Chapter 2
Cancer and Angiogenesis

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Abstract Neoplastic growth is closely linked to neovascularization, as efficient blood supply is necessary to deliver oxygen and nutrients to a tumour. The development of blood vessels in tumours is modulated by pro- and anti-angiogenic factors. Pro-angiogenic factors include those that regulate remodelling of the extracellular matrix (ECM) and changes in perivascular cell structure, as well as those that promote endothelial cell changes and migration, including but not limited to vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and angiopoietin. Anti-angiogenic factors include thrombospondin, 16kDA N-terminal fragments of prolactin and growth hormone, endostatin, vasostatin, and angiostatin. During tumour growth, the balance is tipped in favour of pro-angiogenic factors. This is known as the angiogenic switch and allows for increased tumour progression, a state where proliferation is favoured over apoptosis. The angiogenic switch may thus be considered as the rate-limiting step in the tumour metastasis pathway. Furthermore, this switch is highly dependent on changes in the tumour microenvironment. The tumour microenvironment continues to increase in significance in angiogenesis research and understanding it holds the key to new and more successful anti-angiogenic cancer therapies.

Keywords Angiogenesis · Vascular endothelial growth factor (VEGF) · Endostatin · Basic fibroblast growth factor (bFGF) · Endothelial cells · Microenvironment

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G. Singh (ed.), *Oncodynamics: Effects of Cancer Cells on the Body*,
DOI 10.1007/978-3-319-28558-0_2

Abbreviations

ADM	Adrenomedullin
Ang	Angiopoetin
Akt	Protein kinase B
APC	Antigen-presenting cells
bFGF	Basic fibroblast growth factor
CAF	Cancer-associated fibroblasts
CCL2 CC	Chemokine ligand 2
CSF-1	Colony-stimulating factor-1
ECM	Extracellular matrix
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
ERK	Extracellular signal-regulated kinases
FAK	Focal adhesion kinase
HER	Human epidermal receptor
ICAM-1	Intercellular adhesion molecule-1
IL1β	Interleukin 1β
mCAF	Mammary CAF
MDSC	Myeloid-derived suppressor cell
MMP	Matrix metalloproteinase
MT1-MMP	Membrane-type1 matrix metalloproteinase
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NK cells	Natural killer cells
PA	Tissue-type plasminogen activators
PAI-1	PA inhibitior-1
PDGF	Platelet-derived growth factor
PI3K	Phosphoinositide 3-kinase
PlGF	Placenta growth factor
PMP	Platelet-derived microparticles
SDF-1	Stromal cell-derived factor 1
TEM	Monocytes expressing TIE-2 receptors
TGFα	Transforming growth factor α
TGFβ-1	Transforming growth factor β1
TME	Tumour mircoenvironment
TNF-α	Tumour necrosis factor α
TP	Thymididine phosphorylase
Sema4D	Semaphoring 4D
uPA	Urokinase-type plasminogen activator
VCAM-1	Vascular cell adhesion molecule-1
VEGF	Vascular endothelial growth factor
VPF	Tumour vascular permeability factor

Introduction

Neoplastic growth is closely linked to neovascularization, as efficient blood supply is necessary to deliver oxygen and nutrients to a tumour growing larger than 1–2 mm^3 whilst ensuring waste removal [1]. Neovascularization can be categorized into vasculogenesis (development of new capillaries from endothelial progenitor cells as in embryonic development), angiogenesis (development of new capillaries from pre-existing capillaries), vasculogenic mimicry (development of vessels that do not have endothelial cells), and vessel co-option (a process whereby the tumour co-opts host vasculature) [2–5]. Although all these different forms of neovascularization contribute to neoplastic vessel growth, angiogenesis is considered fundamental. Besides aiding tumour growth through adequate perfusion of the tumour, angiogenesis can also be used as an indicator for the tumour's metastatic potential and is often associated with a poor prognosis [6].

