Chapter 4

Accessory Cells in Tumor Angiogenesis — Tumor-Associated Pericytes

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1. Introduction

In contrast to the normal tissue vasculature, tumor vessels are structurally and functionally abnormal [1-3]. These abnormal tumor vessels are characterized by an irregular, disorganized, and tortuous architecture with a highly dysfunctional and leaky endothelial cell (EC) layer [1, 3]. ECs are often loosely connected with each other and are covered by fewer and abnormal mural pericytes (PCs) [2-4].

Research into the molecular mechanisms and physiology of PCs associated with tumor angiogenesis is a critical field in cancer research. In this chapter, we will focus on the pathophysiology of PCs in tumor angiogenesis, the role of PCs in resistance to anti-angiogenesis therapy, and PCs as a therapeutic target.

2. Pathophysiology of pericytes in tumor angiogenesis

Despite the increasing evidence that PCs plays important roles in the angiogenic process, the origin of PCs is still not fully understood. They are commonly described as originating from various types of progenitors depending on their anatomical location in the body. For example, epicardial, mesenchymal, and neural crest cells are believed to be a source for pericytes in the cardiac coronary vasculature, dorsal aorta, and cardiac outflow tract, respectively [5].

Pericytes play an important role in stabilizing blood vessels in the microvasculature [6, 7]. A feature of pericyte function is their ability to provide vascular stability through crosstalk be-

tween PCs and endothelial cells (ECs). PCs deposit matrix or releasing factors that can promote EC differentiation or quiescence [8].

2.1. Crosstalk between ECs and PCs

In blood vessels, the crosstalk between ECs and PCs plays a critical role in the regulation of vascular formation, maturation, remodeling, stabilization and function [9]. PCs communicate with ECs by direct physical contact and paracrine signaling pathways.

Gap junctions provide direct contact between PCs and ECs that enable the exchange of ions and small molecules. Adhesion plaques anchor PCs to ECs, while peg-and-socket junctions enable the cells to penetrate the vascular basement membrane [10].

A variety of signaling factors mediate PC–EC interactions, including platelet-derived growth factor subunit B (PDGFB) and angiopoietin/Tie 2 [11].

