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Mini review

New molecular mediators in tumor angiogenesis

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Abstract

Angiogenesis is essential for tumor growth and progression. It has been demonstrated that tumor growth beyond a size 1 to 2 mm^3 requires the induction of new vessels. Angiogenesis is regulated by several endogenous stimulators and inhibitors of endothelial cell migration, proliferation and tube formation. Under physiological conditions these mediators of endothelial cell growth are in balance and vessel growth is limited. In fact, within the angiogenic balance endothelial cell turnover is sufficient to maintain a functional vascular wall but does not allow vessel growth. Tumor growth an progression has successfully been correlated to the serum concentration of angiogenic mediators. Furthermore, the vascular density of tumor tissues could be correlated to the clinical course of the disease in several tumor entities. Within the last years several new mediators of endothelial cell growth have been isolated e.g. angiopoietin 1, angiopoietin 2, midkine, pleiotropin, leptin and maspin. In this review we discuss the mechanisms leading to tumor angiogenesis and describe some of the newer mediators of endothelial cell stimulation and inhibition.

Keywords: tumor angiogenesis - angiopoietins - leptin - midkine - pleiotropin - maspin

Introduction

Angiogenesis, the formation of new blood vessels from preexisting capillaries, is essential for tumor growth and progression. Normal tissues are in an angiogenic balance, meaning that the endothelial cells divide to maintain a functional vascular wall, but the proliferation rate does not allow vascular growth. However, normal angiogenesis is tightly controlled and only activated for a defined period of time. In endometrium normal angiogenesis is activated during the proliferative phase while in the secretory phase angiogenesis is inhibited. The angiogenic balance is maintained by several endogenous stimulators and inhibitors of endothelial cell growth. In tumor tissue this angiogenic balance is shifted towards the stimulation of vascular

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Table 1 Endogenous stimulators of angiogenesis.

Acidic fibroblast growth factor (FGF-1)	*Midkine*
Angiogenin	*Platelet-derived endothelial cell growth factor*
Angiopoietins	*Placental growth factor*
Basic fibroblast growth factor (FGF-2)	*Pleiotropin*
Granulocyte colony-stimulating factor	*Proliferin*
Hepatocyte growth factor	*Transforming growth factor alpha*
Insulin like growth factor	*Transforming growth factor beta*
Interleukin-8	*Tumor necrosis factor alpha*
Leptin	*Vascular endothelial growth factors*

growth. In conclusion tumor angiogenesis or pathological angiogenesis can be understood as a disruption of normal control mechanisms of endothelial cell growth.

Several stimulators and inhibitors of angiogenesis have been identified within the last decades (see Table 1 and 2). The expressions of basic-fibroblast growth factor (b-FGF or FGF-2) and vascular endothelial growth factor (VEGF) have been investigated in numerous different tumors and have successfully been correlated to the clinical course of the disease. In this review we will focus on the induction of vessel growth by tumor tissue and will describe some of the newer stimulators and inhibitors of angiogenesis which have not that extensively been investigated in cancers. The factors that are discussed in this review are angiopoietin-1 (Ang-1), angiopoietin-2 (Ang-2), leptin, midkine, pleiotropin and maspin. (Fig. 1)

Tumor Angiogenesis

For more than 100 years, tumors had been observed to be more vascularized than normal tissues [1]. However, vasodilatation of host vessels were thought to be the reason for tumor hyperemia [2]. In 1939 a paper by Ide *et al*. [3] hypothesized for the first time that tumor hyperemia could be related to new blood vessel growth instead of vasodilatation. Today it is widely accepted that vessel growth, induced by tumor cells, leads to hypervascularization of tumor tissue (tumor growth is angiogenic) [4]. Furthermore, in 1971 Judah Folkman proposed that tumor growth depends on the induction of vessel growth (tumor growth is angiogenesis dependent) [5]. This would imply that in principle malignant tumors could be treated by a therapy directed towards the tumor vasculature. Today antiangiogenic agents are in numerous phase I to III clinical trials to test their antitumor activity in humans [6].

