Overview of Angiogenesis: Mechanisms and Predictors

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Introduction

Angiogenesis is an important biological process not only under physiological conditions but in a variety of diseases including cancer [Risau 1997]. It is the sprouting of new blood vessels from the pre-existing ones. This process is important for the growth of new blood vessels during fetal development and tissue repair; however, uncontrolled angiogenesis promotes neoplastic diseases and other disorders. Under these conditions, angiogenesis is a highly regulated process, i.e. turned on for brief periods and then completely inhibited [Folkman and Shing, 1992].

After the primary vascular plexus is formed, more endothelial cells (ECs) are generated, which can form new capillaries by sprouting or by splitting from their vessel of origin in a process termed angiogenesis [Risau 1997]. Angiogenesis depends on the balance between different molecules released by the host and tumor cells, and consists of a series of steps, including separation of ECs from pericytes and the basement membrane, invasion and migration across basement membranes, and eventually resulting with the extension into the tumor body [Carmeliet 2000; Hanahan and Folkman, 1996]. Specific angiogenic molecules can initiate this process. Specific inhibitory molecules can stop it.

Numerous inducers of angiogenesis have been identified, including the members of the vascular endothelial growth factor (VEGF) family, angiopoietins, transforming growth factors (TGF), platelet-derived growth factor, tumor necrosis factor-α, interleukins, and the members of the fibroblast growth factor (FGF) family [Papetti and Herman, 2002; Presta et al, 2005]. In addition, many factors control and influence angiogenesis including soluble growth factors, membrane-bound proteins, cell-matrix and cell-cell interactions, and many interacting systems [Papetti and Herman, 2002].

In this manuscript, we give an overview of important mediators and molecular mechanisms in angiogenesis. Predictors of angiogenesis like hypoxia inducible factor-1 (HIF-1), mast cell density as a surrogate for microvessel density [Acikalin et al, 2005], and CD34, have been actively investigated recently for their potential role as prognostic factors in tumor recurrence, aggressiveness and resistance to chemotherapy. Nevertheless, inhibitors to these molecules are being investigated in different malignancies and different settings. Direct and indirect inhibitors exist. Some of these inhibitors are already in the cilinical use like CCI 779, an mTOR inhibitor responsible for indirect inhibition of HIF-1 alpha [Mellilo 2007]. Further understanding of the tumor microenvironment will lead to development of more effective and more specific targeted therapy consequently leading to better clinical outcomes. In order to facilitate this understanding, we shed light on the role of each of vascular endothelial growth factors and receptors, matrix metalloproteinases system, and plasminogen activator/plasmin system in angiogenesis.

Vascular endothelial growth factors

One of the most specific and crucial regulators of angiogenesis is vascular endothelial growth factor (VEGF) [Gupta and Zhang, 2005]. The VEGF family comprises seven secreted glycoproteins that are designated VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, placental growth factor (PlGF) and VEGF-F [Ferrara et al, 2003; Houck et al, 1991; Suto et al, 2005].

Vascular endothelial growth factor-A (VEGF-A; also referred to as VEGF) is the best characterized and the most studied of the VEGF family members. It is a tumor-secreted cytokine with grave importance in both normal and tumor-associated angiogenesis [Rini and Small, 2005]. VEGF-A exerts its biologic effect through interaction with cell-surface receptors. These are transmembrane protein tyrosine kinase receptors and they include VEGF receptor-1 (VEGFR-1) and VEGFR-2, selectively expressed on vascular ECs, and the neuropilin receptors (NP-1 and NP-2), expressed on neurons and vascular endothelium [Dvorak 2002]. Upon binding of VEGF-A to the extracellular domain of the receptor, a cascade of downstream proteins are activated after the dimerization and autophosphorylation of the intracellular receptor tyrosine kinases. VEGFR-2 appears to be the major receptor responsible for mediating the proangiogenic effects of VEGF-A [Ferrara et al, 2003; Cross et al, 2003]. VEGFR-3 preferentially binds VEGF-C and VEGF-D. VEGFR-3 is up-regulated on blood vascular ECs in pathologic conditions such as in vascular tumors and in the periphery of solid tumors [Partanen et al, 1999]. VEGFR-3 expression was correlated with transient lymphangiogenesis in wound healing and was up-regulated in blood vessel endothelium in chronic inflammatory wounds [Paavonen et al, 2000]. Thus, VEGFR-3 is believed to play various roles in cardiovascular development and remodeling of primary vascular networks during embryogenesis and enhancing lymphangiogenesis in adulthood.

