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Molecular basis of angiogenesis and cancer

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Angiogenesis is a term that describes the formation of new capillaries from a pre-existing vasculature. This process is very important in physiologic conditions because it helps healing injured tissues, and in female populations it helps forming the placenta after fertilization and reconstructs the inside layer of the uterus after menstruation. Angiogenesis is the result of an intricate balance between proangiogenic and antiangiogenic factors and is now very well recognized as a powerful control point in tumor development. In this particular environment, the fine modulation among proangiogenic and antiangiogenic factors is disrupted, leading to inappropriate vessels growth. In this review, we discuss the molecular basis of angiogenesis during tumor growth and we also illustrate some of the molecules that are involved in this angiogenic switch.
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Angiogenesis in cancer

Angiogenesis is a crucial mechanism required for a number of physiological and pathological events. In physiological conditions, angiogenesis is a highly regulated phenomenon. It normally occurs during embryonic development, wound healing, and the menstruation cycle. Unregulated angiogenesis is seen in pathological conditions, such as psoriasis, diabetic retinopathy, and cancer. During tumor growth, angiogenesis is required for proper nourishment and removal of metabolic wastes from tumor sites.

In physiologic conditions, cells are located within 100 and $200\,\mu$m from blood vessels, their source of oxygen. When a multicellular organism is growing, cells induce angiogenesis and vasculogenesis in order to recruit new blood supply. In a pathological condition such as cancer, angiogenesis is required for tumor survival and proliferation. The microenvironment of solid human tumors is characterized by heterogeneity in oxygenation. The proliferation of a network of blood vessels that penetrates into tumors supplies oxygen and nutrients and removes waste products. Formation of solid tumors

requires coordination of angiogenesis with continued tumor cell proliferation. However, despite such neovascularization, hypoxia is persistent and frequently found in tumors at the time of diagnosis. Hypoxia arises early in the process of tumor development because rapidly proliferating tumor cells outgrow the capacity of the host vasculature. Tumors with low oxygenation have a poor prognosis, and strong evidence suggests that this is because of the effects of hypoxia on malignant progression, angiogenesis, metastasis, and therapy resistance. Tumor cells located more than $100\,\mu$m away from blood vessels become hypoxic. Some clones will survive by activating an angiogenic pathway. If new blood vessels do not form, tumor clones will be confined within 1–1.5 mm diameter (Hori *et al.*, 1991; Gastl *et al.*, 1997). Such clones remain dormant from months to years before they switch to an angiogenic phenotype (Folkman, 2002). Vascular cooption is confined only in the tumor periphery and gradual tumor expansion causes a progressive central hypoxia. Hypoxia induces the expression of proangiogenic factors through hypoxia-inducible factor-α, and if proangiogenic factors are in excess of antiangiogenic factors, it may lead to the switch to an angiogenic phenotype (Liotta and Stetler-Stevenson, 1991). The presence of viable hypoxic cells is likely a reflection of the development of hypoxia tolerance resulting from modulation of cell death in the microenvironment. This acquired phenotype has been explained on the basis of clonal selection. The hypoxic microenvironment selects cells capable of surviving in the absence of normal oxygen availability. However, the persistence and frequency of hypoxia in solid tumors raise a second potential explanation. It has also been suggested that stable microregions of hypoxia may play a positive role in tumor growth. Although hypoxia inhibits cell proliferation and eventually cell death, hypoxia also provides angiogenic and metastatic signals, thus allowing prolonged survival in the absence of oxygen and generation of a persistent angiogenic signal (Dulak and Jozkowicz, 2003; Wouters *et al.*, 2003).

The first interest in angiogenesis related to cancer was in 1968 (Ehrmann and Knoth, 1968; Greenblatt and Shubik, 1968), when it was first highlighted that tumor secretes a diffusible substance that stimulates angiogenesis. Since then, several investigators studied the different proangiogenic factors that tumor cells diffuse when the mass has reached the limited size of the early

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tumor (Silverman et al., 1988; Sierra-Honigmann et al., 1998; Buschmann, 2000). Pioneering studies performed by Folkman in 1971 proposed an insightful anticancer therapy by starvation of blood supply (Folkman, 1971, 1985, 1992, 2002; Folkman et al., 1971). Folkman's intuition that tumor growth and metastasis strictly depend on angiogenesis led to the idea that blocking tumor nourishment could be one of the ways to avoid its spread.