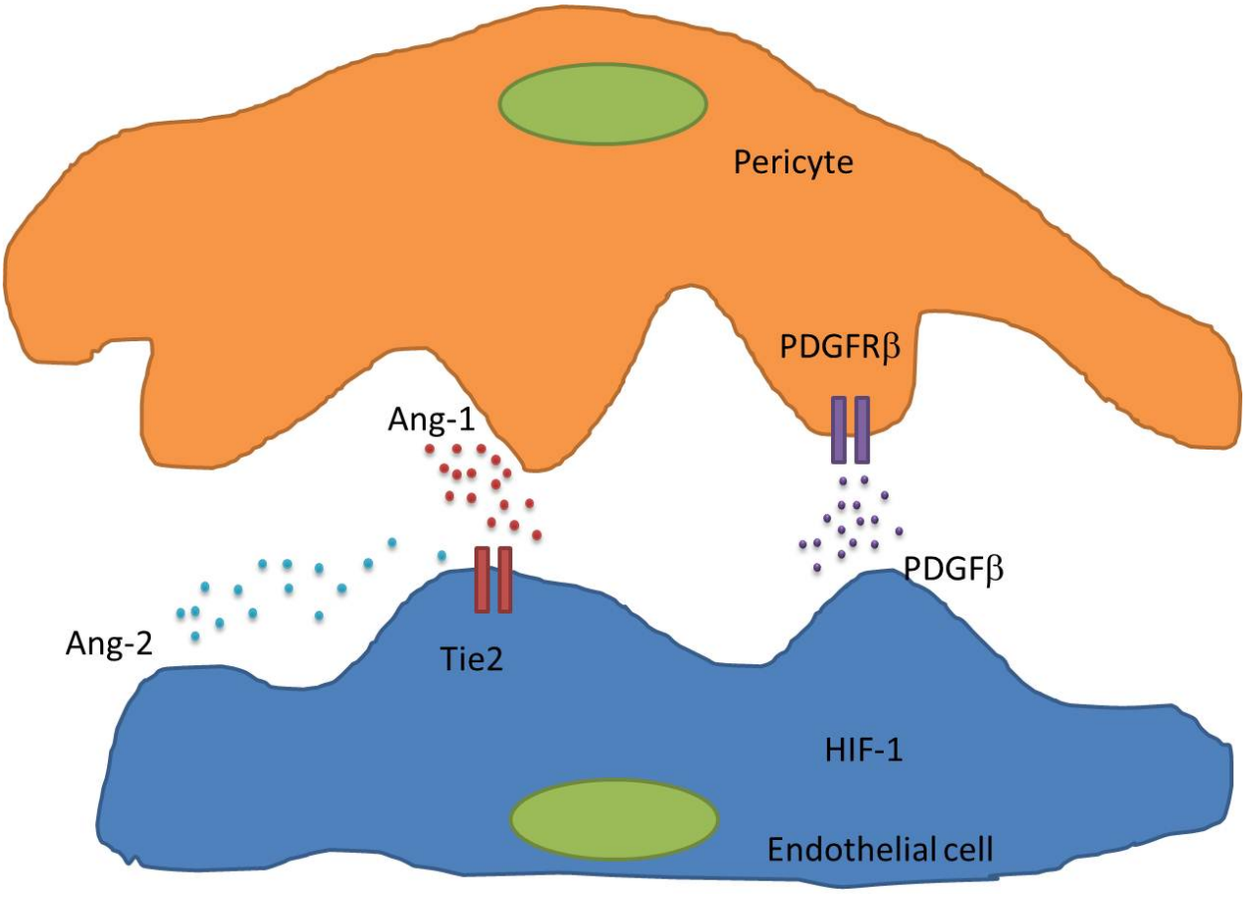

Figure 1. Crosstalk between endothelial cells and pericytes

2.1.1. PDGF/PDGFR family

Pericyte homeostasis in normal biology is regulated in significant part by signaling through the PDGF ligand and receptor system (Fig. 1) [12, 13]. PDGF is a potent mito-

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gen for pericytes and fibroblasts. PDGF consists of A, B, C, and D polypeptide chains, and it forms the homodimers PDGF-AA, BB, CC, and DD, and the heterodimer PDGF-AB [14]. The specific tyrosine kinase receptors of the PDGFR family consist of PDGFR-α and PDGFR-β [15, 16]. PDGFR-α binds to PDGF-AA, BB, AB, and CC, whereas PDGFR-β binds with BB and DD [17].

Previous studies have shown that a <90% reduction in pericyte coverage in mice is compatible with postnatal survival [18], whereas loss of >95% of pericytes is lethal [18, 19], suggesting that a rather low threshold of pericyte density is required for basal function of microvasculature.

Activated ECs secrete PDGF-BB to attract PCs and PC progenitors, which are either tissue-resident cells and/or cells derived from bone marrow, and express PDGFRs [20], suggesting a paracrine signaling circuit [12, 18]. Pericyte deficiency, seen in knockout mice lacking PDGF-BB and its receptor, PDGFR-β, resulted in various changes in microvasculature, including endothelial hyperplasia, vessel dilation, tortuosity, leakage, and rupture, leading to widespread and lethal microhemorrhages and edema in late gestation [19, 21].

Studies of implanted tumors have shown that pericytes initially accumulate at the interface of tumor and host tissue and later around new blood vessels, exhibiting close contacts with ECs. Maturation of the tumor-associated vasculature is accompanied quantitatively by a reduced PC volume and qualitatively by morphological changes in whichPCs become flattened and elongated [22].

There is evidence that overexpression of PDGF-BB in tumor cells dramatically increases the PC coverage [23]. Moreover, Song et al. have also shown that tumor-derived PDGF-BB increases tumor PC coverage by activation of stromal-derived factor 1 alpha (SDF-1α) [24]. Thus, PDGF-BB appears to be a critical player in the recruitment of PCs to newly formed vessels [25].

2.1.2. Angiopoietin/Tie family

The angiopoietin (Ang) family consists of several members including Ang-1, Ang-2, Ang-3 (murine specific), and Ang-4 (human specific), which have two tyrosine kinase receptors, Tie-1 and Tie-2.

Ang-1 was initially identified as an activating ligand for Tie-2, which is expressed by perivascular cells [26]. Genetic deletion of Ang-1 resulted in prenatal lethality, due to severe heart and vascular defects, very similar in phenotype to Tie-2-deficient mice [27]. Ang-1 is predominantly secreted by PCs and can bind with Tie-2 on ECs in a paracrine pattern. Ang-1 enhances PC-EC interactions, represses the proliferation and migration of ECs, and promotes the maturation of newly formed blood vessels [27, 28]. Constitutive Ang-1/Tie-2 signaling is required to maintain the quiescent vasculature [29-31] (Fig. 1).

Ang-2 was initially identified as a homologue of Ang-1 [32]. Ang-2 was found to bind to Tie-2 with an affinity similar to that of Ang-1. However, unlike Ang-1, exogenous Ang-2 produces only a very weak activation of Tie-2 on ECs. When ECs are activated by tumor-derived pro-angiogenic factors, Ang-2 acts as an autocrine antagonist of Ang-1/Tie-2 signaling [33]. More-

over, Ang-2 activates the downstream pathways including Pl3K/Akt, and thus functions as a promoter of angiogenesis [32]. Nasarre et al. have shown that tumors implanted into genetically Ang-2-ablated mice grew more slowly than those implanted into wild-type mice [34], which suggests that Ang-2 is a potent target for anti-tumor therapies (Fig. 1).

Tie-2 receptor expression recently has been identified in mesenchymal cells that are present in the stroma, implicating a repository for tumor vessel pericytes [35].

2.2. PCs in tumor angiogenesis

Many tumors express the pro-angiogenic vascular endothelial growth factor (VEGF) at high levels [36]. In contrast to ECs in normal tissues, ECs in the tumor vasculature are dependent on VEGF for survival [37]. Excessive VEGF signaling through VEGF receptor 2 (VEGFR2) loosens tight junctions of ECs, increasing permeability in the interstitial tumor microenvironment. Interestingly, in tumors with reduced levels of VEGF and other angiogenic regulatory factors, tumor vessels are less torturous, with normalized blood flow due to improved PC coverage, the so-called ''vascular normalization'' [3, 38, 39].

