivo studies are necessary to confirm that EndMT indeed occurs in the retina and that it contributes to the pathogenesis of DR.

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1. Introduction

Diabetes is a worldwide problem which is predicted to affect 380 million people worldwide by 2025 (Christiakov, 2011). Diabetes affects many organs and tissues throughout the body, including the function of several structures of the eye. These structures include the cornea, iris, anterior chamber angle, lens, vitreous, retina, optic nerve, the cranial nerves innervating extraocular muscles and the orbit (Lu, 2004).
This thesis will focus specifically on diabetic retinopathy (DR), a serious condition which is the leading cause of acquired blindness and which 90% of the patients with diabetes develop after 20 years (Christiakov, 2011).
Epithelial-to-mesenchymal transition (EMT) is a process in which epithelial cells lose their epithelial function and phenotype and differentiate into mesenchymal cells. These cells acquire mesenchymal, fibroblast-like properties this way. It has been shown that several types of cells undergo EMT, including retinal pigment epithelial cells. Recently, some studies have found that endothelial cells can also differentiate into mesenchymal cells, a process called endothelial to mesenchymal transition (EndMT) (Ma, 2010) . EndMT has been linked to diabetic diseases, including diabetic nephropathy and diabetes mellitus–induced cardiac fibrosis (Piera-Velázquez, 2011).
It would be interesting to investigate if retinal endothelial cells also undergo EndMT and if this process contributes to DR. As there is not much known about EndMT in DR, this thesis will focus on information that indicates that EndMT might also play a role in DR.
To answer this question, information about the epidemiology and pathophysiology of DR will be discussed first, followed by a paragraph about EndMT and its underlying mechanisms. Next, the very little information that is known about the involvement of EndMT in DR will be discussed, after which the clues that might indicate that EndMT plays an important role in DR will be discussed.
Because the cornea and retina lie very near to each other and the role of EndMT in corneal cells is well described in several studies, this might be a clue that EndMT is also involved in endothelial retinal cells. As said before, EndMT has been linked to diabetic diseases including diabetic nephropathy and diabetes mellitus–induced cardiac fibrosis. This might also be a clue that EndMT plays a role in DR and therefore will also be discussed in this thesis.
In the end, the results will be discussed and summarized and the main question will be answered.

2. Diabetic retinopathy

DR is an ocular microangiopathy and one of the fastest growing causes of acquired blindness in the working age population. Diabetic retinopathy is responsible for 4.8% of the cases of blindness worldwide. As DR remains asymptomatic for a long time, DR is often only noticed in the later stages, because the patient suffers from visual impairment or suddenly goes blind (Lang, 2007). At the moment, there is no effective treatment available for DR (van Geest, 2010), although tight blood glucose regulation can delay the onset and progression of DR in patients with diabetes. The onset and progression of DR has been linked to hyperglycemia in several studies. Not only hyperglycemia is a major risk factor for DR, high blood pressure and duration of diabetes are also important risk factors (Christiakov, 2011). Approximately 90% of the patients with diabetes develop DR after 20 years. At the moment, the prevalence of the disease is 7% (Lang, 2007), but as said in the introduction, the number of people with diabetes is rising to alarming levels and it is expected that the prevalence of DR will rise along with it (Christiakov, 2011).

In the early stage of DR the vascular basal lamina thickens and there is a loss of pericytes and vascular endothelial cells (Lang, 2007). Basement membrane thickening is the result of a disturbed extracellular matrix production in which there is an increased synthesis and/or decreased breakdown of macromolecular proteins such as collagen type IV, fibronectin and laminin (Kuiper, august 2008). The thickening of the vascular basal lamina caused by this disturbed extracellular matrix production is thought to be the result of the metabolic consequences of hyperglycemia (Van Geest, 2012).
In a later stage of the disease, the proliferative stage, the capillaries close with retinal ischemia as a result. Hypoxia causes the production of angiogenic factors such as vascular-endothelial growth factor (VEGF) and insulin-like growth factor-1. These factors contribute to neovascularization.

