PDGF and Vessel Maturation

7

Carina Hellberg, Arne Östman, and C.-H. Heldin

Abstract Pericytes are smooth muscle-like cells found in close contact with the endothelium in capillaries, where they regulate the morphology and function of the vessels. During vessel formation, platelet-derived growth factor-BB (PDGF-BB) is required for the recruitment and differentiation of pericytes. Tumor vessels display abnormal morphology and increased endothelial proliferation, resulting in leaky, tortuous vessels that are often poorly perfused. These vessels typically display decreased pericyte density, and the tumor-associated pericytes often express abnormal markers and show abnormal morphology. Anti-angiogenic therapy targeting pro-angiogenic growth factor pathways has been applied to a broad range of solid tumors with varying results. Studies utilizing mouse models indicate that the presence of pericytes protect endothelial cells against inhibition of vascular endothelial growth factor (VEGF) signaling. Simultaneous inhibition of PDGF receptors on pericytes therefore improves the effect of VEGF inhibitors on endothelial cells and enhances anti-angiogenic therapy.

C.-H. Heldin (✉)
Ludwig Institute for Cancer Research,
Uppsala University, S-751 24, Uppsala, Sweden
e-mail: C-H.Heldin@LICR.uu.e

7.1 Introduction

Newly formed capillaries are stabilized through the recruitment of a specialized form of mural cells termed pericytes. In addition to providing physical stabilization, the presence of pericytes reduces endothelial cell proliferation and promotes differentiation, a process termed vascular maturation. During this process, activation of the platelet-derived growth factor (PDGF) β-receptor plays a crucial role in the recruitment of pericytes to the newly formed vessels. In the present communication, we review the role of PDGF in vessel maturation.

7.2 The PDGF Family

The PDGF family of growth factors is composed of disulfide-bonded homodimers of four polypeptide chains, the classical PDGF-A and -B chains and the more recently described PDGF-C and -D chains (Fredriksson et al. 2004). In addition, the A and B chains heterodimerize to form PDGF-AB. PDGF isoforms exert their biological effects through the

R. Liersch et al. (eds.), *Angiogenesis Inhibition,* Recent Results in Cancer Research,
DOI: 10.1007/978-3-540-78281-0_7, © Springer Verlag Berlin Heidelberg 2010

activation of two tyrosine kinase receptors, PDGF α- and β-receptors (Heldin et al. 1998).

PDGF family are major mitogens for a number of cell types, including mesenchymal cells such as fibroblasts and smooth muscle cells. During the embryonal development, PDGF isoforms are important for the development of mesenchymal cells of different organs, such as mesangial cells of the kidney, alveolar smooth muscle cells of the lung, smooth muscle cells and pericytes of blood vessels, and glial cells of the central nervous system. Overactivity of PDGF has been linked to atherosclerosis, fibrotic diseases, and malignancies. In certain types of rather rare solid tumors, PDGF is involved in autocrine stimulation of tumor cell growth. In addition, PDGF is commonly involved in paracrine recruitment of tumor stroma fibroblasts and stimulation of angiogenesis (Ostman and Heldin 2007).

7.3 Pericytes

7.3.1 Role of Pericytes

Mature blood vessels are composed of endothelial cells and mural cells, including pericytes and smooth muscle cells. Arteries and veins are surrounded by vascular smooth muscle cells, whereas pericytes are present on capillaries, postcapillary venules, and collecting venules throughout the body. Pericytes are smooth muscle-like single cells found in close contact with endothelial cells within the basement membrane, where they are wrapped around the vessel sending out long protrusions that make contact with a number of endothelial cells and other pericytes (Fig. 7.1) (Bergers and Song 2005). The presence of pericytes on these vessel types is plastic, and varies between tissues.

PDGF made by endothelial cells has a crucial role in the recruitment of pericytes to vessels (Betsholtz 2004). Moreover, a basic retention motif in the common C-terminus of PDGF-BB is crucial for this process, since it makes contact with sulfated heparan proteoglycans and ensures that PDGF-BB remains in the close environment of the producing endothelial cells (Lindblom et al. 2003; Abramsson et al. 2007). Since pericytes are contractile cells they presumably exert parts of their morphogenic control of capillary diameter through PDGF-BB-induced pericyte contractility. In addition, pericytes also regulate capillary diameter by regulating endothelial proliferation and differentiation. Absence of pericytes in PDGF-B/PDGF β-receptor null mice coincides with endothelial hyperplasia, suggesting that pericytes negatively control endothelial proliferation (Hellstrom et al. 2001). Absence of pericytes also leads to defects in endothelial junction formation, suggesting that pericytes control endothelial differentiation in vivo.

7.3.2 Identification of Pericytes

Despite their physiological importance, pericytes still remain an understudied cell type. Part of the problem when studying pericytes is the difficulty in identifying them, since there is no convenient pan-pericyte marker. Pericytes reside within the basement membrane; thus morphological identification by electron microscopy remains the most reliable means of identifying a pericyte in mature tissues (Baluk et al. 2005). However, during angiogenic sprouting and vascular remodeling, the basement membrane is not fully developed, making this method of identification more difficult to apply (Baluk et al. 2003). Therefore, several marker proteins are used for studying pericytes during angiogenesis (Bergers and Song 2005).

