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Pericytes in diabetes-associated vascular disease

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Abstract

Pericytes are mural cells that support and stabilize the microvasculature, and are present in all vascular beds. Pericyte-endothelial cell crosstalk is essential in both remodelling and quiescent vasculature, and this complex interaction is often disrupted in disease states. Pericyte loss is believed to be an early hallmark of diabetes-associated microvascular disease, including retinopathy and nephropathy. Here we review the current literature defining pericyte biology in the context of diabetes-associated vascular disease, with a particular focus on whether pericytes contribute actively to disease progression. We also speculate regarding the role of pericytes in the recovery from macrovascular complications, such as critical limb ischemia. It becomes clear that dysfunctional pericytes are likely to actively induce disease progression by causing vasoconstriction and basement membrane thickening, resulting in tissue ischaemia. Moreover, their altered interactions with endothelial cells are likely to cause abnormal and inadequate neovascularisation in diverse vascular beds. Further research is needed to identify mechanisms by which pericyte function is altered by diabetes, with a view to developing therapeutic approaches that normalize vascular function and remodelling.

Keywords

Pericyte; diabetes; vascular; endothelial; angiogenesis

1 Introduction

Pericytes are perivascular or mural cells, which accompany and support the microvasculature. Rouget was one of the first to describe these cells in 1873, hence they were initially referred to as 'Rouget cells' until Zimmermann later introduced the name pericyte (Sims, 1986).

During angiogenesis, vessel maturation and stabilization, endothelial cell (EC) and pericyte crosstalk is essential to form and maintain a functional vasculature (Potente, Gerhardt, & Carmeliet, 2011). Deletion experiments of key components of EC and pericyte crosstalk have demonstrated the importance of this cell interaction, as most disturbances in crosstalk are lethal during embryonic development (Dickson et al., 1995; Hellstrom, Kalen, Lindahl, Abramsson, & Betsholtz, 1999; Lindahl, Johansson, Leveen, & Betsholtz, 1997; Sato et al., 1995).

Diabetes is associated with vascular complications in which pericyte-EC crosstalk is altered and pericyte loss contributes to abnormal EC behaviour. In this review, the role of pericytes in diabetes-associated vascular complications will be summarized, along with a brief overview of pericyte biology.

1.1 Pericyte characteristics

Pericytes, unlike vascular smooth muscle cells (vSMCs), are located in the microvasculature, at terminal arterioles, capillaries and post-capillary venules (van Dijk et al., 2015). They share a common basement membrane with ECs, within which pericytes are usually completely enclosed, and to which both ECs and pericytes contribute in synthesising (Stratman, Malotte, Mahan, Davis, & Davis, 2009). Pericyte cell bodies are often located at capillary branch points and they form large longitudinal processes alongside the capillary, which branch into small circumferential processes (Sims, 1986). Pericytes and ECs are in direct cell contact via peg-sockets, gap junctions and adhesion plaques at the small circumferential braches, where pericytes cross the basement membrane (Armulik, Genove, & Betsholtz, 2011; Sims, 1986).

Pericyte density in the microvasculature and coverage of ECs with pericytes at the abluminal surface varies between tissues and with vascular function. The highest

pericyte density has been described in the retina and central nervous system (CNS) with an EC to pericyte ratio of 3:1 and 1:1, respectively (Sims, 1986). Especially in the CNS, the barrier function of the vasculature is of great importance, and pericytes play a substantial role. During embryogenesis, pericytes are important for blood brain barrier (BBB) formation and experiments with mice lacking critical pericyte signalling mediators have revealed that reduced pericyte abundance is associated with enhanced vascular permeability (Daneman, Zhou, Kebede, & Barres, 2010). In skeletal muscle, EC to pericyte ratio is reported to be around 10:1, resulting in pericyte coverage of ECs exceeding 20%. Coverage of ECs with pericytes in other tissues ranges from 11% in heart muscle, to 30% in CNS and 41% in retina (Sims, 1986; Tilton, Kilo, & Williamson, 1979).