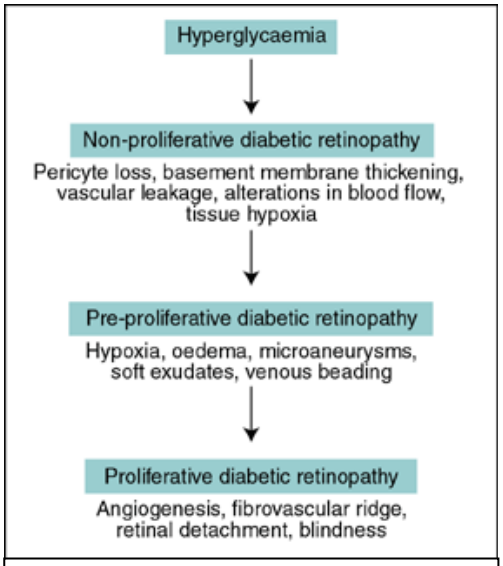

Figure 1: Stages of vascular dysfunction in diabetic retinopathy. Expert Reviews in Molecular Medicine ©2004 Cambridge University Press.

Proliferative DR is a healing-like response to a wound. It is not only characterized by neovascascularization, but it is also accompanied by the increased presence of inflammatory cells and development of myofibroblasts (Kuiper, july 2008).The new vessels can form fibrovascular tissue, which can cause traction on the retina, with retinal detachment and blindness as result. Figure 1 gives an overview of all the stages of vascular dysfunction in DR.

There is a point in DR where there is a switch from neovascularization to fibrosis, the angiofibrotic switch (Figure 2). As said, VEGF is an angiogenetic factor contributing to neovascularization in DR. Connective tissue growth factor (CTGF) on the other hand, is a factor which has primarily a pro-fibrotic activity in the eye and has been linked to fibrosis in vitroretinal disorders, including DR (Van Geest, 2012). A study by Kuiper et al. (July, 2008) showed that the shift in balance between these two factors is associated with the switch from angiogenesis to fibrosis in proliferative DR.
CTGF is the main transforming growth factor-beta (TGF-β) effector in the induction of fibrosis (Gerhardinger, 2009). TGF-β is involved in cellular growth, differentiation and migration, the formation and degradation of extracellular matrix components, immunomodulatory activity, chemotactic processes and apoptosis. It has 5 isoforms, of which TGF- β1 is probably best known and

which shows the strongest relationship with tissue fibrosis (Zorena, 2013). TGF-β is not only linked to fibrosis, but also to EndMT, as will be discussed in the next chapter.

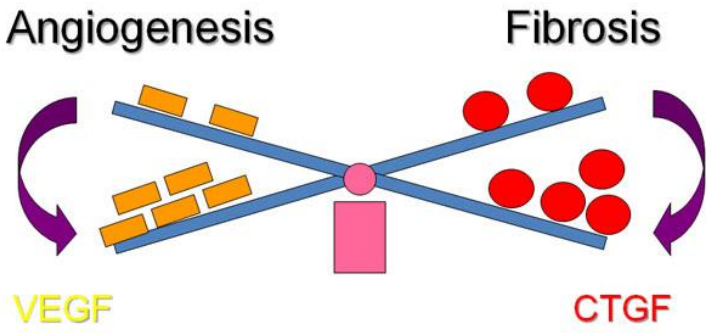

Figure 2: The angiofibrotic switch. The shift in balance between CTGF and VEGF is associated with the switch from angiogenesis to fibrosis in proliferative DR. www. ocular-angiogenesis.nl/ AF-switch/AF-switch.html

3. Endothelial-to-mesenchymal transition

Fibrosis is characterized by the disturbed extracellular matrix production in the affected tissues. This results in the disruption of the normal tissue structure, which leads to the dysfunction and failure of the affected tissue. Myofibroblasts/mesenchymal cells are, among others, responsible for the extracellular matrix production. Persistent activation of these cells distinguishes controlled repair during normal wound healing from uncontrolled fibrosis (Piera-Velazquez, 2011) .
Mesenchymal cells originate from several sources. One of them is epithelial-to-mesenchymal transition (EMT). A process in which epithelial cells lose their epithelial function and phenotype and differentiate into mesenchymal cells. These cells acquire mesenchymal, fibroblast-like properties this way. Recently some studies have found that endothelial cells can also differentiate into mesenchymal cells, called endothelial to mesenchymal transition (EndMT) (Ma, 2010).
The endothelial cells lose specific endothelial cell markers (such as VE cadherin and CD-31) and acquire mesenchymal/myofibroblastic properties, characterized by expression of mesenchymal cell products like α-SMA, vimentin and type I collagen. During EndMT cells lose their cell-cell contacts and become motile and can migrate into the surrounding tissues (Piera-Velazquez, 2011).