The development of blood vessels in tumours is modulated by pro- and anti-angiogenic factors. Pro-angiogenic factors include those that regulate remodelling of the extracellular matrix (ECM) and changes in perivascular cell structure, as well as those that promote endothelial cell changes and migration, including but not limited to vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and angiopoietin [1, 7, 8]. Anti-angiogenic factors include thrombospondin, 16kDA N-terminal fragments of prolactin and growth hormone, endostatin, vasostatin, and angiostatin [8]. During tumour growth, the balance is tipped in favour of pro-angiogenic factors. This is known as the angiogenic switch and allows for increased tumour progression, a state where proliferation is favoured over apoptosis [9, 10]. The angiogenic switch may thus be considered the rate-limiting step in the tumour metastasis pathway. Furthermore, this switch is highly dependent on changes in the tumour microenvironment (TME) [11]. The tumour microenvironment continues to increase in significance in angiogenesis research and understanding it holds the key to new and more successful anti-angiogenic cancer therapies.

Angiogenesis

Angiogenesis can be characterized into sprouting angiogenesis and intussusceptive angiogenesis (see Fig. 2.1) [1, 2]. The sprouting angiogenesis model, whereby new vasculature develops from pre-existing vasculature, was proposed by Folkman and Ausprunk in 1977. This process is initiated when pro-angiogenic factors cause the basement membrane to disintegrate and the intercellular junctions between endothelial cells to loosen. This allows for the rearrangement of endothelial cells, which can now invade the surrounding extracellular matrix (ECM) and produce a new basement membrane lining the new blood vessel (reviewed in [12]). More

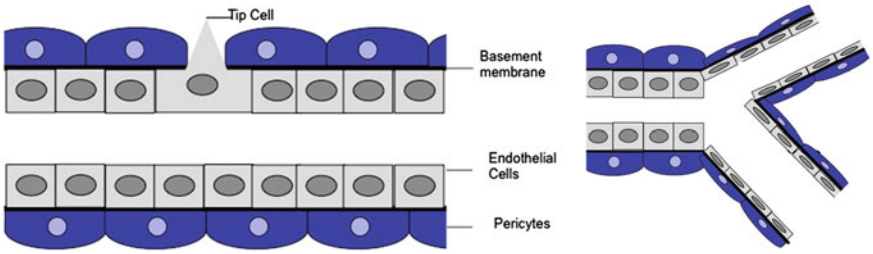

Fig. 2.1 Sprouting (*left*) versus Intussusceptive (*right*) angiogenesis

specifically, endothelial cell function is changed and a single endothelial cell or tip cell is stimulated by pro-angiogenic factors to begin forming a new vessel [13]. Factors secreted by tumour cells and the stroma, including VEGF, FGF, and PDGF, aid this process and serve as chemoattractant signals for the tip cell [1]. The developing sprout maintains its basal–luminal polarity, eventually connects to a neighbouring vessel, and is integrated into the existing vascular system by proliferating pericytes (vascular contractile cells) [14, 15]. The invasive nature of the sprouting process requires the proteolytic action of matrix metalloproteinases (MMPs), which will be discussed in further detail at a later point in this chapter. *In vivo*, sprouting is a slow process and it may take several days for the newly developed capillary to become a functional part of the vascular system [16]. Sprouting angiogenesis is considered the classical model of angiogenesis, but alternative models have been proposed. One such model focuses on intussusceptive or splitting angiogenesis. This type of angiogenesis may occur in response to stress or damage to the vasculature and is achieved by intraluminal growth [reviewed in 12, 17]. In comparison to sprouting angiogenesis, intussusceptive angiogenesis is a much faster process and does not primarily rely on endothelial cell proliferation [17]. Instead, existing vessel walls fold or develop protrusions within the lumen. Opposite endothelial membranes connect, thereby forming the kissing contact, and interendothelial junctions form. At the locus of contact, connective tissue columns or tissue pillars develop and insert themselves between the kissing contact, which results in the formation of two vessels [18]. Tissue pillars are formed when pericytes and myofibroblasts migrate into the collagen pillar core and deposit additional collagenous materials. Tumour cells also have the ability to migrate into developing tissue pillars and contribute to their growth [14]. Intussusceptive angiogenesis is an umbrella term for a process, by which the lumen of an existing vessel is split into two, and includes different subtypes, named for the variable location of the tissue pillars [19].

Although both angiogenic processes have been described separately, it is important to consider that they can occur simultaneously and are not mutually exclusive. Furthermore, a switch from sprouting to intussusceptive angiogenesis may occur, resulting in improved perfusion of the tumour [20].