Pericytes express a wide range of surface markers, of which none is specific in isolation. Moreover, the expression of cell surface markers is dynamic, and changes with developmental state, in pathological conditions, and can be tissue specific. Commonly used markers to identify pericytes include the proteoglycan neuron-glial antigen (NG2), platelet-derived growth factor (PDGF) receptor β (PDGFRβ), the transmembrane glycoprotein CD146 or contractile elements such as α smooth muscle actin (αSMA) (Armulik et al., 2011; Crisan et al., 2008). Additionally, the anatomic location inside the tissue, morphological or structural features, as well as the absence of endothelial or hematopoietic markers should be considered to robustly identify pericytes.

1.2 Pericyte-endothelial cell signalling

The crosstalk between ECs and pericytes is complex and incompletely understood. Here, we focus on three main signalling pathways involved in pericyte-EC crosstalk, which have also been shown to be altered in diabetes associated vascular complications (**Figure 1**).

Extensive studies have implicated PDGFB/PDGFRβ signalling in pericyte recruitment, mesenchymal cell differentiation, and mural cell proliferation along with a recently emergent role of heparin binding epidermal growth factor (HB-EGFs) - EGF receptor (EGFRs, also named ErbBs) interactions (Stratman, Schwindt, Malotte, & Davis, 2010). PDGFB is secreted by endothelial tip cells during angiogenic sprouting to stimulate proliferation and co-migration of pericytes which express PDGFRβ (Abramsson et al.,

2007; Hellstrom et al., 1999). Either PDGFB or PDGFRβ deletion in mice results in lethal haemorrhage and oedema during embryogenesis. Capillaries in PDGFB deficient mice show microaneurysms and a high variability in capillary diameter (Lindahl et al., 1997), which has also been described in PDGFRβ knockout mice (Hellstrom et al., 1999). HB-EGF and EGFRs were recently described to be involved in pericyte recruitment alongside PDGFB. Migration assays using receptor traps to block PDGFB or HB-EGF function showed a decrease in pericyte association with the EC tube compared to control, which was further diminished with the combination of both traps to block PDGFB *and* HB-EGF (Stratman et al., 2010).

Angiopoietin (Ang) signalling (via Tie tyrosine kinase receptors) is important for the delicate balance of vessel maturation and stability and, on the other hand, vessel repair and remodelling (Maisonpierre et al., 1997). Moreover, Ang1/Tie2 signalling may play a role in the maintenance of vascular pericyte coverage, as antagonizing experiments of the Tie2 receptor, or Ang1 knockout, result in pericyte loss (Hammes et al., 2004; Suri et al., 1996). Ablation of the Tie2 receptor is lethal during embryonic angiogenesis. Vessels in these embryos are malformed, dilated and lack a distinction between large and small vessels (Sato et al., 1995). Whereas Ang1 is expressed by mural cells, Ang2, the second ligand of Tie2 is expressed mostly by ECs themselves and antagonizes Ang1 function by blocking Tie2 receptor in an autocrine manner (Maisonpierre et al., 1997). Vascular sprouting requires breakdown of the basement membrane and mural cell detachment.

The role of transforming growth factor β (TGFβ) is controversial, as both, mural cell proliferation and migration, as well as differentiation can be triggered (Pardali, Goumans, & ten Dijke, 2010). TGFβ, as well as the two types of corresponding transmembrane serine/threonine kinase receptors (type I and type II), are expressed in both ECs and mural cells. In EC-mural cell crosstalk, the type I receptors Alk1 and Alk5 appear to be most important. Binding of activated TGFβ to Alk5 on mesenchymal stem cells induces Smad transcription factors 2 and 3, which promote differentiation into vSMC and pericytes, supporting vessel maturation. In contrast, binding to Alk1 receptor instead activates Smad 1 and 5 promoting proliferation and migration of mesenchymal stem cells (MSCs) and inhibition of differentiation (ten Dijke & Arthur, 2007). Accordingly, TGFβ signalling is very complex, with the relative expression of Alk1 and Alk5 on the cell surface, the signal duration, and ligand concentration all potentially

determining mural cell behaviour. Knockout of either TGFβ, or Alk1 and Alk5 in mice is lethal during embryogenesis and arteriovenous malformations and impaired yolk sac vascularisation have been observed in these mice (Dickson et al., 1995; Urness, Sorensen, & Li, 2000).