Under physiological conditions the vasculature is quiescent in the normal adult. Only 0.01% of endothelial cells in a normal adult vessel are in the cell division cycle at any given time [7]. However, in response to an appropriate angiogenic stimulus, the endothelial cells can become activated to grow new vessels. The induction of vessel growth is a complex process, where the basement membrane of the vessel is degraded, the underlying endothelial cells migrate into the surrounding stroma and proliferate at the tip of the migrating column [8]. The time point where angiogenesis is induced in a malignant tumor is named the “angiogenic switch”. Until this stage of tumor progression is reached a tumor is avascular, small (0.5 to 2.0 mm in diame-

Table 2 Endogenous inhibitors of angiogenesis.

Antithrombin	*Matrix metalloproteinases*
Angiostatin	*Plasminogen activator inhibitor 1*
Endostatin	*Prolactin*
Interferon alpha	*Thrombospondin*
Interleukin-12	*Troponin I*
Maspin	

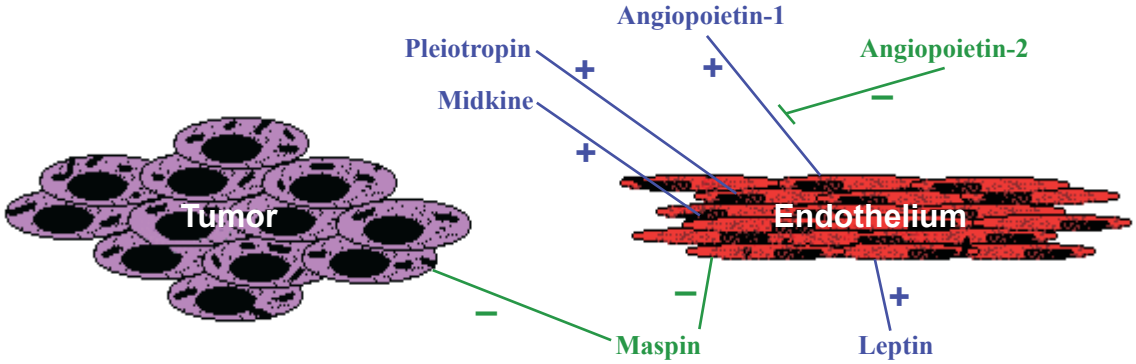

Fig. 1 Effect of the angiogenesis mediators on endothelial and tumor cells. "+" indicates stimulation and "-" indicates inhibition.

ter) and the proliferation and apoptosis of tumor cells is in balance [9]. After going through the angiogenic switch the tumor is vascularized, grows to clinically recognizable size and the balance of tumor cell apoptosis and proliferation are shifted towards proliferation.

The process of metastasis is also angiogenesis dependent. For a tumor cell to metastasize successfully, it needs access to a vessel, survive within the circulation, exit from the vessel in the target organ, seed in the organ stroma and induce angiogenesis [10]. Therefore, angiogenesis is essential at the beginning and at the end of the metastatic cascade.

While tumors are hypervascularized and tumor growth and metastasis are angiogenesis dependent, the question has been raised, if the degree of hypervascularization can be correlated to the prognosis of the disease. Weidner and colleagues in 1991 [11] found that the microvascular density of the primary tumor was a highly significant prognostic marker for human breast cancer [12]. Since then, many studies have found the vascular density to be a prognostic marker for numerous different tumor entities (e.g. prostate cancer, non-small cell lung cancer, colorectal cancer and malignant melanoma) [12-15].

A considerable amount of research has been devoted to the question how tumors induce angiogenesis. The observation that angiogenic tumors could be implanted into an avascular region such as the cornea and than activate the sprouting of new capillaries from distant vessels (e.g. limbus vessels), suggested that tumors release diffusible stimulators of angiogenesis [16]. Today it is widely accepted that tumors (that went through the angiogenic switch) stimulate angiogenesis by the release of angiogenesis stimulators (e.g. basic-fibroblast growth factor and vascular endothelial growth factor). On the other hand, it has been demonstrated that tumors also induce angiogenesis through the down regulation or inhibition of endogenous angiogenesis inhibitors (e.g. thrombospondin-1) [17]. In many studies the serum or tumor levels of angiogenesis stimulators and/or angiogenesis inhibitors could also be identified as a prognostic marker for different tumor entities [18-20].