Differences Between Normal Vessels and Tumour Vessels

Similar to normal vessels, tumour vessels consist of endothelial cells, mural cells (pericytes and smooth muscle cells), and a basement membrane [21]. However, it is important to note that the newly developed vessels in a majority of cancers differ in structure and function from normal vessels (see Fig. 2.2). Such abnormalities can affect the endothelial cells, pericytes, as well as the basement membrane, and result in the formation of tortuous, irregularly shaped, and hyperpermeable vessels [15, 21, 22]. The basement membrane is only loosely associated with the endothelial cells and pericytes, varies in thickness and layer composition, and may invade the stroma of the tumour [21]. However, only the loose association with the endothelial cells and pericytes is unique to tumour cells [23]. Tumour pericytes also play an important role in the abnormality of tumour vessels, as they have abnormal shapes, cannot cover the vessels, and have processes extending away from the vessel wall [24, 25]. This in turn leads to hyperpermeability of the vessels. In addition, the architecture of tumour-derived endothelial cells (TEC) is vastly different from normal endothelial cells, in that they are often leaky with wider junctions and several fenestrations [10, 22]. This architectural change may lead to haemorrhage and increased interstitial fluid pressure, as fluid is no longer contained in the intravascular space. It also hinders cell migration to the tumour site as well as the transport of drugs and oxygen to the tumour, which may aid the cancer in evading detection by the immune system, in becoming less responsive to chemotherapy, and in creating a hypoxic and subsequently acidotic environment. The high interstitial pressure, hypoxia, and low pH in the tumour microenvironment may further alter tumour cells and facilitate the transport of tumour cells through leaky vessels, thus favouring tumour metastasis [22]. In addition to the described differences between normal and abnormal cells, there is also a difference between different tumours in terms of vascular permeability [10]. This complicates targeting angiogenesis in cancer therapy.

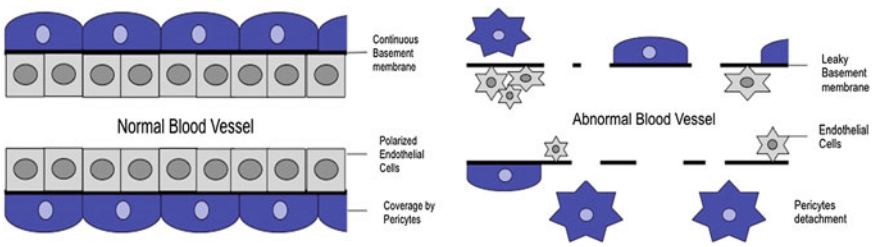

Fig. 2.2 Normal versus tumour vasculature

Molecules Released by Cancer Cells and Tumour Microenvironment

Vascular Endothelial Factor (VEGF)

The most prominent and vastly studied molecule involved in angiogenesis is the vascular endothelial factor (VEGF). VEGF was initially described as an endothelial cell-specific mitogen, while the tumour vascular permeability factor (VPF) was originally shown to increase the permeability of vasculature and to induce fluid accumulation in the peritoneal cavity in a guinea pig tumour model [26, 27]. Senger et al. [26] also stated that the secretion of a permeability factor might be a commonality between all tumour cells [27]. In 1989, cDNA of VPF and VEGF was isolated by two independent research groups [28]. It was subsequently determined that VPF and VEGF were essentially the same molecule and mediated similar biological functions [29]. VEGF can act in a paracrine or an autocrine fashion to regulate normal or abnormal angiogenesis and is expressed in most solid tumours, including cervical cancer, breast cancer, and colon cancer [28, 30, 31].