As expected from its importance for the recruitment of pericytes, the PDGF β-receptor is one of the most widely used markers for pericytes. However, this receptor is also expressed

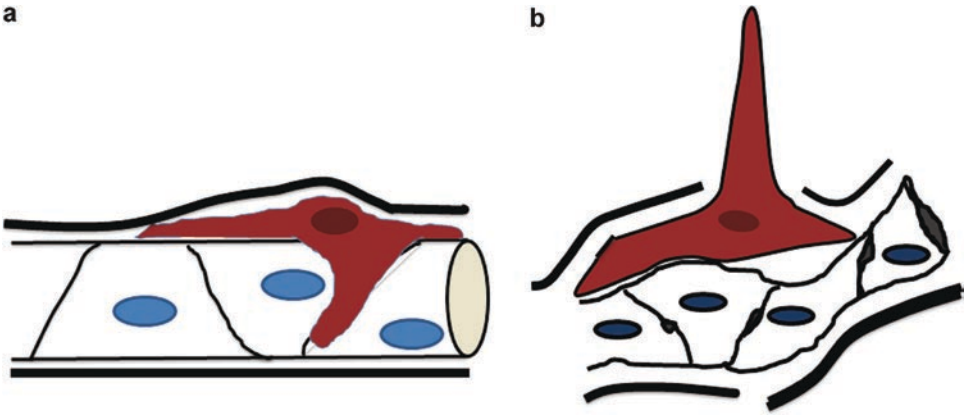

Fig. 7.1 (**a**) Morphology of capillaries of normal tissues. The pericytes reside between the basement membrane and the endothelial cells. Each pericyte makes contact with several endothelial cells and wraps itself around the vessel. (**b**) Morphology of capillaries of tumors. The tumor vessels are contorted and often flattened. Pericytes make contact with the endothelium, but are often partly dethatched and often extend protrusions away from the vessel. The basement membrane varies in thickness and has gaps in it.

on other stromal cells such as smooth muscle cells, fibroblasts and myofibroblasts. Another commonly used marker is α-smooth muscle actin (ASMA) that is expressed by pericytes, smooth muscle cells, and myofibroblasts. The expression of ASMA by pericytes is primarily restricted to sites of vascular remodeling. Desmin is a component of intermediate filaments, and is found in mature skeletal, cardiac, and smooth muscle cells. On pericytes, its expression appears to be restricted to differentiated cells in close physical contact with the endothelium. The expression of ASMA and desmin is likely to reflect the role of pericytes as contractile cells that participate in the regulation of capillary blood flow. The NG2 chondroitin sulfate proteoglycan (also known as high-molecular-weight melanoma-associated antigen, or sometimes AN2 in mice) is expressed on the surface of activated pericytes during vasculogenesis and angiogenesis. The regulator of G-protein signaling-5 (RGS-5), a regulator of signaling pathways downstream of heterotrimeric G-protein coupled receptors, was recently described as a marker for developing pericytes. Its expression overlaps with the expression of NG2 and the PDGF β-receptor.

Not much is known about the regulation of the expression of these markers, and they may represent either distinct or overlapping populations of pericytes. The expressions of pericyte markers are dynamic, and their expression varies, depending on the species and tissue studied. When addressing the involvement of pericytes in vessel maturation, the difficulty to identify pericytes still present a problem, and most studies therefore contain the analysis of several marker proteins.

7.3.3 The Origin of Pericytes

During embryonic development, pericytes and smooth muscle cells, as well as endothelial cells, are believed to be derived from mesenchymal precursors (Bergers and Song 2005). During this process, PDGF-BB/PDGF β-receptor signaling is essential for the recruitment and differentiation of pericytes. Postnatally, pericytes are

presumed to either migrate with the endothelial sprouts during the initiation of angiogenesis, or to differentiate from a local source of mesenchymal cells in response to PDGF and/or transforming growth factor β (TGF-β). However, recent studies have also described the existence of bone marrow-derived pericyte progenitor cells (Lamagna and Bergers 2006) and tissue-specific pericyte progenitors (Howson et al. 2005; Dore-Duffy et al. 2006; Tamaki et al. 2007).

The adult bone marrow contains both hematopoietic and mesenchymal stem and progenitor cells. Therefore, the recruitment of bone marrow-derived progenitors into tumors supports the initiation of tumor angiogenesis by incorporating endothelial and pericyte progenitor cells into the newly formed vessels. It has been proposed that the release of cytokines from tumors induces the mobilization of hematopoietic bone marrow stem cells (Petit et al. 2007). The recruitment of endothelial progenitor cells and their incorporation into tumor vessels have been demonstrated in a number of studies (Lyden et al. 2001; Shirakawa et al. 2002), and was recently shown to have an important role in the vascularization of at least two types of lung metastases. Circulating progenitor cells positive for PDGF β-receptor, the stem cell antigen-1 (Sca-1) and CD11b have been demonstrated to incorporate into blood vessels and to mature into NG2-, ASMA- and desmin-positive pericytes (Song et al. 2005). Circulating cells positive for CD11b and CD45 have also been shown to incorporate into vessels as pericyte-like cells, although these cells only expressed NG2 (Rajantie et al. 2004).