Angiopoietin 1 and Angiopoietin 2

Two families of receptors have been identified whose expression in the human body is mainly restricted to endothelial cells. One family contains the vascular endothelial growth factor (VEGF) receptors (Flt-1, Flt-4 and Flk-1/KDR) which are important during vascular development [21, 22]. The other family contains the tyrosine receptor type ties (Tie1 and Tie2/Tek) and has been found to be essential for vascular formation [23, 24]. Ang-1 has been isolated in 1996, for its ability to bind and activate the Tie2 receptor [25]. Ang-1 is a 70 kDa glycoprotein that during early embryonic life is most prominently expressed in the heart and later during development becomes more widely distributed (but always in vessels) [25]. Ang-1 although activating the Tie2 receptor did not induce endothelial cell proliferation or tube formation *in vitro*. Therefore, it was suggested that Ang-1, in

contrast to VEGF that acts early in blood vessel development, plays a role later during vessel remodeling, maturation and stabilization [25]. However, in an assay where endothelial cells were cultured on beads and embedded in fibrin gels, Ang-1 induced the formation of capillary sprouts [26]. In this assay suboptimal concentrations of VEGF and Ang-1 acted synergistically to induce sprout formation [26]. In contrast to VEGF which was also named VPF (vascular permeability factor) and induces vascular leakage, Ang-1 acts as an antipermeability factor on the vascular wall [27, 28]. Exposure to low oxygen led to a rise of Tie2 protein expression in human endothelial cells [29]. It is very well known that in tumor tissue the oxygen concentration is much lower than in normal tissue, therefore the overexpression of Tie2 in hypoxic tumor endothelium in combination with high Ang-1 could be a mechanism for stimulation of angiogenesis in malignant tumors [29]. Consistent with this theory Takahama and colleagues found an elevated and correlating expression of Tie2, Ang-1, VEGF and CD-31 in 32 non-small cell lung cancers compared to normal lung tissue. The authors concluded that Ang-1 and VEGF are important angiogenic factors in human non-small cell lung cancer [30].

In 1997 an Ang-1 relative, named Ang-2, was identified and shown to be a naturally occurring antagonist of Ang-1 and Tie2 [31]. Ang-2, like Ang-1, binds to the Tie2 receptor but does not send a downstream signal [31]. Therefore, the receptor is occupied and not available for activation through Ang-1. These data suggest that Ang-2 counteracts blood vessel maturation and stabilization mediated by Ang-1. Ang-2 is mainly expressed in microvascular endothelial cells at sits of blood vessel regression and regeneration. It has been shown that endothelial cell levels of Ang-2 were increased by VEGF, b-FGF and hypoxia, whereas Ang-1 decreased Ang-2 expression [32]. This observations led to the suggestion that the angiogenic effect of these factors, at least in part, is achieved by the regulation of an autocrine loop of Ang-2 in endothelial cells [32]. Prevailing view of the initiation of tumor angiogenesis is that the tumor first exists as an avascular tumor cell mass that goes through the angiogenic switch and than attracts vessels by the secretion of angiogenesis stimulators. Two papers by Holash and coworkers suggested a different mechanism for the initiation of tumor angiogenesis [33, 34]. The authors found that malignant cells rapidly coopted host vessels to form an initially well-vascularized tumor mass. The coopted vessel did not undergo angiogenesis but regressed via a mechanism that involved disruption of endothelial cell/smooth muscle cell interactions and endothelial apoptosis. This vessel regression resulted in secondarily avascular tumors and massive tumor cell loss resulting in a necrotic center of the tumor. Ultimately the tumor is rescued by robust angiogenesis that is initiated at the margin of the tumor and that supports rapid tumor growth. The induction of Ang-2 in the coopted host vessel correlates with vessel regression and precedes the induction of VEGF expression in the adjacent tumor cells that leads to the robust angiogenic response. The authors concluded that the previously underappreciated balance between vascular regression and growth might be important for the induction of tumor angiogenesis. Consistent with this observation Wong and coworkers [35] found a significant up-regulation of VEGF in non-small cell lung tumor cells that could be correlated to the number of Ang-2 expressing vessels in the tumor. In contrast, normal lung tissue expressed high and correlating levels of Ang-1 and Tie2 which were significantly reduced in the lung tumors [35]. In 23 samples of hepatocellular carcinomas (HCC) and adjacent normal liver tissue Tanaka et al. investigated the expression of Ang-1 and Ang-2 [36]. They found that Ang-1 was equally expressed in HCC and normal liver tissue. On the other side, Ang-2 was highly expressed in 10 of 12 hypervascular HCC, but only in 2 of 11 hypovascular HCC, suggesting a role for Ang-2 in the development of hypervascularized HCC. Yoshida and colleagues found that colorectal cancers overexpressed Ang-2 (and thrombospondin 2) when compared to normal mucosa [37]. Overexpression of human epidermal growth factor receptor 2 (HER2) is a marker for aggressiveness in breast cancer. Carter and Ward provided evidences that the expression of Ang-2 in breast cancer might be regulated by HER2 [38]. Recently alternative splice variants of Ang-1 and Ang-2 have been identified [39, 40]. The expression and biological relevance of these splice variants in tumor angiogenesis has to be investigated.