Several sequential steps can be highlighted during tumor angiogenesis. In mature (nongrowing) capillaries, the vessel wall is composed of an endothelial cell lining, a basement membrane, and a layer of cells called pericytes, which partially surround the endothelium. The pericytes are contained within the same basement membrane as the endothelial cells and ocassionally make direct contact with them. Angiogenic factors produced by tumoral cells bind to endothelial cell receptors and initiate the sequence of angiogenesis. When the endothelial cells are stimulated to grow, they secrete proteases, heparanase, and other digestive enzymes that digest the basement membrane surrounding the vessel.

Degradation of basement membrane and the extracellular matrix surrounding pre-existing capillaries, usually postcapillary venules, is a mechanism allowed by matrix metalloproteinases (MMPs), a family of metallo-endopeptidases secreted by the tumor cells and the supporting cells. The dissolution of extracellular matrix also allows the release of proangiogenic factors from the matrix (Bhushan et al., 2002). The junctions between endothelial cells become altered, cell projections pass through the space created, and the newly formed sprout grows toward the source of the stimulus. Endothelial cells invade the matrix and begin to migrate and proliferate into the tumor mass. In this location, newly formed endothelial cells organize into hollow tubes (canalization) and create new basement membrane for vascular stability. Fused blood vessels newly established form the blood flow within the tumor. The formation of the lumen during canalization is driven by important interactions between cell-associated surface proteins and the extracellular matrix (ECM). Some of the surface proteins identified in this interaction are hybrid oligosaccharides, galectin-2, PECAM-1, and VE-cadherin (Gamble et al., 1999; Yang et al., 1999; Nangia-Makker et al., 2000). Different situations can provoke an unbalanced shift toward proangiogenic factors such as, for example, metabolic and mechanical stresses, hypoxia, and genetic mutations or altered expression of oncogenes or tumor suppressor genes can stimulate blood vessels growth and the mechanism behind this is still unknown (Carmeliet, 1999; Kerbel, 2000).

Structure of tumor vasculature

In tumors, the normal configuration of blood vessels is generally abolished. Tumor vessels tend to break all the conventional rules of microvasculature, spreading without any organization, following tortuous paths and changing in diameter without any organization. Large-caliber tumor vessels may have thin walls usually belonging to capillaries or an incomplete basement membrane and an unusual pericyte coat (Dvorak et al., 1988; Hobbs et al., 1998; Eberhard et al., 2000). An imbalance in angiogenic factors, like vascular endothelial growth factor (VEGF) and angiopoietins, is the main cause of this chaotic structure in a tumor (Helmlinger et al., 1997; Baish and Jain, 2000). The vessels from which new vessels originate are characterized by degradation of the basement membrane and decreased number of perycites. Moreover, 'mother' vessels have a thinned endothelial cell lining. Tumor vessels are hyperpermeable, mostly described as 'leaky', because of loss of adherence between endothelial junctions as well as a discontinuous basement membrane (Hobbs et al., 1998; Dvorak et al., 1999; Hashizume et al., 2000). The induction of vascular permeability is mediated by vesiculo-vacuolar organelles, the redistribution of platelet endothelial cell adhesion molecules (PECAM-1), and vascular endothelial cadherin (VE-cadherin). Some investigators have also revealed the involvement of Src kinases in this process (Carmeliet, 2000). Vascular permeability allows the extravasation of plasma proteins that constitute a momentary scaffold for migrating endothelial cells. Another very common feature in tumor blood vessels is the presence of focal hemorrhages that occur spontaneously mainly if the tumor cells express VEGF121 or VEGF165 (Cheng et al., 1997). The structural aberrations described so far in tumor vessels are also coupled to molecular and functional disorders such as the overexpression of growth factors, integrins, and the uptake of cationic liposomes (McDonald and Foss, 2000).