PCs stabilize ECs and mediate EC survival and maturation in normal vasculature, through both direct cell contact with ECs and paracrine signaling. It was reported that PCs in tumor vasculature are abnormal [40]. Low PC coverage correlates with poor clinical outcome in several different tumor types [41-43], but so far, the active involvement of PCs in tumor progression remains unclear. PCs are usually absent in tumor vasculature or have loose associations with ECs, leaving most of the tumor microvessels immature, the significance of which has been revealed in studies in which genetic or pharmacologic ablation of PC coverage facilitates metastatic dissemination of tumor cells [43, 44].

Activated PCs loosely attach to microvessels and develop cytoplasmic extensions into the tumor parenchyma [45]. Compared to quiescent PCs, activated PCs can change their genomic expression profiles [9], leading to phenotypes that are highly proliferative with the pluripotency to differentiate into other PCs, matrix-forming cells, smooth muscle cells, or adipocytes.

2.3. Molecular marker of PCs

The challenges of defining a PC have not been made easier by the fact that a general pan-PC molecular marker has not been found. Because of the diverse characteristics, functions, and locations of PCs in various organs, it probably never will be discovered. There are, however, a few dynamic molecular markers that are present in PCs, albeit not exclusively, and that are commonly used for their detection. The expression patterns of these markers can vary in a tissue-specific manner or be dependent on the developmental or angiogenic stage of a blood vessel. Desmin and alpha-smooth-muscle actin (α-SMA) are contractile filaments, and regulator of G protein signaling 5 (RGS-5) is a GTPase-activating protein; all three are intracellular proteins. Neuron-glial 2 (NG2), a chondroitin sulfate proteoglycan, and platelet-derived growth factor receptor beta (PDGFRβ), a tyrosine-kinase receptor, are cell-surface proteins. Antibodies against these proteins (except RGS-5) are commonly used to identify PCs in tissue

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sections (Table 1). Desmin is a muscle-specific class III intermediate filament found in mature skeletal, cardiac, and smooth-muscle cells.

Molecular Marker	Alternative name		Mouse	Human
a-SMA	α-Smooth muscle actin	Expressed only locally by pericytes in tumor vasculature contractile filaments	+	+
PDGFR-β	Platelet-derived growth factor β	Tyrosine kinase receptor	+	+
Desmin		Reactive to developing and developed pericyte contractile filaments	+	+
Nestin	-		+	+
Smooth muscle myosin	-		+	+
Tropomyosin	-		+	+
NG2	Neuron-glial 2 (chondroitin sulfate proteoglycan) High-molecular-weight melanoma-associated antigen (HMWMAA)	Tyrosine kinase receptor Expressed in pericytes in early stages of angiogenesis	+	+
Aminopeptidase A	CD249, BP1		+	+
Aminopeptidase N	CD13		+	+
MMP9	Matrix metalloproteinase-9, gelatinase B		+	+
Sulphatide	3'-sulphogalactosylceramide		-	+
VEGFR1	vascular endothelial growth factor receptor-1		+	+
RGS5	Regulator of G-protein signaling-5	Novel marker for pericytes and vascular smooth muscle cells GTPase-activating protein	+	+
3G5 Ganglioside antigen	-	Specific for a pericyte surface ganglioside	-	+

Table 1. Markers of pericytes for microscopic imaging (antibody availability)

2.4. Role of bone marrow-derived PC progenitors

Bone marrow–derived hematopoietic cells expressing the PC marker NG2 were identified in close contact with tumor blood vessels in animal models of melanoma [46], pancreatic islet

carcinomas [47], and brain tumors [48, 49]. Thus, PC progenitor cells appear to be recruited to sites of angiogenesis from the bone marrow niche; however, intravenously injected PC progenitor cells may fail to migrate and integrate into the tumor vasculature [50].

Tumor hypoxia due to the vascular regression following anti-angiogenic therapy appears to induce recruitment of various bone marrow-derived cells to the tumor microenvironment [51]. Rajantie et al. demonstrated the significant contribution of bone marrow–derived cells using an inducible hypoxia-inducible factor 1 alpha subunit (HIF1-α) animal model. In response to hypoxia in glioblastomas [52, 53], not only Tie-2-, VEGFR1-, CD11b-, and F4/80-positive cells but ECs and PC progenitor cells are released into the circulation from the bone marrow through the HIF1-α signal pathway. Then, they contribute to the neovascularization of glioblastoma [51]. In an HIF1-α knock-down mouse model, fewer bone marrow-derived cells are recruited to the tumors, which severely impairs tumor growth. These data suggest paradoxical induction of tumor angiogenesis via bone marrow-derived vessel progenitor cells after anti-angiogenic therapy.

3. Role of PCs in resistance to anti-angiogenic therapies

Although an anti-VEGF therapy, bevacizumab, has shown clinical efficacy in the treatment of several tumor types, its efficacy will ultimately be limited by acquired drug resistance. [54]. Putative mechanisms of resistance to anti-VEGF therapy include (1) activation and/or up-regulation of alternative pro-angiogenic pathways including PDGF/PDGFR signaling in the tumor [55], (2) recruitment of bone marrow-derived pro-angiogenic cells that differentiate into PCs, and (3) increased PC coverage of tumor microvasculature partially mediated by PDGFR signaling [56, 57].