The human genome contains five genes encoding five VEGF family members: VEGF-A (predominantly known as VEGF), placenta growth factor (PlGF), VEGF-B, VEGF-C, and VEGF-D. These proteins consist of two subunits (120–200 amino acids) and are thus considered homodimers [29]. Out of these five family members, VEGF-A plays a key role in angiogenesis during embryonic development as well as tumour angiogenesis. VEGF-A binds to the Fms-like tyrosine kinase receptor VEGFR-1/Flt-1 and VEGFR-2 [9, 27]. This Fms-like tyrosine kinase receptor contains seven extracellular immunoglobulin domains and exhibits structural similarity to the Fms receptor [29]. VEGF-A can also bind to VEGFR-2 but with lower affinity, despite VEGFR-2 exhibiting a much stronger tyrosine kinase activity than VEGFR-1. VEGFR-2 has been implicated in vascular permeability and is considered a major player in angiogenesis. When VEGF-A (predominantly its splice variants VEGF-A121 or VEGF-A165) binds to VEGFR-2, the receptor is autophosphorylated and a downstream signalling cascade, involving phosphoinositide 3-kinase (PI3K), focal adhesion kinase (FAK), protein kinase B (Akt), and extracellular signal-regulated kinases (ERK), is induced [9]. In particular, the activation of the Rho GTPase pathway seems to be involved in all aspects of angiogenesis ranging from vascular permeability and ECM degradation to lumen formation [9]. Tumour VEGF signalling can be enhanced through inflammatory cytokines, growth factors such as PDGF, as well as hypoxic conditions [9]. In hypoxic areas of tumour masses particularly, VEGF-A expression can directly be upregulated through the hypoxia-inducible factor alpha (HIF-alpha) or indirectly be upregulated through galectin-1 expression, as is the case in human breast cancer [32]. In addition to tumour cells, VEGF-A may be secreted by normal keratinocytes, leukocytes, red blood cells, platelets, and tumour-associated macrophages [33, 34].

VEGF-A was also found to induce the expression of anti-apoptotic proteins Bcl-2 and A1 in human endothelial cells, serine proteases, tissue-type plasminogen activators (PAs), PA inhibitior-1 (PAI-1) in bovine endothelial cells, and metalloproteinases in human umbilical vein endothelial cells [28]. Moreover, VEGF-A is involved in immune responses by promoting the expression of the vascular cell adhesion molecule-1 (VCAM-1) and the intercellular adhesion molecule-1 (ICAM-1) to enhance adhesion of natural killer (NK) cells to endothelial cells via VCAM-1/CD18 and ICAM-1/VLA-4 interactions [28]. VEGF-A also induces monocyte chemotaxis and may inhibit antigen-presenting cells (APCs) from maturing [28]. As such, VEGF-A may facilitate tumour growth by inhibiting antigen-presenting cells from maturing and thus reduce a tumour-targeted immune response. In addition, VEGF-A can aid angiogenesis by increasing the expression of MMPs and plasminogen activators in order to degrade ECM and allow endothelial cells to migrate [35]. It becomes thus quite evident that VEGF-A plays a prominent role in tumour angiogenesis.

PlGF also plays an important role in angiogenesis, as it is secreted in large amounts by activated endothelial cells and in turn regulates the VEGF-mediated angiogenic switch [1]. Furthermore, PlGF has been shown to attract myeloid-derived suppressor cells (MDSCs) and tumour-associated macrophages (TAMs) to the tumour microenvironment (TME) [36]. PIGF is specific to VEGFR-1 and may play a larger role in maintaining tumour blood vessels rather than inducing the development of new vasculature [37].

VEGF-B is arguably the least studied of the VEGF family members. It is a specific ligand to VEGFR-1 as well as neuropilin-1 (NP-1) receptor and has a high sequence homology to VEGF-A but its role in angiogenesis and blood vessel permeability has been controversial [38]. Similar to PIGF, the primary role of VEGF-B seems to centre around the survival of blood vessels in times of stress by inhibiting apoptosis of endothelial cells, smooth muscle cells, and pericytes [37, 38]. These findings suggest that VEGF-B has a survival/anti-apoptotic effect, rather than exhibiting angiogenic activity [37]. Nonetheless, VEGF-B may play a significant role in anti-angiogenic therapy, emphasizing a link between survival/anti-apoptotic and angiogenic activity. As increased coverage by smooth muscles and pericytes confer resistance to anti-angiogenic therapy, limiting the function of VEGF-B may prove to be a viable therapeutic target [38].

Fibroblast Growth Factor (FGF)

Fibroblast growth factors (FGF), among which the basic fibroblast growth factor (bFGF) or FGF2 was the first to be discovered, belong to a family of heparin-binding growth factors and play a key role in the tumour angiogenesis signalling cascade [39]. FGFs are produced by macrophages and tumour cells, among many other cells. They can then bind to their respective receptors, FGR1

and FGR2, on endothelial cells to directly influence the angiogenic process or to indirectly modulate it by inducing the release of pro-angiogenic factors [10, 39]. To be more specific, FGF functions include endothelial cell proliferation, ECM degradation via the upregulation of MMPs, as well as modulation of adhesion proteins including integrins and cadherins [1]. FGFs act synergistically with VEGF-A to promote angiogenesis and may also mediate the resistance to anti-VEGF or anti-EGFR tumour therapy [10, 40].