Proangiogenic and antiangiogenic factors

Knowledge of molecular mediators of angiogenesis is fundamental in understanding the mechanisms that control its pathways and may ultimately be useful in developing therapies for angiogenesis-related diseases. This issue is addressed by many reviews (Denekamp, 1993; Ruiter et al., 1993; Juczewska and Chyczewski, 1997; Pluda, 1997; Klagsbrun and Moses, 1999; Malonne et al., 1999; Soff, 2000; Ryan and Wilding, 2000; Liekens et al., 2001).

Modulators of angiogenesis are secreted by endothelial cells, tumor cells, and by the surrounding stroma. A summarized list of molecules involved in angiogenesis is represented in Table 1.

Opposite effects on adhesion molecules are displayed by VEGF and tumor-necrosis factor-α (TNF-α), which upregulate their expression, fibroblast growth factor (bFGF), and transforming growth factor-β1 (TGF-β1), which downregulates their expression (Jain et al., 1996). These factors act as autocrine or paracrine growth factors to induce angiogenesis (Pluda, 1997).

Table 1 Known angiogenic factors

1-Butyryl glycerol	Interleukin 8 (IL-8)
Acid fibroblast growth factor	Laminin
Adenosine	Leptin
Angiogenin	Midkine
Angiopoietin-1 (Ang1)	Nicotinamide
Collagen	Perlecan
Del-1	Phospholipids (SPP, LPA)
Entactin	Placental growth factor
Epidermal growth factor	Platelet-derived endothelial growth factor (PDGF)
Ephrins	Pleiotropin
Fibroblast growth factor: acid (aFGF) and basic (bFGF)	Proliferin
Fibronectin	Prostaglandins E1 and E2
Follistatin	Scatter factor (SF)
Granulocyte colony-stimulating factor (G-CSF)	Transforming growth factor-alpha (TFG-α) and -beta (TFG-β)
Heparin/heparan sulfate	Tumor necrosis factor-alpha (TNF-α)
Hepatocyte growth factor (HGF)	Vascular endothelial growth factor (VEGF)

Proangiogenic factors

Several proteins are now known to activate endothelial cell growth and movement such as angiogenin, epidermal growth factor, estrogen, interleukin 8, prostaglandin E1 and E2, TNF-α, VEGF, and granulocyte colony-stimulating factor. In addition, there are many other gene products, ranging from transcription factors to the Notch family members that are essential during new vessels formation.

VEGF One of the molecules that play a critical role in vascular formation is VEGF, one of the most potent angiogenic cytokines. It has first been characterized for its ability to induce vascular leakeage and permeability and to promote vascular endothelial cell proliferation (Dvorak *et al*., 1999; Ferrara, 1999).

Different knockout studies have highlighted its importance. Disruption of one VEGF allele in mice results in deadly vascular abnormalities. Disruption of both VEGF alleles in mice results in almost complete absence of vasculature (Carmeliet *et al*., 1996; Ferrara *et al*., 1996; Carmeliet, 2000).

Hypoxia is thought to stimulate VEGF expression in both normal cells and in tumors (Shweiki *et al*., 1992; Minchenko *et al*., 1994), thus causing an increase in the rate of gene transcription (Forsythe *et al*., 1996) and an increased stability of VEGF mRNA (Ikeda *et al*., 1995). In addition, VEGF expression is induced by growth factors and cytokines such as PDGF, EGF, TNF-α, TGF-β1, and IL-1 (Ferrara and Davis-Smyth, 1997; Neufeld *et al*., 1999).