Studies have shown that vessels without PC coverage are more dependent on VEGF signaling for survival [9] and that inhibition of VEGF leads to increased PC coverage of the tumor vasculature [58]. PCs may protect ECs from VEGF withdrawal, leading to PC-mediated resistance to anti-angiogenic therapies.

4. Targeting PCs as an anti-angiogenic therapy

Although a series of anti-angiogenic strategies targeting VEGF or its receptor VEGFR2 have been shown to efficiently prevent the growth of many types of tumors [59, 60], reports have shown that targeting VEGF signaling alone is often ineffective at inducing vascular regression or preventing the rapid regrowth of tumor vessels [58, 61-63]. One possible explanation for this failure is that the anti-angiogenic inhibitors mainly target immature ECs lacking PCs coverage, while showing a limited effect on the PC-associated mature vessels [63-65].

Although tumor PCs are less abundant and more loosely attached to vessels than those in healthy tissues, they have emerged as a critical therapeutic target for anti-angiogenic therapy.

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Preclinical and clinical studies have largely focused on the role of tumor PCs in promoting EC survival and stabilizing the tumor vasculature through a variety of signaling networks. As noted earlier, PC recruitment to tumor neovessels is dependent on signaling through the PDGF-BB/PDGFRβ and Ang-1/Tie-2 networks.

4.1. Targeting PDGF-BB/ PDGFRβ signaling

PDGF-BB/PDGFRβ signaling appears to be critical for maintaining the PC–EC contacts needed for vessel stabilization. Vascular regression could also lead to the normalization of tumor microvessels and the opening of previously collapsed vessels [66] via decreased interstitial fluid pressure [67]. These data suggest that PDGF/PDGFR pathway inhibition is a potent target for anti-tumor therapies by leading to improved drug delivery [68-70].

Drug Name	Target	Type	Clinical stage
Sunitinib (Sutent)	PDGFRs, VEGFRs, FLT-3, CSF1R	Small molecule inhibitor	Approved for metastatic RCC, imatinib-resistant GIST, PNET
Sorafenib (Nexavar)	PDGFRs, VEGFRs, Raf, cKit	Small molecule inhibitor	Approved for metastatic RCC, HPCC
Pazopanib (Votrient)	PDGFRs, VEGFRs, cKit	Small molecule inhibitor	Approved for metastatic RCC
Vandetanib (Caprelsa)	PDGFRs, VEGFRs, EGFR	Small molecule inhibitor	Approved for metastatic medullary thyroid cancer
Axitinib (Inlyta)	PDGFRs, VEGFRs, cKit	Small molecule inhibitor	Approved for metastatic RCC
Motesanib	PDGFRs, VEGFRs, cKit	Small molecule inhibitor	Phase III
Cediranib (Recentin)	PDGFRs, VEGFRs, cKit	Small molecule inhibitor	Phase III
Cabozantinib	PDGFRs, VEGFRs, cMet, RET, cKit	Small molecule inhibitor	Phase III
Tivozanib	PDGFRs, VEGFRs, cKit	Small molecule inhibitor	Phase III
Regorafenib	PDGFRs, VEGFRs, Raf, cKit	Small molecule inhibitor	Phase III

Table 2. PDGF/PDGFR inhibitors that are approved and/or in clinical development

Combining PDGFRβ tyrosine kinase inhibition with VEGF inhibition more efficiently blocked tumor angiogenesis than VEGF inhibition alone in several experimental models [63, 71-74]. Bergers et al. have shown that combined treatment by anti-PDGFR agents together with anti-VEGF significantly reduces PC coverage and increases the success of anti-tumor treatment in the RIP1-TAG2 mouse model [63]. Similarly, PDGF inhibition disrupts PC support and sensitizes ECs to anti-angiogenic chemotherapy, resulting in regression of pre-existing tumor

vasculature in a mouse model [13]. Long-term blockade of PDGF signaling by anti-PDGFRβ antibody reduces the concentration of PCs within the tumor tissue and also increases the apoptosis of ECs [73].

Several studies have tested the effects of combining anti-tumor agents with anti-PC agents that target PDGF or other PC markers, such as NG2 proteoglycan [75]. Involvement of the SDF-1α/CXCR4 axis in PC recruitment within PDGF-BB–overexpressing tumors suggests that a blockade of this axis may provide an additional target in anti-angiogenic tumor therapy [24].

Most recently, treatment of primary tumors in an animal model of breast cancer with combination VEGF and PDGF receptor therapy led to decreased PC coverage and an increased number of metastases. The observed promotion of metastasis by imatinib is consistent with previous reports demonstrating the key role of PDGFRβ signaling in PC recruitment and the importance of PCs in limiting tumor cell metastasis [43]. These findings provide the mechanistic basis for the differential effects these agents have on metastasis promotion.

However, a human clinical trial for renal carcinoma showed that inhibition of both the VEGF and PDGF pathways resulted in no therapeutic benefit when compared to inhibition of the VEGF pathway alone; in fact, the combined regimen exhibited toxicity [76]. Given these results, further preclinical studies are needed to clarify the mechanism(s) by which PDGF-targeted agents affect PC–EC interactions, and additional clinical studies are needed to clarify the potential benefits and risks associated with anti-PC tumor therapy.