Nishishita and Lin (2004) demonstrated how closely these three main signalling pathways are interconnected and tightly regulated by negative feedback loops. This delicate equilibrium is dependent on various external factors and can be disrupted easily (**Figure 1**). Patients with type 2 diabetes were described to have increased vascular endothelial growth factor (VEGF) and Ang2 plasma levels, reducing microvascular stability (Lim, Blann, Chong, Freestone, & Lip, 2004). Moreover, TGFβ expression is enhanced, which has not only been implicated in vessel maturation, but increased Smad 3 signalling also facilitates thickening of basement membrane by excessive fibronectin and collagen deposition (Isono, Chen, Hong, Iglesias-de la Cruz, & Ziyadeh, 2002; Kanwar, Sun, Xie, Liu, & Chen, 2011; Shimizu, Sano, Haruki, & Kanda, 2011). The influence of hyperglycaemia and diabetes on PDGFB/PDGFRβ signalling is less clear. Gene expression of PDGFB in diabetic patients was shown to be elevated (Stoynev et al., 2014), however, the receptor PDGFRβ was found to be inactivated by hyperglycaemia (see also 2.1.1) (Geraldes et al., 2009). Altogether, these findings demonstrate that pericyte-EC crosstalk is altered under diabetic conditions and hyperglycaemia, promoting microvascular instability and pericyte detachment.

2 Pericytes in diabetes

Pericyte loss from the microvasculature under diabetic conditions was first noted in the 1960s, when it was believed to be a phenomenon limited to the retina. Later, pericyte loss was also described in skeletal muscle (Tilton et al., 1985) and the endoneurial microvasculature (Malik et al., 1992). Today, changes in pericyte biology are recognised to be directly associated with biochemical changes in diabetes, which engender diffuse microvascular complications. Here, the involvement of pericytes in diabetes-associated micro- and macrovascular complications will be reviewed to elaborate on the question of whether pericytes are also involved in ‘macrovascular’ pathology, despite the fact that pericytes by definition are located at the microvasculature. Moreover, the question as

to whether pericyte dysfunction contributes actively to disease progression will be evaluated.

2.1 Pericytes in microvascular complications

2.1.1 Diabetic retinopathy

Diabetic retinopathy is the most prevalent diabetes-associated microvascular complication. A third of patients with diabetes will develop ocular complications associated with visual impairment, eventually resulting in blindness. Diabetic retinopathy is characterized by pericyte loss, acellular capillaries, and focal capillary dilatation, leading to microaneurysm formation. Vascular leakage results in the deposition of hard exudates and rupture of microvessels causing haemorrhage (Cheung, Mitchell, & Wong, 2010). Together, these pathologies contribute to retinal hypoxia and inflammation, which can cause macular oedema and proliferative neovascularization into the macula and vitreous (Ejaz, Chekarova, Ejaz, Sohail, & Lim, 2008). However, the underlying mechanism by which diabetes finally effects apoptosis in retinal microvessels remains elusive.

A reduction in retinal pericyte number associated with the microvasculature was first described three decades ago. The term ‘pericyte ghosts’ refers to pericytes which disappeared from the capillary wall thereby leaving pockets in the basement membrane (Robison Jr. et al., 1991). It is believed that pericyte loss in the vascular bed of the retina is one of the earliest hallmarks of diabetic retinopathy (Hammes et al., 2002). Recent research shows that the early pericyte dysfunction, including altered signalling and protein expression, is a major contributor to the later pathophysiological changes in diabetic retinopathy (Braunger et al., 2015; Durham, Dulmovits, Cronk, Sheets, & Herman, 2015). For example, early loss of pericytes is thought to result in endothelial cell loss through a reduction in pericyte-derived pro-survival and quiescence signals, resulting in acellular capillaries which lack blood flow, ultimately causing local ischaemia (Hammes, Feng, Pfister, & Brownlee, 2011). Microaneurysm formation is observed in close proximity to acellular capillaries in human diabetic retinopathy (Kohner & Henkind, 1970), and may represent early ineffective attempts at neovascularization, which is normally suppressed by pericytes. Indeed, substantial pericyte loss in non-diabetic mice

is associated with retinal proliferative retinopathy (Enge et al., 2002). As retinal ischaemia worsens, local VEGF production increases, promoting a proliferative retinopathy which is characterized by sprouting of immature neovessels. These vessels lack adequate pericyte coverage, and thus are prone to leakage and bleeding (Hammes et al., 2011; Wisniewska-Kruk et al., 2014). Hence, pericyte loss and dysfunction appear to contribute to the entire continuum of diabetic retinopathy.