Several studies have also demonstrated the presence of pericyte progenitor cells in various other tissues, which appear to contribute to postnatal vasculogenesis and angiogenesis. Since these progenitor cells give rise to cell populations expressing different pericyte markers (see later in the text), it is unclear as to what extent the presence of these cells on vessels stabilizes the vascular function. Thus, the neonatal rat aorta contains cells positive for the PDGF β-receptor, Tie2 and CD34, but negative for endothelial markers (Howson et al. 2005). These cells differentiates into pericytes when cocultured with endothelial cells or aorta ring explants in vitro. In the microvasculature of the adult rat CNS, cells positive for nestin and NG2 that has the capacity to differentiate into pericyte-like cells in vitro were identified (Dore-Duffy et al. 2006). In mice, skeletal muscle-derived stem cells negative for CD31 and CD45 were reported to differentiate into pericytes, endothelial cells, and smooth muscle in vivo (Tamaki et al. 2007). Although these studies indicate the presence of cells with the capability to differentiate into pericytes in adult tissues, most of the studies were performed in vitro. Information is still scarce about differentiation of tissue pericytes in vivo during vascular remodeling, or during tumor angiogenesis. Further studies addressing the physiological function of vessels that contain pericytes recruited from different sources are required.

7.4 Vessel Maturation

7.4.1 Normal Vessels

During embryonic development, the nascent vascular network is formed through de novo vessel formation from angioblasts or stem cells, a process termed vasculogenesis. From these vessels, new vessels sprout and form bridges by angiogenesis. The vessels are then stabilized through the recruitment of mural cells, and through generation of the basal membrane. The final patterning of the vascular network is determined by signals provided by soluble angiogenesis factors as well as components of the basement membrane and ECM, which stimulates

proliferation, survival, migration, and differentiation of endothelial and mural cells. As already described, PDGF-BB is secreted by the endothelial cells during angiogenesis, presumably in response to vascular endothelial growth factor (VEGF), which facilitates the recruitment of mural cells. The endothelial differentiation sphingolipid G-protein coupled receptor 1 (EDG1 or $S1P_1$) (Allende and Proia 2002), which is activated by sphingosine 1-phosphate, appears to be important for mural-cell migration, and its genetic ablation in mouse led to a similar phenotype as that of PDGF ko mice. It remains to be investigated whether these signals are unrelated or if the EDG1 receptor signals downstream of the PDGF β-receptor.

Angiopoietin (Ang) 1 and 2 both act through the Tie2 receptor, but with different outcomes (Morisada et al. 2006). Ang1 stabilizes the physical contacts between endothelial cells and pericytes, thereby making the vessels less leaky. The role of Ang2 appears to be contextual. When VEGF is absent, Ang2 destabilizes vessels by inhibiting Ang1 signaling, but in the presence of VEGF Ang2 facilitates vascular sprouting. TGF-β is expressed by both endothelial and mural cells. It promotes vessel maturation not only by inducing differentiation of mesenchymal cells to mural cells, but also by stimulating ECM production. As with Ang2, TGF-β can be either pro- or antiangiogenic, depending on the context (Bertolino et al. 2005).

The presence of pericytes on the capillary bed is necessary for normal vessel function. Genetic studies in mice showed that loss of PDGF-BB or the PDGF β-receptor leads to a severe deficiency in pericyte recruitment, causing microvascular leakage and hemorrhage (Lindahl et al. 1997; Hellstrom et al. 1999). When investigating animals chimaeric for the PDGF β-receptor, it was evident that only PDGF β-receptor positive cells populated the vascular smooth muscle and pericyte compartment, directly demonstrating a need for PDGF signaling for the development of these cells (Crosby et al. 1998).

During angiogenic sprouting, PDGF-B is expressed by the endothelial tip cell (Gerhardt et al. 2003). Tissue-specific knockout of PDGF-BB in endothelial cells resulted in a similar phenotype as that of PDGF β-receptor ko, indicating that paracrine signaling between the endothelium and pericytes is required in the process of pericyte recruitment (Bjarnegard et al. 2004). When examining animals chimaeric for endothelial PDGF-BB, they displayed a variation in pericyte coverage and morphology of individual brain capillaries indicative of segments of PDGF-BB, expressing endothelium with normal recruitment of pericytes (Bjarnegard et al. 2004). The PDGF-BB molecule contains a short, basic sequence that functions as a retention motif allowing the secreted growth factor to remain on the tip cell. Mutational loss of the retention sequence resulted in partial detachment of the pericytes from the angiogenic sprout, presumably due to diffusion of PDGF-BB into the surrounding tissue (Lindblom et al. 2003). These findings highlight the need for a paracrine PDGF signal and a physical contact with endothelial cells for pericyte recruitment and differentiation.

Although PDGF β-receptor ko mice die from bleedings due to microanuerysms and a lack of mural cells, loss of pericytes in all tissues is not complete (Hellstrom et al. 1999). It thus appears as if PDGF is critical for the proliferation and migration of both pericytes and vascular smooth muscle cells, but that the initial formation of these cell types can also be induced by other factors. It is presently unclear where these cells originate from and what are the factors that induce their differentiation. It has been proposed that endothelial cells only express PDGF-BB at the sites of the vessels where pericyte recruitment occurs (Lindahl et al. 1997; Hellstrom et al. 1999). This is supported by the finding that in the developing CNS, PDGF-BB is mainly expressed by the endothelial cells situated at the tip of the sprouting vessels (Gerhardt et al. 2003).