4.2. Targeting Ang/Tie signaling

PCs have been shown to stabilize blood vessels and provide EC survival signals through the Ang-1/Tie-2 pathway [73, 77]. Therefore, by targeting tumor PCs it may be possible to overcome PC-mediated resistance to VEGF pathway inhibition and achieve more effective tumor vessel destabilization through disruption of the PC–EC association or directly through PC loss.

Trebananib (AMG 386) is a peptide-Fc fusion protein that inhibits angiogenesis by neutralizing the interaction between the Tie-2 receptor and Ang-1 and Ang-2 [78]. In phase I testing, it was found to be well tolerated in combination with chemotherapy [79] and to reduce tumor blood flow or permeability [80]. In a phase II trial of trebananib in combination with paclitaxel in patients with recurrent ovarian cancer, although a statistically significant improvement in progression-free survival for the treatment arm was not observed, the objective response rates and progression-free survival at the higher dose are suggestive of an antitumor effect [81]. The toxicity profile, including peripheral edema but not bowel perforations, is consistent with a mechanism distinct from that of VEGF inhibitors. Trebananib plus paclitaxel is now being investigated in an ongoing phase III study (TRINOVA-1 [Trial in Ovarian Cancer-1]) for the treatment of recurrent ovarian cancer. Phase II trials in breast, colorectal, kidney, stomach, and liver cancers are underway.

CVX060 (PF-04856884) is a recombinant humanized monoclonal antibody fused to two Ang-2 binding peptides [82, 83]. In preclinical studies, CVX-060 was anti-angiogenic and decreased tumor proliferation. In phase I testing, this agent significantly decreased tumor blood flow and

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affected circulating serum Ang-2 levels. This agent is being evaluated in combination with sunitinib in renal cell carcinoma. Currently, a phase II trial for kidney and a phase I trial for other solid tumors are underway.

Other agents in development include monoclonal antibodies directed against Ang-2 (MEDI-3617, AMG 780, REGN910) and multi-targeted tyrosine kinase inhibitors inhibiting Tie-2 (CEP11981, ARRY614) [84].

4.3. Other approaches

At least two alternative therapeutic approaches appear plausible given the role of PCs in promoting tumor angiogenesis. The first approach is to promote excessive PC recruitment, thereby causing vessel stabilization and restricting vessel sprouting. This approach may limit tumor angiogenesis in blood vessels with normal PC investment of the EC and may prevent the dissemination of tumor cells into the circulation by reducing the leakiness of intratumoral blood vessels and, perhaps, by also blocking extravasation of circulating tumor cells.

The second approach involves the use of PC progenitor cells as a cellular vehicle for gene delivery. This idea is supported by previous work using progenitor ECs [85-87], and more recently, PCs [50] to deliver anti-angiogenic gene therapy.

Neither of these approaches promoting PC recruitment to the tumor vasculature has been tested in preclinical models or clinical trials; both are highly speculative and no proof-of-principle studies have been conducted in animal models.

5. Conclusions

Based on the crucial role of PCs in microvessel maturity and the concomitant histological evaluation of EC-PC interactions and tumor microvessel morphology, combining different chemotherapeutic agents and anti-angiogenic treatments that normalize tumor vasculature seems to be inevitable. Many new angiogenic inhibitors target pathways that are involved in the recruitment of PCs to tumor microvessels. Therefore, it is essential to assess PCs in parallel with ECs when studying tumor vasculature. This evaluation, which can be performed in a diagnostic pathology laboratory, can be used as a decision-making tool to select patients who might benefit from anti-angiogenic therapies.

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References

[1] De Bock, K., et al., Endothelial oxygen sensors regulate tumor vessel abnormalization by instructing phalanx endothelial cells. J Mol Med (Berl), 2009. 87(6): p. 561-9.

[2] Jain, R.K., Determinants of tumor blood flow: a review. Cancer Res, 1988. 48(10): p. 2641-58.

[3] Jain, R.K., Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science, 2005. 307(5706): p. 58-62.

[4] Mazzone, M., et al., Heterozygous deficiency of PHD2 restores tumor oxygenation and inhibits metastasis via endothelial normalization. Cell, 2009. 136(5): p. 839-51.

[5] Bergwerff, M., et al., Neural crest cell contribution to the developing circulatory system: implications for vascular morphology? Circ Res, 1998. 82(2): p. 221-31.

[6] Nehls, V. and D. Drenckhahn, The versatility of microvascular pericytes: from mesenchyme to smooth muscle? Histochemistry, 1993. 99(1): p. 1-12.

[7] Sims, D.E., The pericyte--a review. Tissue Cell, 1986. 18(2): p. 153-74.

[8] Armulik, A., A. Abramsson, and C. Betsholtz, Endothelial/pericyte interactions. Circ Res, 2005. 97(6): p. 512-23.

[9] Benjamin, L.E., I. Hemo, and E. Keshet, A plasticity window for blood vessel remodelling is defined by pericyte coverage of the preformed endothelial network and is regulated by PDGF-B and VEGF. Development, 1998. 125(9): p. 1591-8.

[10] Rucker, H.K., H.J. Wynder, and W.E. Thomas, Cellular mechanisms of CNS pericytes. Brain Res Bull, 2000. 51(5): p. 363-9.