Diabetic retinopathy is associated with many other hyperglycaemia-induced alterations including vasoconstriction of microvasculature, apoptosis and poor quality neovascularization. Nitric oxide (NO) produced by the endothelium is necessary to maintain vasodilation in retinal vessels. Hyperglycaemia induced protein kinase C (PKC) and polyol pathway activation, superoxide generation and advanced glycation endproducts (AGEs) reduce NO bioavailability (Cai & Boulton, 2002; Ishii et al., 1996). Moreover, a reduction in endothelium-derived hyperpolarizing factor and prostacyclin production, which normally promote relaxation of mural cells, further contributes to microvessel vasoconstriction, which leads to ischaemia and apoptosis of vascular cells and pericytes (Ejaz et al., 2008; Mogensen et al., 2011). Pericytes are particularly sensitive to hyperglycaemia induced oxidative stress and aldose reductase activation (polyol pathway), further contributing to pericyte apoptosis (Manea, Constantinescu, Popov, & Raicu, 2004).

Hyperglycaemic conditions have been described to activate NF-κB preferentially in retinal pericytes which triggers a pro-apoptotic program (Romeo, Liu, Asnaghi, Kern, & Lorenzi, 2002). Apoptosis has also been shown to occur independently of NF-κB. Downstream of the hyperglycaemia-induced activation of PKC and protein tyrosine phosphatase SHP1, PDGFRβ in pericytes becomes dephosphorylated and inactivated which decreases pro-survival signals (Geraldes et al., 2009). In contrast, mRNA levels of PDGFB were shown to be increased in diabetic retinas by PKC activation (Yokota et al., 2003), which may reflect an attempt to augment PDGF signalling. Experiments in genetically modified mice have shown the importance of pericyte-EC crosstalk and demonstrated that disturbances of this generate pericyte loss and apoptosis. Haploinsufficiency of PDGFB in mice was sufficient to recapitulate features of retinopathy such as pericyte loss and microaneurysms, even under normoglycaemic conditions (Hammes et al., 2002). Hammes et al. (2004) later showed that Ang2 injection into the retina of normoglycaemic mice causes pericyte loss, and that pericyte

loss in diabetic rats can be prevented by Ang2 deficiency. Moreover, under hyperglycaemic conditions Ang2 initiates apoptosis in pericytes via the p53 pathway, which is not normally induced in normoglycaemia (Park et al., 2014). Deletion of TGFβ postnatally mimics diabetic retinopathy with microaneurysms, vascular leakage and undifferentiated pericytes (Braunger et al., 2015). Moreover, a potential contribution of the immune system to pericyte loss in diabetic retinopathy has been proposed. In a subset of diabetic patients, a pericyte autoantibody was found in sera (Attawia & Nayak, 1999), which has been recently confirmed (Zhang et al., 2016). Zhang et al. (2016) demonstrated that these human serum autoantibodies can induce complement C3a and C5a, contributing to retinal pericyte injury. However, it still needs to be established as to whether reactive autoantibodies are causal for pericyte damage and dysfunction, or, alternatively whether they are activated by pericyte dysfunction and apoptosis.

Recent research offers evidence that pericytes may play an even more central role in the pathogenesis of diabetic retinopathy than previously imagined. Human retinal pericytes from patients with diabetes or control subjects were studied, demonstrating that pericytes from diabetic donors have an altered cytoskeletal organization and contractility which is associated with alterations in the secretome, including increased secretion of pro-angiogenic factors such as VEGF that initiate neovascularization (Durham et al., 2015).