VEGF function is one of the molecules involved and required to start the formation of new vessels by vasculogenesis sprouting, the process by which vessels are formed *de novo* by the assembly of angioblasts of mesodermal origin. There are six known members of the VEGF family: VEGF, placenta growth factor, VEGF-B, VEGF-C, VEGF-D, and VEGF-E (Ogawa *et al*., 1998; Meyer *et al*., 1999; Neufeld *et al*., 1999). All these members have overlapping abilities to interact with different receptors expressed mainly in the vascular endothelium (Eriksson and Alitalo, 1999). They are

initially secreted as dimeric glycoproteins containing the 'cysteine knot' motif composed of regularly spaced eight-cysteine residues (Tischer *et al*., 1989; Potgens *et al*., 1994; Meyer *et al*., 1999). The best described VEGF receptors are VEGFR-1 (Flt-1) and VEGFR-2 (KDR/Flk-1), VEGFR-3 (KDR/Flt-1), VEGF-R2, VEGF-R3 (Ftl-4). Neuropilins like VEGF-R4 (neuropilin-1) are also described as accessory receptors which seem to be involved in modulating binding to the main receptors (Soker *et al*., 1998). VEGF-induced signal, resulting in activation of ERK, p38 MAPK, and p125FAK, and inducing cell progression, is mostly mediated by VEGFR-2 (Waltenberger *et al*., 1994; D'Angelo *et al*., 1995; Landgren *et al*., 1998).

VEGF and bFGF act also as antiapoptotic factors for the newly formed blood vessels, since they induce expression of antiapoptotic molecules, such as Bcl-2, promoting endothelial cell survival (Kim *et al*., 2000b).

Angiopoietins and ephrins Angiopoietin-1 (Ang1) and angiopoietin-2 (Ang2) have been discovered by Yancopoulos and colleagues in 1996 (Davis *et al*., 1996; Suri *et al*., 1996).

Ang1 promotes survival through phosphorylation of the Tie-2 receptor and the signal transduction pathway mediated by phosphatidylinositol 3′-kinase and Akt (Kim *et al*., 2000b). Subsequent development of immature vessels is ruled mainly by Ang1 and ephrin-B2. Ang1 activity is involved in mature vessels maintenance and its alteration is involved in both physiological and pathological neovascularization (Wang *et al*., 1998; Adams *et al*., 1999; Gerety *et al*., 1999).

Ephrin-B2 and EphB4 play an important role during initial distinction between arterial and venous vessels. Ephrin-B2 presence is distributed in the endothelium of primordial arterial vessels, while EphB4 marks the endothelium of primordial venous vessels (Wang *et al*., 1998; Adams *et al*., 1999; Gerety *et al*., 1999). Interesting studies on mice lacking ephrin-B2 and EphB4 highlighted defects on early angiogenic remodeling similar to those shown in mice lacking Ang1 or Tie-2 (Wang *et al*., 1998; Adams *et al*., 1999; Gerety *et al*., 1999). Ephrin-B2

expression continues to mark arteries during later embryonic development and in the adult, underlying that it may be involved in regulating interactions between endothelial and smooth muscle cells implicated in the formation of arterial muscle walls.

Other fundamental molecules that are considered positive regulators of angiogenesis are the transforming growth factor α (TGF-α), transforming growth factor-β (TGF-β), basic-FGF (b-FGF), angiostatin, endostatin, and platelet-derived endothelial cell growth factor (PDGF) among others (Table 1).

Different studies reported a controversial role of another important molecule involved in angiogenesis, TSP-1. TSP-1 is a trimeric 450 kDa glycoprotein that can interact with several matrix components, cell receptors, proteolytic enzymes, and soluble growth factors. Its involvement has been shown in embryogenesis as well as in angiogenesis and tumorigenesis. Both inhibition and promotion of angiogenesis have been reported, depending on the functional status of TSP-1 domains/fragments (Jimenez *et al.*, 2000; Taraboletti *et al.*, 2000). Several proteolytic enzymes, including the neutrophil elastase, thrombin plasmin, trypsin, and cathepsin G, generate two fragments of TSP-1 of 25 and 140 kDa (Hogg *et al.*, 1993a, b). Most of the antiangiogenic activity of TSP-1 has been located in the 140 kDa carboxy-terminal fragment of TSP-1 and it occurs through the TSP-1 receptor CD36 (Tolsma *et al.*, 1993; Taraboletti *et al.*, 1997). A positive effect on angiogenesis has been detected on the heparin binding 25 kDa fragment of TSP-1 (Taraboletti *et al.*, 2000).