[11] Jain, R.K. and M.F. Booth, What brings pericytes to tumor vessels? J Clin Invest, 2003. 112(8): p. 1134-6.

http://dx.doi.org/10.5772/54523

[12] Hellstrom, M., et al., 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, 1999. 126(14): p. 3047-55.

[13] Pietras, K. and D. Hanahan, A multitargeted, metronomic, and maximum-tolerated dose "chemo-switch" regimen is antiangiogenic, producing objective responses and survival benefit in a mouse model of cancer. J Clin Oncol, 2005. 23(5): p. 939-52.

[14] Andrae, J., R. Gallini, and C. Betsholtz, Role of platelet-derived growth factors in physiology and medicine. Genes Dev, 2008. 22(10): p. 1276-312.

[15] Heldin, C.H., A. Ostman, and L. Ronnstrand, Signal transduction via platelet-derived growth factor receptors. Biochim Biophys Acta, 1998. 1378(1): p. F79-113.

[16] Kelly, J.D., et al., Platelet-derived growth factor (PDGF) stimulates PDGF receptor subunit dimerization and intersubunit trans-phosphorylation. J Biol Chem, 1991. 266(14): p. 8987-92.

[17] Betsholtz, C., L. Karlsson, and P. Lindahl, Developmental roles of platelet-derived growth factors. Bioessays, 2001. 23(6): p. 494-507.

[18] Enge, M., et al., Endothelium-specific platelet-derived growth factor-B ablation mimics diabetic retinopathy. EMBOJ, 2002. 21(16): p. 4307-16.

[19] Lindahl, P., et al., Pericyte loss and microaneurysm formation in PDGF-B-deficient mice. Science, 1997. 277(5323): p. 242-5.

[20] Lamagna, C. and G. Bergers, The bone marrow constitutes a reservoir of pericyte progenitors. J Leukoc Biol, 2006. 80(4): p. 677-81.

[21] Hellstrom, M., et al., Lack of pericytes leads to endothelial hyperplasia and abnormal vascular morphogenesis. J Cell Biol, 2001. 153(3): p. 543-53.

[22] Verhoeven, D. and N. Buyssens, Desmin-positive stellate cells associated with angiogenesis in a tumour and non-tumour system. Virchows Arch B Cell Pathol Incl Mol Pathol, 1988. 54(5): p. 263-72.

[23] McCarty, M.F., et al., Overexpression of PDGF-BB decreases colorectal and pancreatic cancer growth by increasing tumor pericyte content. J Clin Invest, 2007. 117(8): p. 2114-22.

[24] Song, N., et al., Overexpression of platelet-derived growth factor-BB increases tumor pericyte content via stromal-derived factor-1alpha/CXCR4 axis. Cancer Res, 2009. 69(15): p. 6057-64.

[25] Betsholtz, C., Insight into the physiological functions of PDGF through genetic studies in mice. Cytokine Growth Factor Rev, 2004. 15(4): p. 215-28.

[26] Davis, S., et al., Isolation of angiopoietin-1, a ligand for the TIE2 receptor, by secretion-trap expression cloning. Cell, 1996. 87(7): p. 1161-9.

[27] Suri, C., et al., Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis. Cell, 1996. 87(7): p. 1171-80.

[28] Iivanainen, E., et al., Angiopoietin-regulated recruitment of vascular smooth muscle cells by endothelial-derived heparin binding EGF-like growth factor. FASEB J, 2003. 17(12): p. 1609-21.

[29] Jones, N., et al., Tie receptors: new modulators of angiogenic and lymphangiogenic responses. Nat Rev Mol Cell Biol, 2001. 2(4): p. 257-67.

[30] Dumont, D.J., et al., Dominant-negative and targeted null mutations in the endothelial receptor tyrosine kinase, tek, reveal a critical role in vasculogenesis of the embryo. Genes Dev, 1994. 8(16): p. 1897-909.

[31] Thurston, G., Role of Angiopoietins and Tie receptor tyrosine kinases in angiogenesis and lymphangiogenesis. Cell Tissue Res, 2003. 314(1): p. 61-8.

[32] Maisonpierre, P.C., et al., Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. Science, 1997. 277(5322): p. 55-60.

[33] Feng, Y., et al., Impaired pericyte recruitment and abnormal retinal angiogenesis as a result of angiopoietin-2 overexpression. Thromb Haemost, 2007. 97(1): p. 99-108.

[34] Nasarre, P., et al., Host-derived angiopoietin-2 affects early stages of tumor development and vessel maturation but is dispensable for later stages of tumor growth. Cancer Res, 2009. 69(4): p. 1324-33.

[35] De Palma, M., et al., Tie2 identifies a hematopoietic lineage of proangiogenic monocytes required for tumor vessel formation and a mesenchymal population of pericyte progenitors. Cancer Cell, 2005. 8(3): p. 211-26.

[36] Senger, D.R., et al., Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science, 1983. 219(4587): p. 983-5.

[37] Zhang, Z., et al., VEGF-dependent tumor angiogenesis requires inverse and reciprocal regulation of VEGFR1 and VEGFR2. Cell Death Differ, 2010. 17(3): p. 499-512.