VEGF-A is the most potent pro-angiogenic protein described to date. It induces proliferation, sprouting and tube formation of ECs [Ferrara et al, 2003]. In addition, it causes vasodilatation by inducing the endothelial nitric oxide synthase and so increasing nitric oxide production [Hood et al, 1998]. VEGF-A binds many receptors on hematopoietic stem cells (HSCs), monocytes, osteoblasts and neurons [Ferrara et al, 2003]. In vivo, VEGF-A expression has been shown to be associated with significant steps in angiogenesis and physiologic vasculogenesis [Jakeman et al, 1993; Shweiki et al, 1993]. In mice, deletion of the VEGF-A gene is lethal, resulting in vascular defects and cardiovascular abnormalities [Carmaliet et al, 1996]. VEGF-A affects an important number of angiogenic processes including

wound healing, ovulation, maintenance of blood pressure, menstruation and pregnancy [Brown et al, 1992]. In humans, VEGF-A is expressed in practically all solid tumors studied as well as in some hematological malignancies [Ferrara et al, 2003]. Recently, VEGRF-A (specifically VEGF189) was over-expressed in patients with distant metastases of pulmonary adenocarcinoma [Nishi et al, 2005].

Matrix Metalloproteinases System in Angiogenesis

The degradation of the basement membranes is an essential requirement for the formation of new vessels. Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes that degrade various components of the ECM. They can be divided into two structurally distinct groups, namely secreted MMPs and membrane-type MMPs (MT-MMPs) [Pepper 2001]. The secreted MMPs include collagenases (MMP-1, MMP-8, and MMP-13), stromelysins (MMP-3, MMP-10, and MMP-11), gelatinases (gelatinase A or MMP-2; gelatinase B or MMP-9) and other MMPs [Pepper 2001].

The role of several MMPs has been characterized in ECs and in the context of angiogenesis. In particular, gelatinases A and B, MMP-2 and MMP-9, play an important role in the angiogenic response as demonstrated in ECs as well as in in vivo animal models deficient in these proteases [Genis et al, 2006]. Thus, MMP-9 is critical for the angiogenic switch required during tumorigenesis as shown in a model of pancreatic cancer; MMP-9 acts by releasing VEGF from the proteoglycan matrix [Bergers et al, 2000]. The role of other MMPs in angiogenesis may depend on the tissue/organ from which ECs are derived. In this regard, it has been shown recently that MT3-MMP is preferentially required for the formation of new capillaries by endometrial ECs [Plaisier et al, 2004]. Recently it has become apparent that inhibition of MMP activity is essential for vessel stabilization during the resolution phase of angiogenesis; unchecked proteolysis results in regression of newly formed vessels [Kraling et al, 1999; Zhu et al, 2000].

MT1-MMP, the first matrix metalloproteinase identified that was anchored to the cell membrane instead of being soluble, seems to be a key player in the angiogenic response. MT1-MMP was identified as the fibrinolysin responsible for degrading and remodeling the fibrin matrix often deposited during vascular injury [Hiraoka et al, 1998]. The generation of MT1-MMP-deficient mice has supported the requirement of MT1-MMP for angiogenesis. MT1-MMP-deficient mice exhibit severe skeletal deformities which lead to death by 3-16 weeks of age [Holmbeck et al, 1999; Zhou et al, 2000]. MT1-MMP participates in several of the steps of the angiogenic response including degradation of the ECM and endothelial invasion [Hiraoka et al, 1998; Chun et al, 2004; Oblander et al, 2005], endothelial migration [Galvez et al, 2001; Galvez et al, 2002], and formation of capillary tubes [Galvez et al, 2001; Langlois et al, 2004; Robinet et al, 2005].