The term metabolic memory describes chronic changes in gene expression, cell signalling, and metabolism, that persist even after loss of the initiating stimulus. Retinal tissue has been demonstrated to acquire a metabolic memory in response to hyperglycaemia. For example, miRNA-23b3p is expressed in hyperglycaemia and remains upregulated under normal glucose conditions, resulting in increased NF-κB expression by reducing sirtuin 1 (SIRT1) expression (Zhao et al., 2016). This metabolic memory may contribute to retinal cell apoptosis and diabetic retinopathy, demonstrating that glycaemic control may not prevent disease progression.

2.1.2 Diabetic nephropathy

In the kidney, pericytes are located in the tubular system (peritubular pericytes) and additionally, specialized pericyte-like cells called mesangial cells and podocytes stabilize the glomeruli (Geevarghese & Herman, 2014; Lenoir et al., 2015). Mesangial cells are believed to be pericyte-like cells which differentiated from immature pericytes

during development to adapt to their function (the regulation of glomerular blood flow, and accordingly glomerular filtration rate (Diaz-Flores et al., 2009; van Dijk et al., 2015)), whereas podocytes arise from post-mitotic pericytes (Lenoir et al., 2015). As with pericytes, mesangial cells express PDGFRβ, are contractile, and generate mesangial basement membrane molecules, such as collagen IV and fibronectin (Isono et al., 2002). Podocytes are also contractile cells, but they are located as an epithelial layer outside the glomerular filtration barrier in contact with the glomerular basement membrane (Borza & Pozzi, 2012).

Diabetic nephropathy is the main cause of end-stage renal disease in humans and is characterized by various pathological alterations within glomeruli and the tubular system, leading to disruption of the filtration barrier (the intersection between blood and urine) and proteinuria (Lenoir et al., 2015). The pathological features include: glomerulosclerosis, mesangial matrix expansion, glomerular basement membrane thickening and podocyte loss, as well as interstitial fibrosis and thickening of the tubular basement membrane (Borza & Pozzi, 2012; Lenoir et al., 2015; van Dijk et al., 2015).

In glomeruli, mesangial cells and podocytes are believed to mediate hyperglycaemia- and hypertension-induced glomerulosclerosis. Angiotensin II and hyperglycaemia increase glomerular capillary pressure and the production of AGEs and reactive oxygen species (ROS) (Campbell, Raij, & Mundel, 2011). These stimuli enhance expression and secretion of growth factors like TGFβ in mesangial cells by activation of PKC, causing mesangial matrix expansion and podocyte detachment and apoptosis (Koya et al., 2000). This TGFβ stimulus is conveyed via Smad3, resulting in enhanced fibronectin expression in mesangial cells (Isono et al., 2002), indicating that pericytes actively contribute to matrix expansion. Moreover, mesangial cell expansion was shown to be ROS induced via the NF-κB pathway (Yang, Wang, & Gao, 2015). However, this generally accepted view of hyperglycaemia-induced pathology in diabetic nephropathy has been challenged by the recent work of Welsh et al. (2010). They demonstrated that normoglycaemic podocyte-specific insulin receptor knockout mice developed diabetic nephropathy, suggesting a role of diminished insulin signalling in the pathogenesis of diabetic nephropathy.

In the tubulointerstitial system, oxidative stress and increased TGFβ expression (by PKC activation) promotes tubular membrane accumulation of collagen and osteopontin, and angiotensin II also directly causes tubular vasoconstriction-induced ischaemia

(Kanwar et al., 2011). Under pathological conditions, pericytes were shown to differentiate into myofibroblasts with contractile, but not vessel stabilizing, abilities. In non-diabetic murine kidney injury models, pericyte originating myofibroblasts have also been shown to substantially add to extracellular matrix formation, vessel destabilisation and fibrogenesis (Kennedy-Lydon, Crawford, Wildman, & Peppiatt-Wildman, 2013; Lin, Kisseleva, Brenner, & Duffield, 2008). With the onset of proteinuria, tubulointerstitial injury is exaggerated, as the excess of proteins damage the tubule, further inducing inflammation (Kanwar et al., 2011). Likewise, disruption of Ang1 signalling in podocytes and mesangial cells was shown to worsen diabetic nephropathy, but only if Ang1 was knocked down in both cell types, suggesting a complementary mechanism involving podocytes and mesangial cells (Jeansson et al., 2011).