[38] Goel, S., et al., Normalization of the vasculature for treatment of cancer and other diseases. Physiol Rev, 2011. 91(3): p. 1071-121.

[39] De Bock, K., S. Cauwenberghs, and P. Carmeliet, Vessel abnormalization: another hallmark of cancer? Molecular mechanisms and therapeutic implications. Curr Opin Genet Dev, 2011. 21(1): p. 73-9.

[40] Raza, A., M.J. Franklin, and A.Z. Dudek, Pericytes and vessel maturation during tumor angiogenesis and metastasis. Am J Hematol, 2010. 85(8): p. 593-8.

[41] Yonenaga, Y., et al., Absence of smooth muscle actin-positive pericyte coverage of tumor vessels correlates with hematogenous metastasis and prognosis of colorectal cancer patients. Oncology, 2005. 69(2): p. 159-66.

http://dx.doi.org/10.5772/54523

[42] O'Keeffe, M.B., et al., Investigation of pericytes, hypoxia, and vascularity in bladder tumors: association with clinical outcomes. Oncol Res, 2008. 17(3): p. 93-101.

[43] Cooke, V.G., et al., Pericyte depletion results in hypoxia-associated epithelial-to-mesenchymal transition and metastasis mediated by met signaling pathway. Cancer Cell, 2012. 21(1): p. 66-81.

[44] Xian, X., et al., Pericytes limit tumor cell metastasis. J Clin Invest, 2006. 116(3): p. 642-51.

[45] Morikawa, S., et al., Abnormalities in pericytes on blood vessels and endothelial sprouts in tumors. Am J Pathol, 2002. 160(3): p. 985-1000.

[46] Rajantie, I., et al., Adult bone marrow-derived cells recruited during angiogenesis comprise precursors for periendothelial vascular mural cells. Blood, 2004. 104(7): p. 2084-6.

[47] Song, S., et al., PDGFRbeta+ perivascular progenitor cells in tumours regulate pericyte differentiation and vascular survival. Nat Cell Biol, 2005. 7(9): p. 870-9.

[48] Bababeygy, S.R., et al., Hematopoietic stem cell-derived pericytic cells in brain tumor angio-architecture. Stem Cells Dev, 2008. 17(1): p. 11-8.

[49] Jodele, S., et al., The contribution of bone marrow-derived cells to the tumor vasculature in neuroblastoma is matrix metalloproteinase-9 dependent. Cancer Res, 2005. 65(8): p. 3200-8.

[50] Bexell, D., et al., Bone marrow multipotent mesenchymal stroma cells act as pericyte-like migratory vehicles in experimental gliomas. Mol Ther, 2009. 17(1): p. 183-90.

[51] Du, R., et al., HIF1alpha induces the recruitment of bone marrow-derived vascular modulatory cells to regulate tumor angiogenesis and invasion. Cancer Cell, 2008. 13(3): p. 206-20.

[52] Li, Z., et al., Cardiovascular lesions and skeletal myopathy in mice lacking desmin. Dev Biol, 1996. 175(2): p. 362-6.

[53] Milner, D.J., et al., Disruption of muscle architecture and myocardial degeneration in mice lacking desmin. J Cell Biol, 1996. 134(5): p. 1255-70.

[54] Jubb, A.M. and A.L. Harris, Biomarkers to predict the clinical efficacy of bevacizumab in cancer. Lancet Oncol, 2010. 11(12): p. 1172-83.

[55] Sasaki, T., et al., Administration of VEGF receptor tyrosine kinase inhibitor increases VEGF production causing angiogenesis in human small-cell lung cancer xenografts. Int J Oncol, 2008. 33(3): p. 525-32.

[56] Bergers, G. and D. Hanahan, Modes of resistance to anti-angiogenic therapy. Nat Rev Cancer, 2008. 8(8): p. 592-603.

[57] Crawford, Y., et al., PDGF-C mediates the angiogenic and tumorigenic properties of fibroblasts associated with tumors refractory to anti-VEGF treatment. Cancer Cell, 2009. 15(1): p. 21-34.

[58] Benjamin, L.E., et al., Selective ablation of immature blood vessels in established human tumors follows vascular endothelial growth factor withdrawal. J Clin Invest, 1999. 103(2): p. 159-65.

[59] Hanahan, D. and J. Folkman, Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell, 1996. 86(3): p. 353-64.

[60] O'Reilly, M.S., et al., Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. Cell, 1997. 88(2): p. 277-85.

[61] Baluk, P., H. Hashizume, and D.M. McDonald, Cellular abnormalities of blood vessels as targets in cancer. Curr Opin Genet Dev, 2005. 15(1): p. 102-11.

[62] Bergers, G. and S. Song, The role of pericytes in blood-vessel formation and maintenance. Neuro Oncol, 2005. 7(4): p. 452-64.

[63] Bergers, G., et al., Benefits of targeting both pericytes and endothelial cells in the tumor vasculature with kinase inhibitors. J Clin Invest, 2003. 111(9): p. 1287-95.

[64] Bono, A.V., et al., Sorafenib's inhibition of prostate cancer growth in transgenic adenocarcinoma mouse prostate mice and its differential effects on endothelial and pericyte growth during tumor angiogenesis. Anal Quant Cytol Histol, 2010. 32(3): p. 136-45.