7

7.4.2 Tumor Vessels

The growth of large, solid tumors is directly dependent on the presence of a tumor vasculature (Folkman 1971). As the tumor grows, hypoxia induces the expression of VEGF by tumor cells. This induces angiogenesis by activating the endothelial cells on surrounding vessels, thereby stimulating the branching and growth of new blood vessels into the tumor (Bergers and Benjamin 2003) (Fig. 7.2). During this process, the connections between the endothelial cells are loosened. Matrix metalloproteases are activated, leading to the degradation of the basement membrane. This serves two purposes. First, it allows the detachment of both endothelial cells and pericytes from the basement membrane, facilitating their migration into the tumor. Second, it may also activate proangiogenic factors within the basement membrane. Endothelial cells and pericytes migrate into the tumor tissue, where they proliferate and reconnect to form new vessels. Although most studies suggest that the endothelial cells lead the formation of new vessels, there are also studies suggesting that the pericytes migrate into the tissue first.

Tumor blood vessels have a number of structural and functional abnormalities (Jain 2003). They are dynamic, and vessels are continuously being initiated, remodeled, and regressed. The vessels are irregular in size and shape, tortuous and lack the normal hierarchical arrangement of arterioles, capillaries, and venules. The structure of the vessel wall is also abnormal. The tumor vessel diameter varies greatly, and the endothelial cells form an imperfect lining and contain a large number of fenestrations (Baluk et al. 2005). Although the presence of a basement membrane surrounding tumor vessels have been reported, it appears to be morphologically and functionally altered due to the vessel remodeling (Baluk et al. 2003). As a result of the

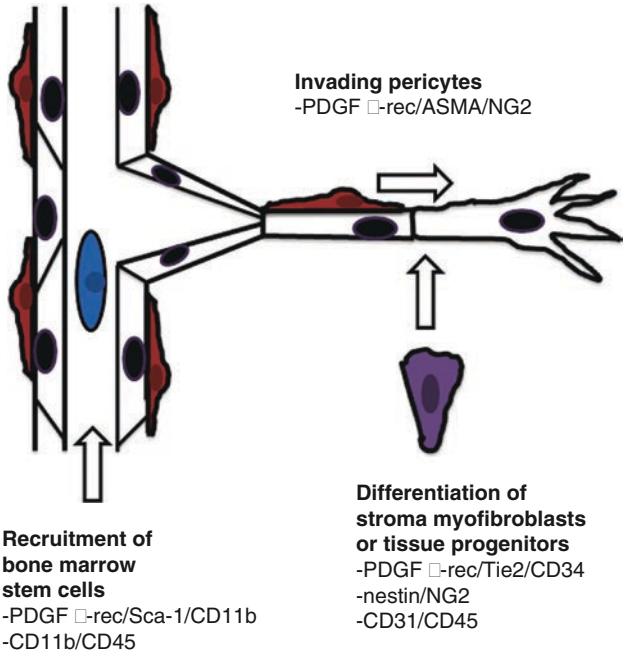

Fig. 7.2 Angiogenic sprouting. A new capillary is formed as a tip cell leads the sprouting capillary into the tissue. Pericytes may be recruited through the invasion and proliferation of pericytes from the mother vessel, by differentiation of stromal precursors, or through the recruitment of bone marrow precursor cells. Different molecular markers described for pericytes derived from mother vessels (Gerhardt et al., 2003), bone marrow-derived progenitors (Song et al., 2005; Rajantie et al., 2004, respectively) and tissue progenitors (Dore-Duffy et al., 2006; Howson et al., 2005; Tamaki et al., 2007, respectively) are given.

abnormal organization and ultrastructure of the vessels, the blood flow in the tumor vessels is chaotic and the vessels are leaky (Hashizume et al. 2000), resulting in an increase the interstitial fluid pressure (IFP) in the tumor. Furthermore, because of continuous remodeling of the vasculature, the blood flow and permeability varies not only between tumors, but also between regions of the same tumor, between the same region over time, and between the tumor and metastases. This is at least partly due to an imbalance between various pro- and antiangiogenic factors such as VEGF and Ang1/2. Taken together, the vessel abnormalities render the tumor vasculature inefficient at delivering not only oxygen and nutrients, but also drugs.

The density of pericytes covering normal capillaries varies between tissues, which is the case also for the pericyte coverage of tumor vessels (Baluk et al. 2005). In some studies no pericytes were found around the tumor capillaries, whereas other studies found the vessels covered to varying extents. It is unclear as to what extent these variations are due to differences between the tissues or the tumor types studied. Since PDGF-BB expression by endothelial cells is heterogeneous (Hellstrom et al. 1999), this may explain the uneven pericyte coverage of tumor vessels. Also, as discussed earlier, there may also be differences in the expression of the various molecular markers between tumor types, making an accurate pericyte count difficult. It is not just the pericyte density that is abnormal. Tumor-associated pericytes often display both an abnormal expression of markers, as well as abnormal morphology (Baluk et al. 2005). In tumors, pericytes are often found to express both ASMA and NG2, which are known to be expressed during vascular remodeling. Where normal pericytes are in close contact with the endothelium and reside within the basement membrane, tumor pericytes are often loosely associated with the endothelium, and may extend long protrusions into the surrounding tissue. At present, it is not clear whether the cells displaying the abnormal morphology are truly pericytes, if they are in the process of differentiation from myofibroblasts, or if they represent pericytes that are partially detaching from the endothelium to divide. Furthermore, it is unclear as to what extent these pericytes are able to participate in vessel function.

There are several sources described for pericyte progenitors during normal vascular remodeling, but it is currently unclear where tumor pericytes originate from. In angiogenesis in the retina, pericytes have been shown to migrate along the angiogenic sprout in response to the PDGF-BB expressed by the endothelial cells (Gerhardt et al. 2003). In tumors, it is also possible that components of the tumor microenvironment induce the differentiation of fibroblasts into myofibroblasts. Such cues may be TGF-β or PDGF expressed by tumor cells or the surrounding stroma. The incorporation of bone marrow-derived circulating pericyte progenitors into tumors have also been described (Song et al. 2005). The relative importance of these sources of pericytes is yet to be determined.