2.1.3 Diabetic neuropathy

Diabetic neuropathy is characterized by sensory loss particularly in the distal lower limb, which can lead to foot ulceration and ultimately to lower limb amputation (Cameron, Eaton, Cotter, & Tesfaye, 2001). In patients with diabetes, significant pathological alterations of endoneurial microvessel basement membranes and demyelination of axons have been described (Kaku, Vinik, & Simpson, 2015). Wall thickening is associated with cellular debris, a decrease in pericyte coverage and endothelial hyperplasia, resulting in degenerative changes in the microvasculature and disruption of the blood-nerve barrier (BNB) (Malik et al., 1992; Shimizu, Sano, Haruki, et al., 2011; Shimizu, Sano, Abe, et al., 2011). Basement membrane thickening and reduplication, along with loss of pericytes is more pronounced in patients with diabetic polyneuropathy, compared with diabetic controls, and is associated with disease severity (Giannini & Dyck, 1995). Shimizu, Sano, Haruki, et al. (2011) also demonstrated that the thickening of the basement membrane and the concomitant disruption of the blood-nerve barrier is pericyte-driven, as a response to AGEs. AGEs accumulate in ECs, pericytes, basement membrane and other tissues under diabetic conditions, and stimulate the secretion of TGFβ in peripheral nerve pericytes, and VEGF in both pericytes and ECs. TGFβ and VEGF in turn stimulate the secretion of fibronectin, collagen type IV and tissue inhibitor of metalloproteinase (TIMP-1) by peripheral nerve pericytes, resulting in thickening of the basement membrane.

The vascular changes in endoneurial microvessels are associated with nerve pathology, axon demyelination and degeneration, most likely due to impaired nerve blood flow and hypoxia (Cameron et al., 2001; Malik et al., 1992). Diabetes-associated complications, such as hypertension and coagulation abnormalities, are further suggested to contribute to endoneurial vessel occlusion and hypoxia, or may even cause reduced blood flow before other pathological changes occur (Cameron et al., 2001).

2.2 Pericytes in macrovascular complications

2.2.1 Peripheral vascular disease

Peripheral vascular disease (PVD), also referred to as peripheral artery disease (PAD) is usually an atherosclerotic angiopathy of the lower limbs which results in muscular ischaemia, cutaneous ulcers and impaired wound healing (Caporali et al., 2008). In the 1980s Tilton et al. (1985) first described differences between microvascular characteristics of diabetic and non-diabetic muscle samples. Whereas pericyte coverage was not significantly different between the groups, basement membrane width, number of acellular capillaries and pericyte debris was significantly higher in diabetic skeletal muscle.

The contribution of altered pericyte biology to the pathogenesis of peripheral vascular disease *per se* (i.e. independent of diabetes) also remains incompletely explored. However, it has been shown that transplantation of a combination of human pluripotent stem cell derived ECs and pericytes (Dar et al., 2012), EPCs and MSCs (Lasala, Silva, Gardner, & Minguell, 2010) and even multipotent pericytes from adventitial capillaries alone (Kabara et al., 2014) can rescue limb ischemia by stimulating angiogenesis and improving blood flow recovery, indicating that pericytes play a central role in PVD. The relationship between pericytes and MSC has become a new field of research, yet many uncertainties remain, possibly because cell-surface markers for these lineages are overlapping and non-specific, and also due to the heterogeneity of cell populations studied. For example, whereas Crisan et al. (2008) and Kabara et al. (2014) demonstrated that pericytes can play a role as MSC-like progenitor cells, Dar et al., (2012) described that MSCs can be expanded to pericyte-like cells expressing established pericyte marker such as CD146, NG2 and PDGFRβ. Whether lineage

switching between MSC and pericyte (or vice versa) is relevant to the mechanisms of flow recovery remains unproven. However, Lasala, Silva, & Minguell (2012) showed in a phase II clinical trial that administration of a combination of MSCs and EPCs is safe and improves walking time and ankle-brachial index in severe limb ischaemia.