[65] Huang, J., et al., Vascular remodeling marks tumors that recur during chronic suppression of angiogenesis. Mol Cancer Res, 2004. 2(1): p. 36-42.

[66] Padera, T.P., et al., Pathology: cancer cells compress intratumour vessels. Nature, 2004. 427(6976): p. 695.

[67] Rodt, S.A., et al., A novel physiological function for platelet-derived growth factor-BB in rat dermis. J Physiol, 1996. 495 (Pt 1): p. 193-200.

[68] Baranowska-Kortylewicz, J., et al., Effect of platelet-derived growth factor receptor-beta inhibition with STI571 on radioimmunotherapy. Cancer Res, 2005. 65(17): p. 7824-31.

[69] Jayson, G.C., et al., Blockade of platelet-derived growth factor receptor-beta by CDP860, a humanized, PEGylated di-Fab', leads to fluid accumulation and is associated with increased tumor vascularized volume. J Clin Oncol, 2005. 23(5): p. 973-81.

[70] Pietras, K., et al., Inhibition of PDGF receptor signaling in tumor stroma enhances antitumor effect of chemotherapy. Cancer Res, 2002. 62(19): p. 5476-84.

[71] Shaheen, R.M., et al., Tyrosine kinase inhibition of multiple angiogenic growth factor receptors improves survival in mice bearing colon cancer liver metastases by inhibition of endothelial cell survival mechanisms. Cancer Res, 2001. 61(4): p. 1464-8.

[72] Reinmuth, N., et al., Induction of VEGF in perivascular cells defines a potential paracrine mechanism for endothelial cell survival. FASEB J, 2001. 15(7): p. 1239-41.

[73] Erber, R., et al., Combined inhibition of VEGF and PDGF signaling enforces tumor vessel regression by interfering with pericyte-mediated endothelial cell survival mechanisms. FASEB J, 2004. 18(2): p. 338-40.

[74] Farhadi, M.R., et al., Combined inhibition of vascular endothelial growth factor and platelet-derived growth factor signaling: effects on the angiogenesis, microcirculation, and growth of orthotopic malignant gliomas. J Neurosurg, 2005. 102(2): p. 363-70.

[75] Maciag, P.C., et al., Cancer immunotherapy targeting the high molecular weight melanoma-associated antigen protein results in a broad antitumor response and reduction of pericytes in the tumor vasculature. Cancer Res, 2008. 68(19): p. 8066-75.

[76] Hainsworth, J.D., et al., Treatment of advanced renal cell carcinoma with the combination bevacizumab/erlotinib/imatinib: a phase I/II trial. Clin Genitourin Cancer, 2007. 5(7): p. 427-32.

[77] Abramsson, A., P. Lindblom, and C. Betsholtz, Endothelial and nonendothelial sources of PDGF-B regulate pericyte recruitment and influence vascular pattern formation in tumors. J Clin Invest, 2003. 112(8): p. 1142-51.

[78] Coxon, A., et al., Context-dependent role of angiopoietin-1 inhibition in the suppression of angiogenesis and tumor growth: implications for AMG 386, an angiopoietin-1/2-neutralizing peptibody. Mol Cancer Ther, 2010. 9(10): p. 2641-51.

[79] Mita, A.C., et al., Phase 1 Study of AMG 386, a Selective Angiopoietin 1/2–Neutralizing Peptibody, in Combination with Chemotherapy in Adults with Advanced Solid Tumors. Clin Cancer Res, 2010. 16(11): p. 3044-3056.

[80] Herbst, R.S., et al., Safety, Pharmacokinetics, and Antitumor Activity of AMG 386, a Selective Angiopoietin Inhibitor, in Adult Patients With Advanced Solid Tumors. J of Clin Oncol, 2009. 27(21): p. 3557-3565.

[81] Karlan, B.Y., et al., Randomized, Double-Blind, Placebo-Controlled Phase II Study of AMG 386 Combined With Weekly Paclitaxel in Patients With Recurrent Ovarian Cancer. J of Clin Oncol, 2012. 30(4): p. 362-371.

[82] Huang, H., et al., Specifically Targeting Angiopoietin-2 Inhibits Angiogenesis, Tie2-Expressing Monocyte Infiltration, and Tumor Growth. Clin Cancer Res, 2011. 17(5): p. 1001-1011.

[83] Doppalapudi, V.R., et al., Chemical generation of bispecific antibodies. Proc Nat AcadSci, 2010. 107(52): p. 22611-22616.

[84] Cascone, T. and J.V. Heymach, Targeting the Angiopoietin/Tie2 Pathway: Cutting Tumor Vessels With a Double-Edged Sword? J Clin Oncol, 2012. 30(4): p. 441-444.

[85] Dudek, A.Z., et al., Systemic inhibition of tumour angiogenesis by endothelial cell-based gene therapy. Br J Cancer, 2007. 97(4): p. 513-22.

[86] Somani, A., et al., The establishment of murine blood outgrowth endothelial cells and observations relevant to gene therapy. Transl Res, 2007. 150(1): p. 30-9.

[87] Milbauer, L.C., et al., Blood outgrowth endothelial cell migration and trapping in vivo: a window into gene therapy. Transl Res, 2009. 153(4): p. 179-89.