Tumor cells secrete cytokines and angiogenic molecules that can alter the expression of adhesion molecules and of different surface markers on endothelium growing in tumors. During tumor angiogenesis, a selective expression of adhesion receptor integrin $\alpha_v\beta_3$ has been detected (Brooks *et al.*, 1994). The survival of new endothelial cells is increased by a specific signal following the recognition of integrin $\alpha_v\beta_3$ from its receptor.

Activated endothelial cells also secrete growth factors that maintain their activated state and facilitate their invasion as well as tumor cells proliferation.

Some oncogenes function directly as angiogenic stimuli (Rak *et al.*, 1995a). For example, H-ras and v-src upregulate VEGF expression in fibroblasts and epithelial cells (Rak *et al.*, 1995a, b; Arbiser *et al.*, 1997; Jiang *et al.*, 1997; White *et al.*, 1997; Charvat *et al.*, 1999; Casanova *et al.*, 2002). Similarly, upregulation of insulin-like growth factor 1 (Lahm *et al.*, 1996; Valentinis *et al.*, 1997; Werner *et al.*, 2000) and plateled-derived growth factor B (Sonobe *et al.*, 1991) is induced by a wide range of oncogenes. Furthermore, some oncogenes also have inhibitory effects on anti-angiogenic factors (Fotsis *et al.*, 1999; Breit *et al.*, 2000).

Basic FGF The FGF family consists of nine distinct members, while the FGF receptor family consists instead of four transmembrane receptor tyrosine kinases. FGF-1 (acidic) and FGF-2 (basic) are described

as inductors of angiogenesis. Binding of FGF to their receptors leads to activation of a tyrosine kinase signal transduction pathway to downstream signaling cascades. It is not yet very clear how the signaling cascade could lead to endothelial cell differentiation. However, the interactions of bFGF with heparin-like molecules result in transcriptional changes and biological responses. Many FGFs contain signal peptides for secretion and are secreted into the extracellular environment, where they can bind to the heparan-like glycosaminoglycans (HLGAGs) of the ECM. From this reservoir, FGFs may act directly on target cells, or they can be released through digestion of the ECM or the activity of a carrier protein, a secreted FGF binding protein. FGFs bind specific receptor tyrosine kinases in the context of HLGAGs and this binding induces receptor dimerization and activation, ultimately resulting in the activation of various signal transduction cascades. Some FGFs are potent angiogenic factors and most play important roles in embryonic development and wound healing. FGF signaling also appears to play a role in tumor growth and angiogenesis, and autocrine FGF signaling may be particularly important in the progression of steroid hormone-dependent cancers to a hormone-independent state.

Antiangiogenic factors

The presence of angiogenic factors is not enough to initiate the new vasculature growth. The influence of proangiogenic factors is counterbalanced by a number of inhibitory agents. The net result of these opposing factors on the vascular endothelial cell determines the outcome of angiogenesis homeostasis.

Several inhibitory factors known in angiogenesis are described in Table 2.

Dormant tumors secrete inhibitory factors such as endostatin, angiostatin, thrombospondins, and tissue inhibitors of metalloproteinases that prevents the tumors from increasing their size (Folkman, 2002).

Several investigators emphasize that different angiogenic inhibitors like TSP-1, angiostatin, and endostatin induce apoptosis in cultured endothelial cells (Jimenez *et al.*, 2000; Nor *et al.*, 2000; Chavakis and Dimmeler, 2002; Volpert *et al.*, 2002). Additionally, it has been shown that TSP1 mediates endothelial cell apoptosis and inhibits angiogenesis in association with increased expression of Bax, decreased expression of Bcl-2, and processing of caspase-3 into smaller proapoptotic forms. TSP1 also attenuated VEGF-mediated Bcl-2 expression in endothelial cells *in vitro* and angiogenesis *in vivo*. Furthermore, TSP1 inhibited neovascularization in sponge implants in SCID mice, implying that these molecules are able to eliminate and prevent new vessel formation by altering the expression profile of survival genes (Volpert *et al.*, 2002).