Like EMT, EndMT can be induced by TGF- β. As described earlier, TGF-β plays a crucial role in the pathogenesis of tissue fibrosis. Figure 3 shows the process of EndMT in tissue fibrosis. Inflammatory cells like lymphocytes and macrophages secrete TGF- β. TGF- β induces the differentiation of the endothelial cells into myofibroblasts. These 'new' myofibroblast are motile and migrate into the interstitium and participate in fibrosis.
One of the first studies suggesting this process was from Arciniegas et al. (1992). They showed that TGF-β1 induces the differentiation of mature bovine aortic endothelial cells into a smooth muscle-like phenotype. Following this early study, further studies investigated if EndMT was also involved in the pathogenesis of tissue fibrosis, just like EMT (Piera-Velazquez, 2011).

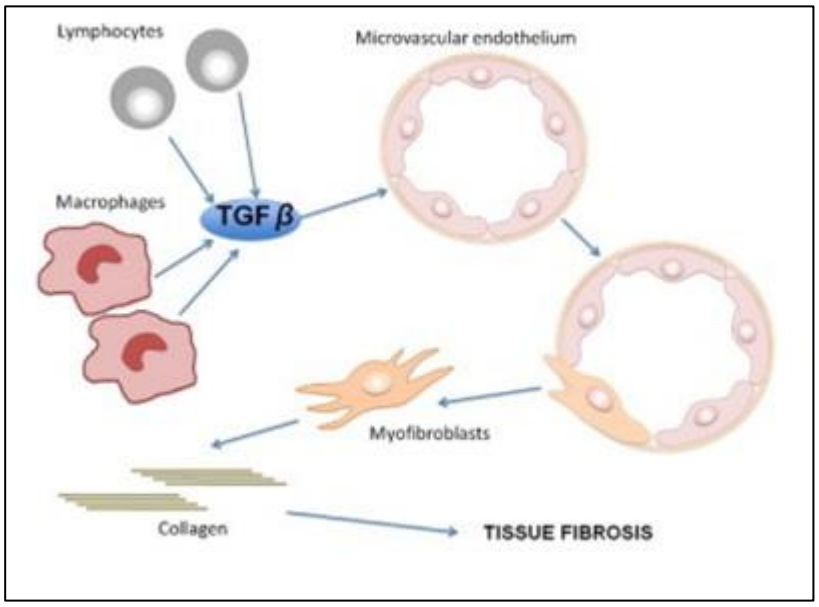

Figure 3: EndMT in tissue fibrosis. Am J Pathol. 2011 September, 197 (3): 1074-1080.

4. EndMT in diabetic retinopathy

EMT was first described by Hay in 1982 in the culture of corneal epithelial cells. EndMT can be seen as a specialized form of EMT, as the endothelium can be seen as a specialized form of epithelial tissue (Ma, 2010). The process of EMT has already been demonstrated to occur in retinal pigment epithelium (Saika, 2009), but the occurrence of EndMT in the retina has not been investigated thoroughly.
So far known, only one study (Ma, 2010) investigated the occurrence of EndMT in the retina. In this study the relationship between the presence of advanced glycation end products and the occurrence of EndMT in monkey choroid-retinal endothelial was investigated. It is known that AGEs are involved in the pathogenesis of several diabetic pathologies, especially the pathogenesis of diabetic microvascular and macrovascular complications. Their results showed a loss of endothelial markers VE-cadherin and β-catenin and a gain of mesenchymal markers vimentin and N-cadherin induced by AGEs. The cells also showed increased motility, enhanced tube formation and decreased polarity, all features of EndMT (Ma,2010). There must be said that this study used monkey endothelial cell lines. Human cell line studies and in vivo studies are necessary to confirm if EndMT indeed occurs in the retina and if it contributes to the pathogenesis of DR.