Plasminogen Activator/Plasmin System in Angiogenesis

Plasmin is a broad-spectrum protease which is presumed to hydrolyse many extracellular proteins, most notably fibrin. Urokinase-type plasminogen activator (uPA) and tissue plasminogen activator (tPA) are serine proteases that mainly activate plasminogen; plasminogen is their specific substrate. uPA is a serine protease and binds to a specific glycosylphosphatidylinositol-anchored cell surface receptor (uPA receptor-uPAR) [Pepper 2001].

Assessing the role of the individual components of the PA/plasmin system in angiogenesis in vivo was made possible by the generation of mice deficient in these components [Carmeliet and Collen, 2000]. Plasminogen activator inhibitor type-1 (PAI-1) is considered one of the key regulators of tumor invasion, metastasis, as well as cancer-related angiogenesis [Chorostowska-Wynimko et al, 2004]. Hypoxia which is a chief stimulus for angiogenesis was reported to increase uPAR [Kroon et al, 2000] and PAI-1 [Uchiyama et al, 2000] in ECs. In vivo, several studies have demonstrated a necessity for the PA/plasmin system in angiogenesis and tumor cell invasion [Bajou et al, 1998; Heymans et al, 1999].

Anti-angiogenesis: a medical revolution in therapeutic approach

In the last decade, there has been a tremendous advancement in the treatment of many malignancies using anti-angiogenic medications. This has impacted on the outcome and survival of cancer patients.

Among the most effective anti-angiogenic medications introduced into the armamentarium of cancer treatment are Bevacizumab and selected tyrosine kinase inhibitors (TKIs). Bevacizumab (Avastin) is a recombinant, humanized monoclonal antibody against VEGF that is used to inhibit VEGF function in vascular endothelial cells and thereby inhibit tumour angiogenesis. The addition of bevacizumab to irinotecan or oxaliplatin in metastatic colorectal cancer significantly increased median progression-free survival and overall survival in most randomized clinical trials. Overall survival advantage due to bevacizumab was 4.7 months when used as first-line therapy in phase III trials in metastatic colorectal carcinoma [McCormack and Keam, 2008]. In metastatic renal cell carcinoma (RCC), bevacizumab resulted in a significantly longer progression-free survival when compared to the traditional treatment of IFN-α (10.2 versus 5.4 months) [Escudier et al, 2007]. In addition to their role as kinase inhibitors, TKIs have an anti-angiogenic effect. For instance, Sunitinib and Sorafenib have a significant activity in RCC through their anti-angiogenic properties. Sunitinib, an oral TKI, resulted in a median progression-free survival of 11 month which was significantly longer than that in the interferon alfa treatment group (5 months) [Motzer et al, 2007].

Angiogenesis: Clinical significance and predictors

One of the most important and independent predictors of survival in cancer patients is angiogenesis [Weidner et al, 1999]. This has been noticed in a variety of malignancies like breast, lung, bladder, prostate and skin cancers [Weidner et al, 1999; Bosari et al, 1992; Weidner et al, 1993; Macchiarini et al, 1992; Srivastava et al, 1988; Beatrice et al, 1998; Williams et al, 1994]. Angiogenesis has been correlated not only with survival, but rather with distant metatstasis and chemotherapy resistance.Microvessel density, for instance has been proven to be a very significant factor in predicting tumour recurrence and time to recurrence in colorectal cancer [Engel et al, 1996]. Tanigawa et al. reported a strong association between vascularity and overall survival in 133 patients with colorectal adenocarcinoma [Tanigawa et al, 1997]. In another study on the same type of colorectal cancers, Zheng et al reported a strong correlation between microvessel density on one hand and stage and tumour grade on the other hand [Sasaki et al, 2001]. Based on these facts, it is crucial to look for predictors of angiogenesis and finding potential inhibitors for these molecules. Since micovessel density is a reliable means for evaluation of the severity of angiogenesis, several markers have been introduced into the diagnostics. Different endothelial cell markers, such as CD31, CD34 and von Willebrand factor were used for evaluating neovascularisation. Monoclonal antibodies were developed in order to detect these markers in tumor tissues [Singhal et al, 2005]. In addition to PDGF, bFGF, and IL-8 are considered to play a major role in the angiogenic process. Basic fibroblast growth factor (bFGF) which originates from extracellular matrix under the effect of proteolytic enzyme increases the expression of other proteolytic molecules leading to proangiogenic and increase tumor growth [Cox et al, 2000]. Similarly there is evidence that IL8 was associated with increased microvessel density in NSCLC and eventually with a worse outcome [Yuan et al, 2000].