7.5 Tumor Therapy Targeting PDGF Receptors on the Vasculature

The genetic instability of tumor cells poses a serious problem when developing specific drugs targeting mutated proteins in tumors. Tumors that initially respond well to targeted therapies often develop resistance to the therapy, partly because of the enrichment of cells with new mutations. It has been proposed that targeting of tumor stroma, tumor vessels, and fibroblasts could be a means to avoid drug resistance since these tissues are more genetically stable (Hofmeister et al. 2008). Given the importance of PDGF in tumor stroma formation, the PDGF receptors are interesting targets in antistromal therapy.

7.5.1 Antiangiogenic Therapy Targeting Pericytes

Targeting of tumor angiogenesis has long been an attractive idea for the treatment of solid tumors (Folkman 1971; Cao 2004). Although therapies targeting the VEGF pathway are efficient in mouse tumor models, the results of several clinical trials suggests that therapies targeting VEGF alone may not be enough for efficient antiangiogenic therapy (Cobleigh et al. 2003; Yang et al. 2003).

When revisiting the mouse models, studies indicated that the response to VEGF-targeting therapy may be dependent on the maturity of the tumor vessels. Established, mature tumor vessels with richer pericyte coverage appear to be less sensitive to the antiangiogenic therapy than vessels with fewer pericytes. The notion that PDGF-BB may protect endothelial cells from antiangiogenic therapy was further supported by a study by Huang et al. (Huang et al. 2004), where an increased expression of PDGF-BB was detected around vessels that became resistant to anti-VEGF therapy. These observations initiated the idea that antiangiogenic therapy may be more efficient if both endothelial cells and pericytes are targeted using both VEGF receptor and PDGF receptor kinase inhibitors. This notion has recently been corroborated in a study of vessels in a nonmalignant tissue, chicken chorioallantoic membranes, displaying different stages of vascular maturity during development (Hlushchuk et al. 2007). In tumors, this type of combination therapy have been shown to give synergistic antiangiogenic and antitumor effects (Bergers et al. 2003; Erber et al. 2004; Hasumi et al. 2007), although the magnitude of the effect of PDGF inhibitors on the vasculature may be contextual (Sennino et al. 2007). It is currently not known why tumor pericytes are targeted during this treatment, while the vast majority of mural cells in the body are unaffected. It is possible that only a subset of abnormal, activated pericytes that are in the process of being remodeled, are targeted while more mature subsets are unaffected (Hasumi et al. 2007).

7.5.2 Improving the Efficacy of Conventional Therapies

As a consequence of the poor perfusion and abnormal functions of tumor vessels, the delivery of nutrients and oxygen into the tumor is poor (Jain 2005). This also affects the therapeutic outcome of both chemotherapy and radiotherapy. In the clinic, hypoxia strongly correlates with tumor radioresistance (Moeller et al. 2007), and preclinical data showed that increased tumor oxygenation improved the response to radiation therapy. Different approaches to modify the tumor vasculature have been taken to correct for this problem.

The abnormal tumor vasculature and stroma results in an elevated IFP in tumors compared to normal tissues. In addition to forming a barrier to transcapillary transport of nutrients and oxygen, the elevated IFP also results in inefficient uptake of therapeutic agents, especially macromolecules such as liposomes and antibodies (Heldin et al. 2004). Thus, lowering of IFP could be used to improve the therapeutic efficiency of both chemotherapy and radiation therapy. There are several factors affecting the IFP in tumors. The excess VEGF produced by hypoxic tumor cells induce capillary leakiness, resulting in an increased outflow of plasma proteins into the interstitium. Tumors are typically void of functional lymph vessels, which impair the fluid drainage from the tumor. Also, fibrosis and contraction of interstitial matrix mediated by stroma fibroblasts participates in the elevation of tumor IFP. PDGF contributes to the regulation of IFP through the phosphatidylinositol-3’ kinase (PI3K) signaling pathway (Heuchel et al. 1999). Activation of PDGF β-receptors on stromal fibroblasts induce αvβ3 intergrin-mediated contraction of the extracellular matrix thereby controlling the dermal IFP (Liden et al. 2006). The PDGF

β-receptor may also participate in the regulation of vessel leakiness by recruiting pericytes that stabilizes the capillaries (Lindahl et al. 1997). Lowering of IFP by VEGF inhibitors (Lee et al. 2000; Tong et al. 2004) as well as PDGF inhibitors (Pietras et al. 2001) have been demonstrated. At present, it is not clear if the PDGF inhibitors reduce IFP by acting on pericytes or fibroblasts, or on both cell types. The reduction in IFP has been correlated to increased drug uptake (Pietras et al. 2001; Willett et al. 2004) and increased effect of chemotherapy (Pietras et al. 2002).