In diabetic retinopathy, NF-κB was reported to trigger a pro-apoptotic program (Romeo et al., 2002) and also in PVD NF-κB activation was described to have negative effects on pericyte biology. Caporali et al. first reported the association of p75 neurotrophin receptor ($p75^{NTR}$) (Caporali et al., 2008) and miRNA503 (Caporali et al., 2011) with impaired endothelial function in diabetes. $p75^{NTR}$ expression, which is enhanced in capillary ECs under diabetic conditions, depresses the VEGFA/Akt/NO axis and angiogenesis. They further elucidated that $p75^{NTR}$ activates NF-κB, and thereby increases the expression of miRNA503 in ECs, which is transferred to pericytes via microparticles and disrupts VEGFA expression (Caporali et al., 2015). Subsequently, migration and proliferation of pericytes is impaired resulting in a reduction of pericyte coverage, an increase in capillary permeability and defective angiogenesis under ischemic conditions in the limb muscle.

2.2.2 Ischaemic stroke

The role of pericytes in ischaemic stroke, one of the major macrovascular complications of diabetes, has not been studied in detail. In the CNS, pericytes not only stabilize the microvasculature, but also contribute a substantial part of the blood brain barrier, and regulate blood flow (Daneman et al., 2010; Hall et al., 2014). Pericyte density, which is highest in the CNS, is reduced in diabetes (Prakash et al., 2012). Diminished pericyte density may reduce the Ang1/Tie2 signal of pericyte-EC crosstalk and occludin expression of ECs, which can reduce the number of tight junctions in the BBB and increases vascular permeability (Hirase et al., 1997; Hori, Ohtsuki, Hosoya, Nakashima, & Terasaki, 2004). Moreover, the ratio of non-perfused vasculature to total vasculature in CNS is elevated in diabetes, as is VEGF expression (Prakash et al., 2012). Pathological secretion of VEGF by pericytes has recently been demonstrated to facilitate disruption of the BBB during stroke, which contributes and worsens the progression of stroke (Bai et al., 2015). Furthermore, ROS have been described to worsen ischaemic stroke. Under pathological conditions, pericytes are able to produce high amounts of ROS, which are also known to be elevated under diabetic conditions.

Oxidative stress and ROS production is suggested to be responsible for cerebral pericyte loss in diabetes (Shah, Morofuji, Banks, & Price, 2013). Additionally, it has been shown that ROS increase intracellular Ca^{2+} which leads to contraction of brain pericytes, especially in response to hypoxia, so the perfusion deficit in an ischaemic event is even more prolonged (Ergul, Valenzuela, Fouda, & Fagan, 2015; Yemisci et al., 2009). Pericyte constriction upon ischemia eventually leads to pericyte death which further exacerbates ischaemic injury (Ergul et al., 2015; Hall et al., 2014). However, whether this particular process of prolonged pericyte constriction is enhanced under diabetic conditions needs to be investigated.

2.2.3 Coronary heart disease

The importance of cardiac pericytes has long been disregarded, even though Tilton et al. (1979) confirmed their presence over 30 years ago in rat hearts. Pinto et al. (2016) demonstrated that pericytes account for around 5% of non-cardiomyocyte cardiac cells, using fluorescence-activated cell sorting to robustly identify pericytes. Pericytes were also confirmed in the whole coronary system, even in large arteries and veins by studying human explant hearts (Juchem, Weiss, Hagl, & Nees, 2013). Interestingly, pericytes were found to coat pre-capillary arterioles in the absence of vSMC and these pericytes are interconnected, which suggests an important barrier function and role in blood flow regulation (Nees, Weiss, & Juchem, 2013). As with pericytes from other tissues, cardiac pericytes have been also shown to exhibit MSC-like properties and angiogenic potential under conditions of hypoxia, but which appear more pronounced than pericytes of other origins (Chen et al., 2015). Moreover, Chen et al. (2015) demonstrated that cardiac pericytes have no skeletal myogenic potential but some may show a cardiomyogenic potential, which may point towards a specific role of pericytes in the heart, compared to pericytes in other tissues, which already has been proposed by Tilton et al. (1979) based on the different shape of secondary pericyte processes. Further evidence that cardiac pericytes have a particular role in myocardial homeostasis, different from pericytes in other vascular beds, comes from the use of Sunitinib, a receptor tyrosine kinase inhibitor, which is used as anti-cancer drug and was reported to be cardiotoxic by inducing coronary microvascular dysfunction and pericyte loss in the heart. However, Sunitinib may not affect pericytes of other origin to such an extent, which implies a possible cardiac specific property of these pericytes (Chintalgattu et al., 2013).