Angiopoietin

Angiopoietin signalling plays a major role in angiogenesis as well, as it is involved in vasculature development and maturation. In humans, this growth factor family contains three members: angiopoietin-1 (Ang-1), angiopoietin-2 (Ang-2), and angiopoietin-4 (Ang-4) [1]. Ang-1 is produced by pericytes, smooth muscle cells, fibroblasts, and monocytes. Ang-2 is solely produced by endothelial cells, as is the case with cells of the tumour endothelium [41]. The tumour endothelium also facilitates further recruitment of monocytes expressing TIE-2 receptors (TEM) to the tumour site [42]. All members of the angiopoietin family have the ability to bind to TIE-2, a tyrosine kinase receptor expressed on endothelial cells, but can elicit opposing effects. Ang-1 activates TIE-2 signalling and is involved in endothelial cell migration and adhesion as well as pericyte and smooth muscle cell recruitment, whereas Ang-2 serves to inhibit this signalling cascade and functions to decrease vessel stability [43]. Although Ang-1 activates TIE-2 signalling, the role of Ang-1 in tumour angiogenesis is unclear. Depending on tumour type, Ang-1 may enhance or limit growth. Thus, targeting Ang-1 in tumour therapy may not be effective. Ang-2 on the other hand seems to be a more promising target. It is overexpressed in mammary carcinoma, melanoma, and metastatic colorectal cancer leading to non-functional and abnormal blood vessel formation [44, 45]. It has also been suggested that Ang-2 acts together with VEGF-A to further increase tumour vessel permeability and vessel sprouting [7]. It has been thus suggested that VEGF-A anti-angiogenic therapy resistance may be modulated by the upregulation of Ang-2 [46]. In contrast to the hypothesis that VEGF-A and Ang-2 work together to promote angiogenesis, TIE-2 expression on endothelial cells was found to be downregulated via VEGF-A signalling [41]. Therefore, further research is warranted to elucidate the interaction between Ang-2 and VEGF-A. In addition to being a potential therapeutic target, Ang-2 may also serve as a clinical screening marker, as patients with non-small cell lung cancer in comparison to healthy subjects were shown to have higher plasma Ang-2 levels [47]. Plasma levels of Ang-2 may also be used as a biochemical outcome marker to determine metastasis potential and prognosis, as was demonstrated in patients with melanoma and metastatic colorectal cancer [44, 45].

Matrix Metalloproteinases (MMPs)

Matrix metalloproteinases (MMPs), such as MT1-MMP, MMP-2, and MMP-9, belong to a family of zinc-containing calcium-dependent endopeptidases active at neutral pH and have proteolytic functions in the extracellular matrix [48]. The extracellular matrix consists of collagen, proteoglycans, and glycoproteins, and is constantly remodelled by MMPs. MMPs are responsible for the degradation of the basement membrane and matrix proteins in the ECM to allow for new vessel development, as well as modulating the balance between pro- and anti-angiogenic factors. The latter is mainly accomplished by producing protein fragments, which can either inhibit or activate angiogenesis [13]. Thus, MMPs are essential in regulating sprouting angiogenesis. In particular, MMP-9 plays a significant role in angiogenesis, as it can release VEGF-A through the degradation of the ECM [49].

MMP activity is controlled by a membrane-anchored glycoprotein, called the reversion-inducing cysteine-rich protein with Kazal motifs (RECK). RECK expression was found to be decreased in tumours of the liver, pancreas, breast, colon, lung, and skin [49]. In cancer, Ras proteins may downregulate RECK, which may result in an increased secretion of MMP-9 but not MMP-2 and thus inhibit VEGF-A induced angiogenesis [50, 51].

Cells in the Tumour Microenvironment (TME) Involved in Angiogenesis

Although signalling cascades involved in angiogenesis have been studied in the past, it is essential to note that pro-angiogenic factors are not solely secreted by tumour cells. A significant role is played by components of the tumour microenvironment (TME), as pointed out throughout this chapter. Tumour cells have been shown to interact with surrounding inflammatory cytokines, the ECM, platelets, cancer-associated fibroblasts (CAF), tumour endothelial cells (TEC), and tumour-associated macrophages (TAM).