TSP-1 As mentioned previously, TSP-1 has also been considered an antiangiogenic factor. The 140 kDa fragment of TSP-1 has an inhibitory effect on angiogenesis through its interaction with CD36, a receptor that

Table 2 Known antiangiogenic factors

2-Methoxy-estradiol	Maspin
1,25-Dihydroxyvitamin D_3	Metalloproteinase inhibitor (TIMPs)
ADAMTS-1	METH-1
Angiopoietin-2	PEDF
Angiostatin	Pex
Antiangiogenic antithrombin III	Pigment-epithelium-derived factor
Calreticulin	Placental ribonuclease inhibitor
Canstatin	Plasminogen fragment Kringle 5
Cartilage-derived inhibitor (CDI)	Platelet factor 4 (PF4)
CD59 complement fragment	Prolactin 16 kda fragment
Decorin	Proliferin-related protein (PRP)
Endostatin	Retinoids
Fibronectin fragment	Soluble VEGF receptor
Gro-β	Tetrahydrocortisol-S
Heparinases	Thrombospondin-1 (TSP-1) and -2 (TSP-2)
Heparin hexasaccharide fragment	Tissue inhibitor of metalloproteinases-1 (TIMP-1)
Human chorioic gonadotropin (hCG)	Tissue inhibitor of metalloproteinases-2 (TIMP-2)
Interferons α, β, γ	Tissue inhibitor of metalloproteinases-3 (TIMP-3)
Interferon-inducible protein (IP-10)	Vascural endothelial growth inhibitors (VEGI)
Interleukin-1 (IL-1), -4 (IL-4), -12 (IL-12)	Vasculostatin
Ligands of PPARγ	Vasostatin

marks the surface of endothelial cells. The interaction between TSP-1 and its receptor activates a sequence of events that finally results in endothelial cell apoptosis, thus inhibiting angiogenesis. Among several steps, many of them not yet completely identified, is the induction of p38 mitogen-activated protein kinase (MAPK) that changes the gene expression pattern leading to an antiangiogenic biological effect (Sargiannidou et al., 2001).

Endostatin Endostatin is generated by the cleavage of a 20 kDa fragment at the C-terminus of collagen XVIII, a proteoglycan/collagen found in vessel walls and basement membranes. It contains a region particularly rich in arginine residues acting as a heparin-binding epitope. It is a potent antiangiogenic molecule that inhibits endothelial cell migration, induces endothelial cell apoptosis and cell cycle arrest *in vitro* (Hanai et al., 2002). Endostatin decreases the hyperphosphorylated and inactive form of the retinoblastoma gene product and downregulates cyclin D1 mRNA and protein. Importantly, endostatin is unable to arrest cyclin D1 overexpressing endothelial cells, suggesting that cyclin D1 is a critical target for endostatin action.

It has been reported that endostatin binds directly to KDR/Flk-1 interfering with VEGF signaling. The mechanism by which endostatin inhibits endothelial cell proliferation and migration is unknown. It inhibits endothelial cell migration *in vitro* and appears to be highly effective in murine *in vivo* studies (Hanai et al., 2002; Kim et al., 2000a). Its inhibitory effects on tumors are mediated by its interaction with tropomyosin, which results in disruption of microfilament integrity (Mac-Donald et al., 2001). This leads to inhibition of cell motility, induction of apoptosis, and ultimately inhibition of tumor growth. Endostatin significantly reduces endothelial as well as tumor cellular invasion into reconstituted basement membrane *in vitro* (Kim et al.,

2000a). Kim and co-workers revealed that endostatin inhibited the activation of promatrix metalloproteinase-2 (proMMP-2). This finding would partly explain the mechanism of the potent antiangiogenic and antitumor activities of endostatin.