However, there are other indications that EndMT might play a role in DR. As said before, EndMT is a process in which endothelial cells acquire mesenchymal, fibroblast-like properties which contribute to fibrosis. TGF- β is the main factor that contributes to fibrosis and it has been shown that it can induce EndMT.
A study from Gerhardinger et al. (2009) found that the complete loop of TGF-β signaling, including Smad2 phosphorylation, was enhanced in the retinal vessels of streptozotocin induced rats. They also showed that sorbinil and aspirin, drugs that protect the retinal vessels of diabetic rats, both reduced (respectively 55% and 40%) the upregulation of genes of the TGF-β pathway.
Loukovaara et al. (2012) showed that TGF- β1 concentrations are higher in the vitreoretinal intersurface in adult type 1 diabetes mellitus with proliferative DR, which may be linked to retinal tissue fibrosis.
Zorena et al. (2013) demonstrated that TGF- β1 serum levels were 10 times higher in patients with non-proliferative DR and type 1 diabetes mellitus as compared to healthy controls and about 4 times higher in diabetic juvenile without DR as compared to healthy controls.

These studies indicate that there is a link between the overexpression of TGF- β1, the presence of DR and retinal tissue fibrosis and they might indicate that EndMT also occurs in the retina and contributes to the pathogenesis to DR.
Moreover, EndMT has been found in other diabetic diseases including diabetic nephropathy and diabetes mellitus–induced cardiac fibrosis and in other structures in the eye, like the cornea. This might indicate that EndMT also occurs in DR and will be discussed next.

5.1. EndMT in the cornea

Diabetes affects many organs and tissues throughout the body, including the function of several structures of the eye (Lu, 2004). Because the cornea and retina lie very near to each other and the role of EndMT in corneal cells is well described in several studies, this might indicate that EndMT is also involved in the retina.

Endotelial cells in the cornea undergo EndMT when chemical, mechanical, or other injury cause inflammation (Ko, 2005). EndMT seems to play an important role in corneal fibrosis after such an injury. TGF-β and FGF2 associated pathways have been shown to induce EndMT in the cornea. (Nakano, 2008).

Petroll et al. (1998) wanted to determine the potential role of TGF- β on corneal endothelial transformation using the cornea of 28 adult cats. Organ cultures in TGF- β1/- β2/ -β3 induced EndMT, including development of extracellular fibronectin fibrils. This shows that TGF- β plays an important role in EndMT in the cornea and in the abnormal extracellular matrix production that causes fibrosis.
Indeed, it has been shown in many tissues that blocking TGF-β signaling by targeted deletion of Smad3 or gene introduction of Smad7 (an inhibitory Smad that blocks phosphorylation of TGF-β driven Smad2/3 activation) prevents EMT and pathological tissue fibrosis. It was however never fully investigated in EndMT in corneal endothelial cells yet. Sumioka et al. (2008) tried to suppress the pathological fibrotic reaction after an induced injury in corneal endothelial cells in vivo in rats. They did this by Smad7 gene introduction, using the Cre/LoxP of adenoviral gene transfer of Smad7. The overexpression of Smad7 did indeed abrogate the expression of αSMA and the accumulation of collagen I. Thus, EndMT and subsequent endothelial fibrosis was blocked by Smad7. Moreover, they showed that overexpressed Smad7 blocked the Smad signal, as much less phospho-Smad2 labeled cell nuclei were observed. This shows that blocking the TGF-β/Smad signaling might have a therapeutic effect.

Although this thesis mainly focusses on TGF-β, there are other mediators of EndMT, such as FGF-2. The purpose of a study by Lee et al. (2012) was to determine the role of nuclear factor-κB (NF-κB) during FGF-2–mediated endothelial mesenchymal transformation in response to interleukin (IL)-1β stimulation. IL-1β is a cytokine that plays an important role in acute and chronic inflammatory diseases, but also in the regulation of inflammation and wound healing on the ocular surface. For this study they used rabbit corneal endothelial cells. Their findings showed that when the cells where stimulated with IL-1β, the canonical signaling components, IRAK and TRAF6, were transiently expressed.
IRAK activates PI 3-kinase, which subsequently phosphorylates IKK complex, causing degradation of IκB. Degradation of IkB leads to phosphorylation of the transcription factor NF-κB. NF-κB binds in the nucleus to the binding site of the promoter of the FGF-2 gene, after which FGF-2 induces EndMT, leading to irreversible fibrosis (Figure 4).