Leptin

Obesity is characterized by an excess of fat mass as a consequence of adipocyte hypertrophy and hyperplasia. In 1995, leptin, the product of the ob gene was identified [41]. Leptin is a cytokine, generated and secreted by adipocytes, that regulates many physiological processes, for example body weight, energy homeostasis, ovarian development, steroidogenesis and serves as a permissive regulator of sexual maturation [42, 43]. Because adipocytes possess angiogenic activity [44] and the growth of adipose tissue requires the formation of new blood vessels, it has been suggested that leptin might stimulate endothelial cell proliferation and angiogenesis [45]. It could be demonstrated that endothelial cells in vitro express at least two forms of leptin receptors (Ob-Ra amd Ob-Rb) and that these could be activated by the treatment of cultured endothelial cells with leptin (10 ng/mL) [45, 46]. Furthermore, it has been shown that leptin promotes endothelial cell survival and proliferation *in vitro* [45, 46] and stimulates angiogenesis *in vivo* when using the chicken chorioallantoic membrane assay (CAM) [45, 46]. Uckaya and colleagues found that patients suffering from diabetic retinopathy (an angiogenic disease) had higher plasma leptin levels the more advanced the retinopathy was [47]. Leptin as an angiogenesis factor is almost not investigated in tumor angiogenesis, however, Bertolini and coworkers found no differences for circulating leptin levels in patients with non-Hodgkin's lymphoma that had complete remission or were in progression [48]. It is interesting that cancer patients with advanced diseases often suffer from extensive weight loss (tumor cachexia). In breast cancer patients elevated plasma levels and adipose tissue expression of leptin have been found [49], while in other cancers e.g. colorectal and lung cancer this correlation could not be seen [49, 50]. Especially no correlation for tumor cachexia and elevated leptin levels have been found so far. However, the interaction of leptin expression, tumor growth and cachexia are not understood today, but one has to keep in mind that stimulation and inhibition of angiogenesis is a local process and is not necessarily mirrored by elevated serum levels of angiogenic mediators.