It has been proposed that restoring the balance between pro-and antiangiogenic factors could temporary normalize the vasculature, allowing for drug delivery (Jain 2005). According to this notion, removal of VEGF signaling would target the immature vasculature, pruning the less functional vessels and promoting the blood flow through the remaining vessels. Part of the normalization of vessel function seen after anti-VEGF therapy can be explained by decreased vessel leakiness, which reduces the osmotic pressure of the interstitium and consequently reduces the tumor IFP. The reported effects of anti-VEGF therapy have been transient, which would be expected if prolonged treatment disrupt also mature vessel function. Studies have demonstrated that targeting VEGF receptors alters vessel morphology (Miller et al. 2005; Taguchi et al. 2008), with temporary improvements in tumor oxygenation and response to radiotherapy (Dings et al. 2007). Another study demonstrated decreased effects of chemotherapy on glioblastomas, presumably due to restoration of the blood–brain barrier (Claes et al. 2008).

7.6 Future Perspectives

It is increasingly clear that the properties of the tumor vasculature are important for the outcome of both antiangiogenic targeted therapies and conventional cancer therapies. The presence of pericytes on the vessels gives structural stability to capillaries, resulting in improved perfusion, and provides endothelial cells with protection to anti-angiogenic therapy targeting the VEGF pathway. Understanding the precise mechanisms underlying vascular maturation should provide cues for refining therapies targeting the vasculature, whether to inhibit tumor angiogenesis or to normalize the vascular function for more efficient drug delivery. To achieve this, further studies evaluating the effects of antiangiogenic inhibitors on vessel function, both in animal models and in patients, are needed. The approval of several PDGF receptor inhibitors for use in the clinic (Ostman and Heldin 2007) will be important for further validation of the concept of vascular normalization after antiangiogenic therapy and the concomitant increase in drug uptake in patients. Such studies should reveal the extent to which different tumor types vary regarding their response to the combined targeting of VEGF- and PDGF receptor signaling, and may provide markers for the prediction of the therapeutic outcome in subsets of tumors.

The pericytes on tumor vessels are abnormal, both in the expression of molecular markers and in morphology. Nevertheless, B16 mouse melanoma tumors expressing PDGF-BB display increased pericyte coverage of the vessels, and an increased growth rate (Furuhashi et al. 2004), which correlates to an increased perfusion of small vessels and increased blood-flow rate (Robinson et al. 2008), indicating that the presence of abnormal pericytes exert effects on tumor vessel function. So how do these pericytes differ from the function of a fully differentiated pericyte? Further studies are required to understand the molecular mechanisms underlying pericyte differentiation and their interactions with endothelial cells. It will also be important to determine the source of tumor pericytes and the signaling pathways involved in their recruitment into the tumor vasculature. These studies require further development of more refined methods for studying vessel function in vivo.

7

References

Abramsson A, Kurup S, Busse M, Yamada S, Lindblom P, Schallmeiner E, Stenzel D, Sauvaget D, Ledin J, Ringvall M, Landegren U, Kjellen L, Bondjers G, Li JP, Lindahl U, Spillmann D, Betsholtz C, Gerhardt H (2007) Defective N-sulfation of heparan sulfate proteoglycans limits PDGF-BB binding and pericyte recruitment in vascular development. Genes Dev 21:316–331

Allende ML, Proia RL (2002) Sphingosine-1-phosphate receptors and the development of the vascular system. Biochim Biophys Acta 1582: 222–227

Baluk P, Hashizume H, McDonald DM (2005) Cellular abnormalities of blood vessels as targets in cancer. Curr Opin Genet Dev 15:102–111

Baluk P, Morikawa S, Haskell A, Mancuso M, McDonald DM (2003) Abnormalities of basement membrane on blood vessels and endothelial sprouts in tumors. Am J Pathol 163: 1801–1815

Bergers G, Benjamin LE (2003) Tumorigenesis and the angiogenic switch. Nat Rev Cancer 3: 401–410

Bergers G, Song S (2005) The role of pericytes in blood-vessel formation and maintenance. Neuro-Oncol 7:452–464

Bergers G, Song S, Meyer-Morse N, Bergsland E, Hanahan D (2003) Benefits of targeting both pericytes and endothelial cells in the tumor vasculature with kinase inhibitors. J Clin Invest 111:1287–1295

Bertolino P, Deckers M, Lebrin F, ten Dijke P (2005) Transforming growth factor-beta signal transduction in angiogenesis and vascular disorders. Chest 128:585S–590S

Betsholtz C (2004) Insight into the physiological functions of PDGF through genetic studies in mice. Cytokine Growth Factor Rev 15:215–228

Bjarnegard M, Enge M, Norlin J, Gustafsdottir S, Fredriksson S, Abramsson A, Takemoto M, Gustafsson E, Fässler R, Betsholtz C (2004) Endothelium-specific ablation of PDGFB leads to pericyte loss and glomerular, cardiac and placental abnormalities. Development 131:1847–1857

Cao Y (2004) Antiangiogenic cancer therapy. Semin Cancer Biol 14:139–145

Claes A, Wesseling P, Jeuken J, Maass C, Heerschap A, Leenders WP (2008) Antiangiogenic compounds interfere with chemotherapy of brain tumors due to vessel normalization. Mol Cancer Ther 7:71–78

Cobleigh MA, Langmuir VK, Sledge GW, Miller KD, Haney L, Novotny WF, Reimann JD, Vassel A (2003) A phase I/II dose-escalation trial of bevacizumab in previously treated metastatic breast cancer. Semin Oncol 30:117–124

Crosby JR, Seifert RA, Soriano P, Bowen-Pope DF (1998) Chimaeric analysis reveals role of Pdgf receptors in all muscle lineages. Nat Genet 18:385–388