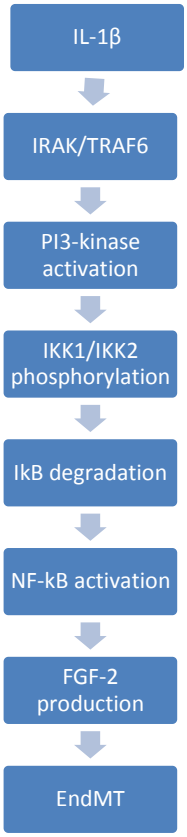

Figure 4: Signaling pathway of the IL-8 inductive activity of IL-1β on FGF-2, the direct mediator of EMT. (Lee, 2012)

These studies demonstrated that EndMT occurs in the cornea and that signaling pathways toward EndMT originate from TGF-β and FGF-2 and they may be independent of each other (Sumioka, 2008).

5.2. EndMT in diabetes mellitus–induced cardiac fibrosis

Diabetes cardiomyopathy is among other things characterized by fibrosis. Cardiac fibrosis is the result of a disturbed extracellular matrix production, caused by the recruitment of fibroblasts. However it was not clear what the source of these fibroblast was.

Zeisberg et al. (2007) examined if EndMT might contribute to the development of cardiac fibrosis. They used two strains of transgenic mice. Cardiac fibrosis was induced in these mice by aortic banding. They used Tie1-Cre;R26Rosa-lox-STOP-lox-LacZ double transgenic mice in which the endothelial cell-specific promoter Tie controls the Cre-recombinase. Cre-recombinase removes the STOP cassette which results in constitutive expression of the β-galactosidase in endothelial cells. In Tie1cre;R26Rosa-lox-STOP-lox-LacZ mice, β-galactosidase–positive cells (which also express fibroblast markers) were present in the fibrotic areas (but not in nonfibrotic hearts), which shows that EndMT has occurred. The second strain to investigate the occurrence of cardiac EndMT were *FSP1-GFP*transgenic mice in which green fluorescent (GFP) expression was under the control of fibroblast-specific protein 1 (FSP-1). GFP staining was found within cardiac endothelial cells in fibrotic hearts. The coexpression of fibroblast markers and endothelial markers suggest the occurrence of EndMT in these cells. These experiments demonstrated that cardiac fibrosis is indeed linked to the emergence of fibroblasts originating from endothelial cells. Zeisberg et al. also investigated the role of TGF-β1 in fibrotic hearts. They used aortic banded *Smad3*$^{+/-}$ transgenic mice in which the TGF-β response was blunted due to the deficiency of Smad3. They found a significant reduction in EndMT-derived mesenchymal cells, which suggests that TGF-β induces endothelial cells to undergo EndMT.
They also showed that recombinant human bone morphogenic protein 7, a TGF-β antagonistic protein, preserved the endothelial cell type and significally inhibited EndMT and the progression of cardiac fibrosis in mouse models.
In total, 27% to 35% of all (α-SMA– or FSP1-positive) fibroblasts in mice with aortic binding were of endothelial origin. This study provided the evidence that endothelial cells can differentiate into fibroblast-like cells and that the accumulation of these fibroblast contributes to cardiac fibrosis. Moreover, they showed that TGF-β induces the endothelial cells to undergo EndMT (Zeisberg, 2007; Zeisberg, 2010; Piera-Velazquez, 2011).

A review from Goumans et al (2008) proposed a mechanism (Figure 4) in which oxidative stress is the initiator of EndMT by increasing TGF- β. In diabetes, hypertension, chronic renal failure, but also after myocardial infarction, substances can accumulate and lead to oxidative stress, limiting the production of NO by uncoupling of endothelial nitric oxide synthase (NOS). This leads to the overexpression of TGF-β, causing fibrosis via EndMT. EndMT may also attribute to the loss of microvascular endothelial cells which results in rarefaction, a reduction of the density of capillaries and lead to a state of chronic hypoxia. This in turn could lead to up-regulation of TGF- β expression, causing fibrosis via EndMT.
As said before, TGF- β levels are increased in DR. Above studies might indicate that these high TGF- β levels induce EndMT in the retina, which contributes to fibrosis.