Midkine and Pleiotropin

Midkine (MK) and pleiotropin (PTN) are heparin-binding cytokines that are involved in neurogenesis, cell migration and mesoderm-epithelial interactions [51]. In 1997 Choudhuri et al. investigated the role of these two growth factors in tumorigenesis [52]. Employing different *in vitro* and *in vivo* assays they found that *in vitro* tumor cell growth is not affected by these growth factors. However, *in vivo* tumor cell growth was stimulated and this growth advantage correlated to an increase of the vascular density of the tumors [52]. In the same study the angiogenic activity of MK and PTN was confirmed in an rabbit corneal assay. In 47 primary bladder tumors and 7 normal bladder samples MK expression was identified in 98% of tumor samples and 70% of normal bladder tissues. However, median MK expression was 4-fold higher in tumors than in normal bladders [53]. Furthermore, overexpression of MK predicted a poor outcome in patients with invasive bladder cancer [53]. In thyroid papillary carcinomas the expression of MK has been shown to relate to the invasion of these carcinomas [54]. Two other studies have demonstrated an overexpression of MK in hepatocellular carcinomas and Sternberg Reed cells of Hodgkin's disease [55, 56]. Ikematsu et al. investigated serum MK levels in 135 normal individuums and 150 patients with different types of cancer [57]. In this study the serum MK level for normal individuums has been 0.154 ± 0.076 ng/mL. Using a cut-off value of 0.5 ng/mL, 87% of cancer patients showed elevated MK serum levels. Because serum MK levels did not correlate to serum C-reaktive protein levels, a marker for inflammation, the authors stated that MK could be used as a general tumor marker [57]. The expression of MK and PTN was examined in normal and malignant human breast tissue. The majority of both malignant and normal breast tissues expressed PTN, whereas MK was frequently expressed in the malignant breast tissue but in only one of the normal specimens [58]. Studies correlating MK and PTN expression to angiogenic characteristics like vascular density are missing.

Maspin

Maspin, a tumor suppressing serpin (serin protease inhibitors), has been isolated from mammary

epithelial cells [59]. The maspin protein is expressed in normal mammary epithelial cells but not in most mammary carcinoma cell lines [59]. The loss of maspin expression occurred most frequently in advanced cancers [59]. Rat maspin mRNA was detected in rat mammary gland, vagina, urinary bladder, thymus, small intestine, skin, ventral prostate, seminal vesicles, and thyroid but not in heart, lung, liver, brain and kidney [60]. Maspin has been shown to inhibit cell motility, tumor invasion, and development of metastasis [61, 62, 63]. In a recent publication by Zhang and colleagues demonstrated that maspin is a potent inhibitor of angiogenesis [64]. *In vitro*, maspin inhibited b-FGF and VEGF stimulated endothelial cell (migration towards b-FGF and VEGF), and reduced endothelial cell proliferation and tube formation. *In vivo*, maspin blocked neovascularization in the rat cornea pocket model [64]. In 44 cases of human oral squamous cell carcinomas the expression of maspin has been correlated to patient survival. Sixtysix % of cases expressed low to intermediate levels of maspin and 34% of cases expressed high levels of maspin. High maspin levels were associated with the absence of lymph node metastasis and, more importantly, were significantly correlated with a better overall survival [65]. As mentioned above, maspin belongs to the superfamily of serpins (serine protease inhibitor). Recently two other serpins, antithrombin (AT-III) and plasminogen activator inhibitor I (PAI-I), have been demonstrated to exert antiangiogenic activity [66, 67].

Conclusion

In this review we described some of the newer molecular mediators of angiogenesis. Most of the factors described here, have originally been isolated for other biological functions, but later have been demonstrated to be angiogenic or antiangiogenic. While other angiogenesis mediators, e.g. b-FGF and VEGF, are extensively characterized in tumor angiogenesis, these factors are not that well investigated. As described above, interactions for angiopoietins and VEGF in tumor angiogenesis have been found, but in general the interactions and functional mechanisms of angiogenesis mediators are not that well understood. Furthermore, the therapeutic potential of the regulation of angiogenesis in malignant and other diseases is of utmost importance. The dependence of tumor growth and progression from the induction of vessel growth builts a potential new strategy in the fight against cancer. To design effective and save antiangiogenic regimes against cancer investigation of the mechanisms of tumor angiogenesis and the interactions of its mediators are essential.

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