Dings RP, Loren M, Heun H, McNiel E, Griffioen AW, Mayo KH, Griffin RJ (2007) Scheduling of radiation with angiogenesis inhibitors anginex and Avastin improves therapeutic outcome via vessel normalization. Clin Cancer Res 13:3395–3402

Dore-Duffy P, Katychev A, Wang X, Van Buren E (2006) CNS microvascular pericytes exhibit multipotential stem cell activity. J Cereb Blood Flow Metab 26:613–624

Erber R, Thurnher A, Katsen AD, Groth G, Kerger H, Hammes HP, Menger MD, Ullrich A, Vajkoczy P (2004) Combined inhibition of VEGF and PDGF signaling enforces tumor vessel regression by interfering with pericyte-mediated endothelial cell survival mechanisms. FASEB J 18:338–340

Folkman J (1971) Tumor angiogenesis: therapeutic implications. N Engl J Med 285:1182–1186

Fredriksson L, Li H, Eriksson U (2004) The PDGF family: four gene products form five dimeric isoforms. Cytokine Growth Factor Rev 15:197–204

Furuhashi M, Sjöblom T, Abramsson A, Ellingsen J, Micke P, Li H, Bergsten-Folestad E, Eriksson U, Heuchel R, Betsholtz C, Heldin CH, Östman A (2004) Platelet-derived growth factor production by B16 melanoma cells leads to increased pericyte abundance in tumors and an associated increase in tumor growth rate. Cancer Res 64: 2725–2733

Gerhardt H, Golding M, Fruttiger M, Ruhrberg C, Lundkvist A, Abramsson A, Jeltsch M, Mitchell C, Alitalo K, Shima D, Betsholtz C (2003) VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. J Cell Biol 161:1163–1177

Hashizume H, Baluk P, Morikawa S, McLean JW, Thurston G, Roberge S, Jain RK, McDonald DM (2000) Openings between defective endothelial cells explain tumor vessel leakiness. Am J Pathol 156:1363–1380

Hasumi Y, Klosowska-Wardega A, Furuhashi M, Östman A, Heldin CH, Hellberg C (2007) Identification of a subset of pericytes that respond to combination therapy targeting PDGF and VEGF signaling. Int J Cancer 121:2606–2614

Heldin CH, Rubin K, Pietras K, Östman A (2004) High interstitial fluid pressure – an obstacle in cancer therapy. Nat Rev Cancer 4:806–813

Heldin CH, Östman A, Rönnstrand L (1998) Signal transduction via platelet-derived growth factor receptors. Biochim Biophys Acta 1378:F79–F113

Hellström M, Gerhardt H, Kalen M, Li X, Eriksson U, Wolburg H, Betsholtz C (2001) Lack of pericytes leads to endothelial hyperplasia and abnormal vascular morphogenesis. J Cell Biol 153:543–553

Hellström M, Kalén M, Lindahl P, Abramsson A, Betsholtz C (1999) Role of PDGF-B and PDGFR-beta in recruitment of vascular smooth muscle cells and pericytes during embryonic blood vessel formation in the mouse. Development 126: 3047–3055

Heuchel R, Berg A, Tallquist M, Åhlen K, Reed RK, Rubin K, Claesson-Welsh L, Heldin CH, Soriano P (1999) Platelet-derived growth factor beta receptor regulates interstitial fluid homeostasis through phosphatidylinositol-3' kinase signaling. Proc Natl Acad Sci U S A 96:11410–11415

Hlushchuk R, Baum O, Gruber G, Wood J, Djonov V (2007) The synergistic action of a VEGF-receptor tyrosine-kinase inhibitor and a sensitizing PDGF-receptor blocker depends upon the stage of vascular maturation. Microcirculation 14:813–825

Hofmeister V, Schrama D, Becker JC (2008) Anticancer therapies targeting the tumor stroma. Cancer Immunol Immunother 57:1–17

Howson KM, Aplin AC, Gelati M, Alessandri G, Parati EA, Nicosia RF (2005) The postnatal rat aorta contains pericyte progenitor cells that form spheroidal colonies in suspension culture. Am J Physiol Cell Physiol 289:C1396–C1407

Huang J, Soffer SZ, Kim ES, McCrudden KW, New T, Manley CA, Middlesworth W, O'Toole K, Yamashiro DJ, Kandel JJ (2004) Vascular remodeling marks tumors that recur during chronic suppression of angiogenesis. Mol Cancer Res 2:36–42

Jain RK (2003) Molecular regulation of vessel maturation. Nat Med 9:685–693

Jain RK (2005) Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science 307:58–62

Lamagna C, Bergers G (2006) The bone marrow constitutes a reservoir of pericyte progenitors. J Leukoc Biol 80:677–681

Lee CG, Heijn M, di Tomaso E, Griffon-Etienne G, Ancukiewicz M, Koike C, Park KR, Ferrara N, Jain RK, Suit HD, Boucher Y (2000) Anti-vascular endothelial growth factor treatment augments tumor radiation response under normoxic or hypoxic conditions. Cancer Res 60:5565–5570

Liden A, Berg A, Nedrebo T, Reed RK, Rubin K (2006) Platelet-derived growth factor BB-mediated normalization of dermal interstitial fluid pressure after mast cell degranulation depends on beta3 but not beta1 integrins. Circ Res 98:635–641

Lindahl P, Johansson BR, Leveen P, Betsholtz C (1997) Pericyte loss and microaneurysm formation in PDGF-B-deficient mice. Science 277: 242–245