Besides TGF- β, Endothelin-1 (ET-1) also plays an important role in EndMT. ET-1 is a vasoconstrictor with proliferative, profibrotic, and proinflammatory properties. Plasma ET-1 levels are increased in patients with diabetes (Ergul, 2011). A study from Widyantoro et al. (2010) showed that endothelial-1 (ET-1) promotes cardiac fibrosis in diabetic hearts through stimulation of EndoMT. They induced diabetes mellitus in mice with Streptozotocin, which resulted in an increased ET-1 expression. The elevated ET-1 levels stimulated severe cardiac fibrosis via EndMT. Moreover, the hearts of transgenic mice with endothelial cell–specific ET-1 deletion showed none of these results (Widyantoro, 2010; Piera-Velazquez, 2011).
Kawamura et al (1992) showed that plasma ET-1 levels in diabetic patients with retinopathy are

increased. It could be hypothesized that these high levels also induce EndMT in the retina and stimulate fibrosis this way.

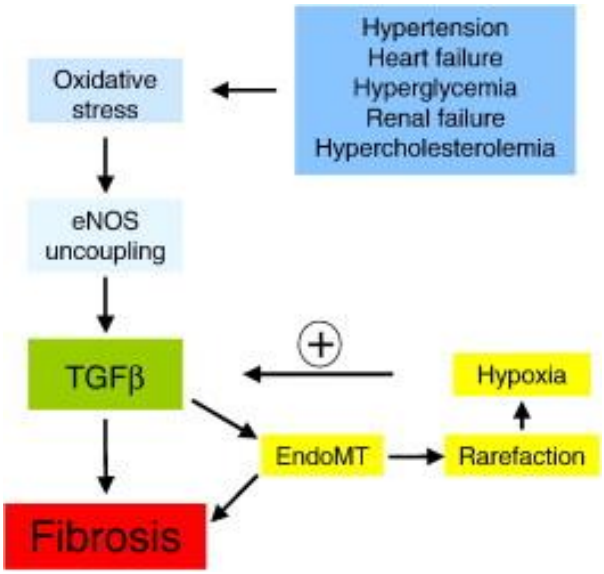

Figure 5: EndMT in disease. Oxidative stress may be the initiator of EndMT, increasing TGF β in a NO-dependent manner leading to fibrosis via EndMT. A loss of microvascular endothelial cells due to EndMT may contribute to rarefaction of capillaries and lead to a state of chronic ischemia (Goumans, 2008)

5.3. EndMT in diabetic nephropathy

EndMT has been linked to diabetic diseases including diabetes mellitus–induced cardiac fibrosis, as discussed earlier, but has also been linked to diabetic nephropathy (DN). The process of EndMT in peritubular capillary endothelial cells is shown in Figure 6.
DN is morphologically characterized by glomerular and interstitial fibrosis. Myofibroblasts play an important role in the extracellular matrix production and thus in the development and progression of renal fibrosis. In DN, the number of myofibroblasts is inversely correlated with the renal function (Li, August 2010).

After the finding that EndMT occurs in diabetes mellitus-induced cardiac fibrosis, Zeisberg et al. (2008) investigated if endothelial cells contribute to the emergence of myofibroblasts in kidney fibrosis via EndMT. They investigated three mouse models of chronic kidney disease: unilateral ureteral obstructive nephropathy, streptozotocin-induced diabetic nephropathy, and a model of Alport renal disease. The occurrence of EndMT was assessed by the presence of CD-31 (endothelial specific cell surface marker) and the presence of FSP-1 and α-SMA (specific markers for respectively fibroblasts and myofibroblasts). Co-expression of CD-31 along with FSP-1 and α-SMA was found in approximately 30%-50% of the fibroblasts in the fibrotic kidneys. The true percentage could even be higher as double labeling does not detect EndMT-derived fibroblasts that have already lost their endothelial markers. Although fibrosis was mild in the streptozotocin-induced diabetic nephropathy model compared to the Alport mice, they found a very high percentage of EndMT-derived fibroblasts in the streptozotocin mice (40-50%). The ureteral obstructive nephropathy had a similar amount of fibrosis compared with the diabetic nephropathy model, but showed a lower percentage of EndMT-derived fibroblasts (Zeisberg, 2008; Piera-Velazquez, 2011). This study demonstrates the contribution of EndoMT to the formation of activated fibroblasts and myofibroblasts in fibrotic kidneys, including diabetic nephropathy.