Platelets

Platelets are physiologically involved in hemostasis and wound healing. Upon activation, platelets also secrete pro-angiogenic proteins, such as VEGF-A, FGF, insulin-like growth factor 1 (IGF-1), PDGF, angiopoietins, stromal cell-derived factor-1 (CXCL12), MMP-1, MMP-2, and MMP-9 [33, 52]. Platelets may contain VEGF-A for two reasons: (1) synthesis of VEGF-A and (2) endocytosis of

circulating VEGF-A [33]. The activation of platelets can be mediated by thrombin [53]. The involvement of thrombin in angiogenesis and its link to platelets was further strengthened by Yuan and Liu [54], who found that carcinoma patients had a shorter thrombin time (increased levels of thrombin). Besides being involved in coagulation and wound healing, thrombin can induce angiogenesis by binding to its receptor PAR-1 on endothelial and tumour cells [55]. Targeting PAR-1 in ovarian carcinoma led to a decreased expression of pro-angiogenic factors, including VEGF-A, IL-8, and MMP-2 [54]. This further highlights the role of the tumour microenvironment in angiogenesis.

It was also shown that platelets shed small plasma membrane vesicles, called microparticles. These platelet-derived microparticles (PMP) play a role in angiogenesis and have been associated with tumour aggressiveness and poor prognosis. Their angiogenic function may be mediated by stored growth factors and survival factors [52]. Platelets also express markers on their surface, which mediate tumour angiogenesis. One such marker is CD41, which is involved in mediating the adhesion of platelets to endothelial cells and promoting endothelial-dependent angiogenesis. The expression of CD41 was correlated with increased levels of VEGF-A in ovarian carcinoma, further suggesting that platelets play a role in inducing angiogenesis [54].

Cancer-Associated Fibroblasts (CAF)

Fibroblasts are spindle-shaped cells and are physiologically involved in ECM synthesis, inflammation, and wound healing. Fibroblasts are also found in the tumour microenvironment, where they play a role in tumour growth, angiogenesis, and ECM remodelling [56–58]. These fibroblasts are termed cancer-associated fibroblasts (CAF). In particular, mammary CAF (mCAF) were shown to secrete pro-angiogenic factors, such as bFGF, EGF, VEGF, and a variety of MMP [59, 60]. mCAF also enhance angiogenesis by releasing stromal cell-derived factor 1 (SDF-1) to recruit endothelial progenitor cells into carcinomas [57]. Furthermore, mCAF recruit immune cells to the tumour microenvironment, further contributing to the development of a niche favourable for tumour progression and metastasis [58]. mCAF were also shown to interact with tumour cells for metabolic purposes, as mCAF secrete pyruvate and lactate to provide energy metabolites for the tricarboxylic acid cycle to cancer cells in hypoxic environments, a process termed the reverse Warburg effect [56, 61]. The presence of CAF in the tumour microenvironment is strongly associated with resistance to cancer therapy. CAF mediates resistance to the chemotherapy drug tamoxifen via MMP, among other factors [62]. Despite its key role in cancer and therapy resistance, targeting CAF for cancer therapy is challenging. One reason for this is that CAF express a variety of

molecular markers but none of them are unique to CAF [63]. Targeting CAF may thus produce undesired off-target effects.

Tumour Endothelial Cells (TEC)

Tumour Endothelial Cells (TEC) may have their origin in normal endothelial cells. It is postulated that these endothelial cells change their phenotype as a result of the tumour microenvironment [24]. In renal carcinoma, melanoma, and liposarcoma, TEC have also been found to be aneuploid (containing an abnormal number of chromosomes), which may be the result of factors secreted by the tumour microenvironment [64]. Aneuploidy affected TEC structure, causing the loss of normal apical–basal polarity. Furthermore, TEC showed evidence for altered function and were able to proliferate without senescence *in vitro* [65]. TEC may also be derived from the differentiation of bone marrow-derived circulating or tissue-resident stem cells, as well as tumour cells [24]. Again, the tumour microenvironment may play a significant role in this differentiation, which may occur in a VEGF-dependent or VEGF-independent manner [66].