Several other growth factors also play a role in the development of tumoral blood supply. For instance a newly discovered cytokine produced by mesenchymal cells known as hepatocyte growth factor exerts its effect on epithelial and endothelial cells in NSCLC through binding its receptor. Tissue factor and c-met protein have a major role in angiogenesis in NSCLC as well though details about its mechanism of action are not yet well understood. There is some evidence that hepatocyte growth factor, c-met, and tissue factor may have prognostic role in NSCLC cases [Takanami et al, 1996; Siegfried et al, 1998; Tokunou et al, 2001; Koomagi and Volm, 1998].

HIF-1 α : a marker and a target

Among the aforementioned factors, HIF-1 seems to be the major player with avid evidence on its significant role in angiogenesis in different cancers in humans. This role has been persued since the 1990's.

HIF-1 is composed of HIF-1 alpha and HIF-1 beta [Wang et al, 1995]. Under normal

condition HIF-1 alpha activity is induced in response to hypoxia and this induction of HIF-1 α gene transcription was shown to directly regulate VEGF expression in xenograft tumors [Maxwell et al, 1997]. Since malignant cells has hypoxic conditions HIF-1 α is overexpressed in these cells which leads to VEGF and SDF-1 (stromal –derived growth factor 1 protein that are known to induce angiogenesis [Schofield and Ratcliffe, 2005; Semenza 2003]. HIF-1 alpha accumulation in hypoxic conditions is mainly due to inhibition of 26S proteosome-mediated HIF-1 alpha degradation during hypoxia [Schofield and Ratcliffe, 2005]. Degradation of HIF-1 α could be inhibited by decreased binding of mutated VHL to protein to as in renal cell carcinoma condtion leading to its accumulation and increased neovascularization [Blancher et al, 2001]. This increase in HIF-1 α expression has been documented by histochemical analysis of tissue biopsies of different human cancers [Semenza 2003]. In addition to VEGF-1 and SDF-1, HIF-1 α controls the expression of other growth factors responsible for angiogenesis; these include ANGPT1, ANGPT2, PLGF and PDGF-B [Ravi et al, 2000]. Increased HIF-1 α leads to increase in insulin like growth factor 2 (IGF-2) resulting in enhancing IGF-1 receptor tyrosine kinase and activation of PI3K and MAP kinase pathways responsible for cell survival and proliferation [Fukuda et al, 2002]. At the animal level using mice models, tumor neovascularization was found to be impaired in HIF-1 α –deficient endothelial [Tang et al, 2004].

HIF-1 α and therapeutic options

Inhibitors of HIF-1 α could be either selective or non selective with most of the inhibitors being non-selective ones [Rapisarda and Melillo, 2008]. Examples of direct inhibitors include Chetamine which is known to block recruitment of coactivator P300/CBP [Kung et al, 2004], echinomycin [Kong et al, 2005] and synthetic polyamides [Olenyuk et al, 2004] which directly inhibits binding of HIF-1 α to DNA. There are new efforts for targeting the dimerization of HIF-1 α to HIF-1 β .