Li et al. (2009) also confirmed the occurrence of EndMT in streptozotocin-induced diabetic mice and the contribution of EndMT to the early development of renal interstitial fibrosis, independently of microalbuminuria. The same researchers (Li, October 2010) investigated if the blockage of EndMT would reduce the development of streptozotocin-induced diabetic nephropathy. Firstly, they showed that advanced glycation end-products induced EndMT in a mouse pancreatic microvascular endothelial cell line and in an endothelial lineage-traceable mouse line Tie2-Cre;Loxp-EGFP. Secondly, they used a specific inhibitor of Smad3 (SIS3) that blocks Smad3 phosphorylation and TGF-β1–induced fibrotic response in fibroblasts. The TGF-β pathway inhibition with SIS3 stopped the process of EndMT, reduced renal fibrosis and slowed the progress of nephropathy down. These results show that EndMT can be induced by advanced glycation end-products and that Smad3 is an important mediator in this process. Moreover, SIS3 may provide a new drug therapy against diabetic nephropathy and other fibrotic processes. (Li, 2009; Li, October 2010; Piera-Velazquez, 2011).

All together, these studies identify the occurrence of EndMT in diabetic nephropathy and the contribution of EndMT to fibrogenesis. Smad3, which transduces signals from TGF-β, may play an important mediator in this process. As TGF- β concentrations are shown to be increased in DR, it can be hypothesized that these high concentrations may induce EndMT in the retina.

Besides the fibrotic effect of EndMT, Basile et al. (2011) have analyzed the phenotypic alterations of endothelial cells after ischemia-reperfusion in Tie2-Cre;YFP transgenic mice . The results of this study suggested that EndMT might contribute to rarefaction, the reduced density of endothelial cells (Guerrot, 2012). This reduced density of endothelial cells is also seen in DR and might be another indication that EndMT plays a role in DR.

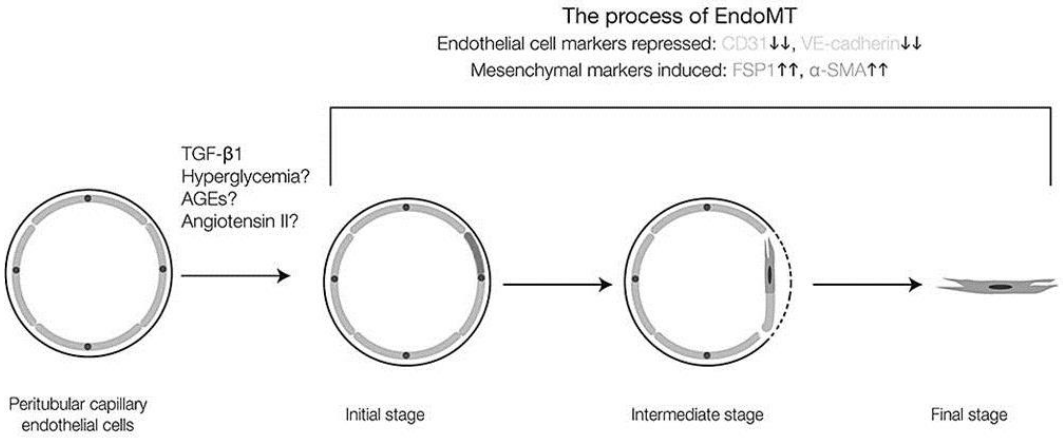

Figure 6: The process of EndMT in peritubular capillary endothelial cells.
Nephrology, Volume 15, issue 5, pages 507-512, 19 Mar 2010
DOI:10.1111/j.1440-1797.2010.01319.x

6. Conclusion

DR is a serious condition which is the leading cause of acquired blindness and which 90% of the patients with diabetes develop after 20 years. As diabetes is predicted to affect 380 million people by 2025 (Christiakov, 2011), research on the pathogenesis of DR is very important. However, the occurrence of EndMT in the retina and the possible contribution to the pathogenesis of DR has not been investigated thoroughly.

The studies discussed here in this thesis strongly suggest the participation of EndMT in DR.
It was demonstrated that EndMT occurs in the cornea and that signaling pathways toward EndMT originate from TGF-β and FGF-2, leading to irreversible fibrosis. TGF-β levels were shown to be increased in DR. This and the fact that the cornea and retina lie very near to each other, could suggest that EndMT is also involved in the retina.
The studies also showed that EndMT is linked to several diabetic diseases, including diabetic nephropathy and diabetes mellitus–induced cardiac fibrosis. As well as in diabetes mellitus–induced cardiac fibrosis and diabetic nephropathy, TGF-β induces, just like in the cornea, the endothelial cells to undergo EndMT.
Goumans et al. (2008) proposed a mechanism in which, among others, hyperglycemia causes oxidative stress. Oxidative stress may be the initiator of EndMT, increasing TGF-β, leading to fibrosis via EndMT. Besides TGF-β, ET-1 levels are elevated and stimulate severe cardiac fibrosis via EndMT. Plasma ET-1 levels are increased in patients with diabetes (Ergul, 2011). These findings suggests that the high levels of TGF-β and ET-1 might also induce EndMT in the retina, which could contribute to fibrosis in DR.
Besides this, Ma et al. (2010) showed the occurrence of EndMT in monkey choroid-retinal endothelial.