Furthermore, TEC can express EGFR. Interestingly, EGFR is also expressed by a variety of solid tumours of the breast, colon, lung, pancreas, head and neck, bladder, and brain [67]. The epidermal growth factor receptor (EGFR) belongs to the human epidermal receptor (HER) family, which consists of four receptor tyrosine kinases, and can be bound by its ligands—epidermal growth factor (EGF) and transforming growth factor alpha (TGFα). This elicits a pro-angiogenic response, which is likely mediated by the upregulation of VEGF-A and MMP [68]. As such, EGFR and TEC serve as another target for anti-angiogenic therapy in cancer.

Tumour-Associated Macrophages (TAM)

Tumour-associated macrophages (TAM) are monocytes that have been recruited to the tumour site and that have been primed by molecules secreted by the tumour, including colony-stimulating factor-1 (CSF-1), VEGF-A, and CCL2 [69, 70]. Recruitment may occur in response to hypoxic conditions. Hypoxia can upregulate the expression of pro-angiogenic chemokines secreted by macrophages, including CXCL12, C-C chemokine ligand 2 (CCL2), CXCL8, CXCL1, CXCL13, and CCL5 [71]. TAM phenotype is highly dependent on the tumour microenvironment with a predominance of pro-tumour M2-polarized TAM over antitumour M1-polarized TAM. In models of polyoma virus middle T oncogene (MMTV-PyMT) induced mammary adenocarcinoma, increased presence of TAMs correlated with conditions favourable for angiogenesis and metastasis [6]. TAMs may secrete factors

regulating angiogenesis, including but not limited to VEGF-A, bFGF, EGF, tumour necrosis factor alpha (TNF-α), cytokines (IL-1, IL-2, IL-6, IL-8, IL-12, and IL-17), PDGF, matrix metalloproteinases (MMP-9), nitric oxide, thymididine phosphorylase (TP), urokinase-type plasminogen activator (uPA), adrenomedullin (ADM), and semaphoring 4D (Sema4D) [6, 42]. In particular, TAM expressing CSF-1 receptors infiltrate the stroma of the primary solid tumour, promote increased vessel density, and are thus actively involved in promoting the angiogenic switch [6]. In addition to breast cancer, TAM involvement has been established in animal models of ovarian cancer, melanoma, prostate cancer, cervical cancer, gliomas, lymphomas, and other solid tumours [42]. The importance of TAM research also translates to human cancers, as TAM density has been associated with increased levels of VEGF-A, which is the result of TAM expressing the hypoxia-inducible factor HIF-1α [42]. Besides hypoxia, interleukin 1β (IL1β) and transforming growth factor β1 (TGFβ-1) were shown to induce the HIF-1 controlled VEGF-A expression. Alternatively, tumour-released M-CSF could induce the expression of VEGF-A by TAM through the activation of the nuclear factor kappa-light-chain-enhancer of activated B cells, NF-κB [72, 73]. The release of VEGF-A from the extracellular matrix was also found to be facilitated by MMP-9, another molecule secreted by TAM. Although VEGF-A seems to be highly important in angiogenesis, other factors are equally involved in promoting angiogenesis. One such example is TP, a molecule involved in endothelial cell migration, which in high levels is accociated with poor prognosis in human glioma, gastric cancer, breast cancer, and pancreatic cancer. Interestingly, it has been shown that tumour cells and TAM may act synergistically to amplify the production of pro-angiogenic factors in the tumour microenvironment and thus facilitate the angiogenic switch [42].

Conclusion

Angiogenesis is mediated by a variety of factors, including VEGF-A, FGF, angiopoietin, and MMP. Due to the importance of the angiogenic process in tumour progression and metastasis, it has been long considered as a target for cancer therapy. However, anti-angiogenic therapy has encountered significant challenges due to therapy resistance, which may be facilitated by altered cell characteristics or re-neovascularization. It has been suggested that tumour cells, which have acquired pericyte or smooth muscle coverage confer increased anti-angiogenic therapy resistance [38]. Tumours may also adapt to anti-angiogenic therapy and circumvent the need for angiogenesis by primarily invading locally or co-opting existing blood vessels [43]. Instead of being a cell autonomous process, it is important to remember that tumour progression and angiogenesis is co-mediated by the tumour microenvironment, a process termed oncodynamics. This means that future therapies targeting angiogenesis will have to consider off-target effects and the role of the microenvironment in order to be successful.

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Oncodynamics: Effects of Cancer Cells on the Body
Singh, G. (Ed.)
2016, XIV, 215 p. 21 illus., 4 illus. in color., Hardcover
ISBN: 978-3-319-28556-6