The exact function of cardiac pericytes has not yet been revealed and extensive studies are necessary to address this important topic. Nevertheless, pericytes are suggested to be involved in coronary no-reflow, an impairment of adequate microvascular reperfusion after myocardial infarction (MI) treatment (O'Farrell & Attwell, 2014). As in stroke, sustained pericyte constriction may worsen MI outcome.

With respect to diabetes, no studies have been performed to assess whether pericyte biology is altered in the diabetic heart, which may further elaborate on the wider role of pericytes in diabetes-associated vascular disease. However, PDGFB/PDGFRβ signalling, which is dysregulated by diabetes in other vascular beds was shown to play a role in cardiac development (Bjarnegard et al., 2004), suggesting that diabetes could affect cardiac pericyte biology.

3 Summary

There is clear evidence that pericytes are important in the pathogenesis of at least some diabetes-associated vascular complications (**Figure 2**). It is now apparent that pericytes are not just passively altered by diabetes (e.g. hyperglycaemia induced apoptosis), but also contribute actively to progression of vascular dysfunction. Pericytes secrete pro-angiogenic factors such as VEGF and TGFβ, initiate neovascularization, contribute to basement membrane thickening and worsen ischaemia by vasoconstriction. In other words, pericyte dysfunction is a major contributor to disease progression. Additionally, the literature clearly suggests the involvement of pericytes in macrovascular complications such as PVD and ischaemic stroke. This finding is interesting as it underlines the importance of the microvasculature in tissues and organ systems affected by macrovascular disease. However, further research is needed to fully understand pericyte dysfunction, how pericytes contribute to vascular disease, and finally, how pericyte biology can be a potential therapeutic target to prevent diabetes-associated vascular disease.

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Figure legends

Figure 1: Pericyte-endothelial cell signalling during angiogenesis, pericyte recruitment and in quiescent vessel. a) In health, during angiogenesis, Ang2 secretion blocks Tie2 receptor and inhibits Ang1 signal for pericyte detachment. Pericyte recruitment including mesenchymal cell differentiation and mural cell proliferation and migration is PDGFB/PDGFRβ, HB-EGF/EGFR and TGFβ/Alk1 driven, whereas pericyte coverage in a maturing and quiescent vessel is mainly maintained by TGFβ/Alk5 and Ang1/Tie2 signalling. **b)** In diabetes, VEGF and Ang2 expression is increased, PDGFRβ is inactivated and enhanced TGFβ signalling promotes excessive basement membrane molecule deposition, altogether contributing to microvascular instability and pericyte detachment. Ang - Angiopoietin , Tie - Tie tyrosine kinase receptor, PDGFB - platelet-derived growth factor B, PDGFRβ - PDGF receptor β, HB-EGF - heparin binding epidermal growth factor, EGFR - EGF receptor, TGFβ - transforming growth factor β, Alk - type 1 transmembrane serine/threonine kinase receptor, VEGF - vascular endothelial growth factor. (Geraldes et al., 2009; Hellstrom et al., 1999; Isono et al., 2002; Lim et al., 2004; Maisonpierre et al., 1997; Pardali et al., 2010; Shimizu, Sano, Haruki, et al., 2011; Stratman et al., 2010)

Figure 2: Hyperglycaemia and hyperinsulinaemia induced changes in pericyte biology. Hyperglycaemia alters pericyte function and phenotype, induces pericyte apoptosis leading to pericyte loss and impaired neovascularization, moreover hyperglycaemia contributes to endothelial dysfunction, which further adds to pericyte loss and impaired neovascularization. However, pericytes themselves play an active role in this pathogenesis (red), by secretion of pro-angiogenic factors, fibronectin, and collagen IV, thereby contributing to basement membrane thickening, neovascularisation and pericyte apoptosis. To which extend hyperinsulinaemia is conducive to pericyte loss remains elusive. (Durham et al., 2015; Geraldes et al., 2009; Ishii et al., 1996; Isono et al., 2002; Lim et al., 2004)