It must be said that almost all of these studies were done on experimental animal models or animal cell lines. Human cell line studies and in vivo studies are necessary to confirm that EndMT indeed occurs in the retina and that it contributes to the pathogenesis of DR. Further research is needed to understand the molecular mechanisms and pathways involved in EndMT. Although this thesis has mostly focused on TGF-β, as it seems to be the main initiator of EndMT, the identification of other factors, like ET-1 and FGF, is also of great value to provide targets for possible therapeutic interventions.
At the moment, there is no effective treatment available for DR. In the cornea, EndMT and subsequent endothelial fibrosis were blocked by overexpression of Smad7. A specific Smad3 inhibitor also stopped the process of EndMT in diabetic nephropathy. This shows that blocking the TGF-β/Smad signaling might have a therapeutic effect. Indeed, Gerhardinger et al. (2009) have showed that the TGF-β pathway is a common target of drugs that prevent experimental diabetic retinopathy. Moreover, Angiotensin II blockers were recently reported to reduce the progression of vascular pathologies caused by excessive TGF-β signaling. This coupled with an overactive TGF- β pathway in DR, may explain the encouraging results obtained with candesartan (an angiotensin II blocker) in the prevention of retinopathy in type 1 diabetic patients (Gerhardinger, 2009).

To conclude, Figure 7 gives an overview of all the indications discussed in the thesis that EndMT might also occur in the retina and contributes to the pathogenesis of DR. All the information together strongly suggest that this is indeed the case.

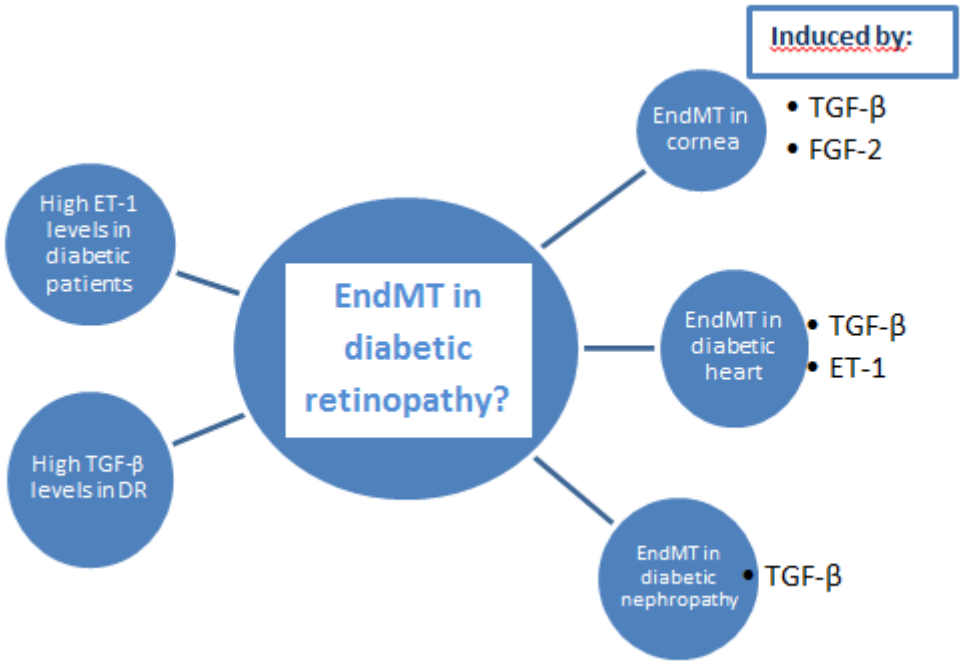

Figure 7: Concluding figure. Shown are the indications that EndMT might also occur in the retina and that EndMT might contribute to diabetic retinopathy.

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