Lindblom P, Gerhardt H, Liebner S, Abramsson A, Enge M, Hellström M, Bäckstrom G, Fredriksson S, Landegren U, Nystrom HC, Bergström G, Dejana E, Ostman A, Lindahl P, Betsholtz C (2003) Endothelial PDGF-B retention is required for proper investment of pericytes in the microvessel wall. Genes Dev 17:1835–1840

Lyden D, Hattori K, Dias S, Costa C, Blaikie P, Butros L, Chadburn A, Heissig B, Marks W, Witte L, Wu Y, Hicklin D, Zhu Z, Hackett NR, Crystal RG, Moore MA, Hajjar KA, Manova K, Benezra R, Rafii S (2001) Impaired recruitment of bone-marrow-derived endothelial and hematopoietic precursor cells blocks tumor angiogenesis and growth. Nat Med 7: 1194–1201

Miller DW, Vosseler S, Mirancea N, Hicklin DJ, Bohlen P, Volcker HE, Holz FG, Fusenig NE (2005) Rapid vessel regression, protease inhibition, and stromal normalization upon short-term vascular endothelial growth factor receptor 2 inhibition in skin carcinoma heterotransplants. Am J Pathol 167:1389–1403

Moeller BJ, Richardson RA, Dewhirst MW (2007) Hypoxia and radiotherapy: opportunities for improved outcomes in cancer treatment. Cancer Metastasis Rev 26:241–248

Morisada T, Kubota Y, Urano T, Suda T, Oike Y (2006) Angiopoietins and angiopoietin-like proteins in angiogenesis. Endothelium 13:71–79

Östman A, Heldin CH (2007) PDGF receptors as targets in tumor treatment. Adv Cancer Res 97: 247–274

Petit I, Jin D, Rafii S (2007) The SDF-1-CXCR4 signaling pathway: a molecular hub modulating neo-angiogenesis. Trends Immunol 28:299–307

Pietras K, Östman A, Sjöquist M, Buchdunger E, Reed RK, Heldin CH, Rubin K (2001) Inhibition of platelet-derived growth factor receptors reduces interstitial hypertension and increases transcapillary transport in tumors. Cancer Res 61:2929–2934

Pietras K, Rubin K, Sjöblom T, Buchdunger E, Sjöquist M, Heldin CH, Östman A (2002)

Inhibition of PDGF receptor signaling in tumor stroma enhances antitumor effect of chemotherapy. Cancer Res 62:5476–5484

Rajantie I, Ilmonen M, Alminaite A, Ozerdem U, Alitalo K, Salven P (2004) Adult bone marrow-derived cells recruited during angiogenesis comprise precursors for periendothelial vascular mural cells. Blood 104:2084–2086

Robinson SP, Ludwig C, Paulsson J, Östman A (2008) The effects of tumor-derived platelet-derived growth factor on vascular morphology and function in vivo revealed by susceptibility MRI. Int J Cancer 122:1548–1556

Sennino B, Falcon BL, McCauley D, Le T, McCauley T, Kurz JC, Haskell A, Epstein DM, McDonald DM (2007) Sequential loss of tumor vessel pericytes and endothelial cells after inhibition of platelet-derived growth factor B by selective aptamer AX102. Cancer Res 67: 7358–7367

Shirakawa K, Shibuya M, Heike Y, Takashima S, Watanabe I, Konishi F, Kasumi F, Goldman CK, Thomas KA, Bett A, Terada M, Wakasugi H (2002) Tumor-infiltrating endothelial cells and endothelial precursor cells in inflammatory breast cancer. Int J Cancer 99:344–351

Song S, Ewald AJ, Stallcup W, Werb Z, Bergers G (2005) PDGFRbeta+ perivascular progenitor cells in tumours regulate pericyte differentiation and vascular survival. Nat Cell Biol 7:870–879

Taguchi E, Nakamura K, Miura T, Shibuya M, Isoe T (2008) Anti-tumor activity and tumor vessel normalization by the vascular endothelial growth factor receptor tyrosine kinase inhibitor KRN951 in a rat peritoneal disseminated tumor model. Cancer Sci 99(3):623–630

Tamaki T, Okada Y, Uchiyama Y, Tono K, Masuda M, Wada M, Hoshi A, Akatsuka A (2007) Synchronized reconstitution of muscle fibers, peripheral nerves and blood vessels by murine skeletal muscle-derived CD34(-)/45 (-) cells. Histochem Cell Biol 128:349–360

Tong RT, Boucher Y, Kozin SV, Winkler F, Hicklin DJ, Jain RK (2004) Vascular normalization by vascular endothelial growth factor receptor 2 blockade induces a pressure gradient across the vasculature and improves drug penetration in tumors. Cancer Res 64:3731–3736

Willett CG, Boucher Y, di Tomaso E, Duda DG, Munn LL, Tong RT, Chung DC, Sahani DV, Kalva SP, Kozin SV, Mino M, Cohen KS, Scadden DT, Hartford AC, Fischman AJ, Clark JW, Ryan DP, Zhu AX, Blaszkowsky LS, Chen HX, Shellito PC, Lauwers GY, Jain RK (2004) Direct evidence that the VEGF-specific antibody bevacizumab has antivascular effects in human rectal cancer. Nat Med 10:145–147

Yang JC, Haworth L, Sherry RM, Hwu P, Schwartzentruber DJ, Topalian SL, Steinberg SM, Chen HX, Rosenberg SA (2003) A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. N Engl J Med 349:427–434