Indirect HIF-1 α inhibitors on the other hand are numerous and target pathways involved in activating HIF-1 α as an end point [Rapisarda and Melillo, 2008]. Among these are Hsp90 inhibitors which downregulate HIF-1 α . Microtubule inhibitors like methoxyestradiol (2ME2), which in addition to downregulating HIF-1 α , inhibits dimerization of microtubule [Melillo 2007]. Topoisomerase I inhibitors like topotecan, due to their small size are able to act as inhibitors of the HIF-1 α at meytreonomic daily doses the fact which lead the NCI to run a prospective trial on the use of topoisomerase I inhibitors in metastatic cancers as a HIF-1 α expression inhibitors.
Histone deacetylase inhibitors and tyrosine kinase receptor inhibitors are known to produce HIF-1 α inhibition, though the efficacy of the former as HIF-1 α therapeutic inhibitor is still controversial [Melillo 2007]. On the other hand, the efficacy of tyrosine kinase inhibitors in treating malignancies is well known and is greatly attributed to HIF-1 α inhibition [Rapisarda and Melillo, 2008]. mTOR inhibitors like CCI 779 which is already in clinical use of RCC affects indirectly HIF-1 α expression as well [Melillo 2007]. Combination of HIF-1 α inhibitors with other antiangiogenic factors, chemotherapy and radiotherapy is to be further investigated in order to determine its efficacy.

Angiogenesis: Radiologic Biomarkers and Future perspective

Sustained angiogenesis is one of the pathophysiological processes in solid tumors that are targeted by different agents [Hanahan and Weinberg, 2000; Gibbs 2000]. Effect of therapeutic agents used in malignancy includes either cytolysis or cytostasis; targeted therapy includes mainly cytostasis maintaining same tumor size though less activity leading to response which is difficult to assess when using traditional CT scan and MRI [Hurwitz et al, 2004; Johnson et al, 2004]. PET and dynamic contrast enhanced MRI (DCE-MRI) can do better evaluation by measuring microvascular characteristics [O'Connor et al, 2008]. Measurement in Hounfield units (HU) using contrast CT scan by calculating the perfusion score through subtracting the post-contrast CT data from the pre-contrast one was proven to be a useful biomarker of tumor blood supply [Koukourakis et al, 2007]. Another important biomarker for tumor response when using PET scan is standardized-uptake value (SUV) [Kelloff et al, 2005], but this was not effective when tried on a population of patients with rectal cancer who received Bevacizumab [Willett et al, 2004]. On the other hand, using DCE-CT and DCE-MRI was able to show rapid antivascular effect in patients with rectal carcinoma who received Bevacizumab [Willett et al, 2004]. This implies that each tumor type and therapeutic approach might need a specific biomarker and these different biomarkers depend mainly on reduced blood flow or permeability [O'Connor et al, 2008].

One of the most recent and interesting approaches for solid tumors assessment is measurement of tumor vascular function by using PET scan [Anderson and Price, 2002]. During this procedure dynamic data is collected within 10 minutes after intravenous injection of short bolus of 15 O-labelled water (^{15}O-labelled water ([^{15}O] H2O) in order to measure blood flow [Anderson and Price, 2002]. Another approach is arterial spin labeling using the MRI, which is an alternative biomarker of tumor blood flow which is promising in assessment of response to blood flow and antiangiogenic therapy [De Bazelaire et al, 2005; Wong et al, 2005]. Its technique depends on using magnetically labeled protons in tissue water and calculating the difference between control and labeled images with no need to administer exogenous contrast [Petersen et al, 2006]. New studies are using vessel size imaging in order to measure capillary diameter as a biomarker, the thing which affects transverse relaxation rate and effective transverse relation rate [Donahue et al, 2000]. All these factors like change in tumor size, HU changes, and change in degree of enhancement and SUV in (18F) FDG PET are very helpful in assessing tumor response, not only through traditional size measurement but rather through assessing vascularity which reflects tumor viability and activity [O'Connor et al, 2008]. In spite of these promising results to assess response by using the above biomarkers, yet criteria for their use as radiological features should be refined through phase III trials. The question whether new agents targeting tumor vasculature could be developed to enhance radiological features in order to assess response to antiangiogensis remains open to be answered.

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