Angiostatin Angiostatin is known to inhibit certain aspects of endothelial function, for example, angiogenesis. It was discovered after the observation of inhibition of tumor growth by tumor mass. In an animal model, a primary tumor inhibits its remote metastases. After tumor removal, metastases neovascularize and grow. When the primary tumor is present, metastatic growth is suppressed by a circulating angiogenesis inhibitor, angiostatin. Angiostatin was purified from the urine of tumor-bearing mice. It is a 38 kDa plasminogen fragment. A corresponding plasminogen fragment in humans has a similar activity. Systemic injection of angiostatin, but not intact plasminogen, has been shown to potently block neovascularization and metastatic growth. Angiostatin functions as an inhibitor of ECM-enhanced and t-PA-catalysed plasminogen activation (Stack et al., 1999). t-PA seem, therefore, to have a binding site for the inhibitor angiostatin, and for its substrate plasminogen that, when occupied, prevents ternary complex formation between t-PA, plasminogen, and matrix protein. The inhibition of matrix-enhanced plasminogen activation leads to a reduced invasive activity, suggesting a crucial involvement of angiostatin in cellular migration and invasion. While the angiostatin mechanism of action seems quite clear, that of endostatin remains unknown, but a synergistic antitumor activity has been reported when these factors are delivered in combination to tumors by retroviral gene transfer (Scappaticci et al., 2001).

Calreticulin Calreticulin is a calcium binding molecule chaperone expressed primarily in the lumen of the

endoplasmic reticulum. It can also localize to the cytoplasm adjacent to the cell membrane where it binds integrins, and to the nucleus (Wheeler *et al*.,. 1995; Kageyama *et al*., 2002). It has been shown to be essential for cardiac and neural development in mice, but the mechanism by which it functions in cell differentiation is not fully understood. The 1–180 amino acids N-terminal domain of calreticulin is vasostatin, which inhibits endothelial cell proliferation, angiogenesis, and tumor growth. Vasostatin binds to laminin, inhibits endothelial cell attachment, and reduces subsequent endothelial cell growth induced by bFGF (Yao *et al*., 2002). Its antiangiogenic capacity that derives from its interference with endothelial cell attachment to components of the ECM makes this recently discovered molecule a potentially novel cancer therapeutic.

Interleukin and interferon To elucidate the unclear mechanisms underlying neovascularization that accompanies certain chronic immune/inflammatory disorders, the effects of interferon molecules on endothelial cell (EC) growth *in vitro* and angiogenesis *in vivo* have been studied studied. Interferons (IFNs) have established antitumor and antiangiogenic actions; however, the mechanism underlying these effects is species specific and not yet clear. IFN-α has the ability to decrease the transcription of VEGF gene expression through an Sp1- and/or Sp3-dependent inhibition of VEGF promoter activity *in vitro* (von Marschall *et al*., 2003). *In vivo* studies demonstrated that the antitumor effect of recombinant IFN-*β* may be mediated, at least in part, via angiogenesis inhibition rather than antiproliferative activity (Hong *et al*., 2000) and that IFNα and retinoic acid have remarkably synergistic antiangiogenic effects able to inhibit both the growth and the neovascularization of head and neck squamous cell carcinoma injected into the floor of the mouth of nude mice (Lingen *et al*.,

1998). Other *in vivo* studies evidenced that mice treated with rat antibody raised against murine IFN-γ results in neutralizing the antiangiogenic effect of murine interleukin-12 (IL-12) (Majewski *et al*., 1996). This phenomenon indicates that IFN-γ is a mediator of the antiangiogenic effect of IL-12 and that the mechanism of antitumor action of IL-12 may depend not only on the immunostimulatory activity of this cytokine but also on its effect on tumor cell-induced angiogenesis.

Conclusions

The ability to mount an angiogenic response is likely present in all tissues, and stimulation of endothelial cells by any one of a wide variety of factors initiates a cascade of events leading to angiogenesis. In most tissues, the overall lack of angiogenesis in physiological situations results from the balance of a complex multifactorial system constituted by stimulators and inhibitors of angiogenesis. An imbalance in any one of these proteins may lead to a switch toward an angiogenetic phenotype. Owing to the multitude of angiogenetic signals triggered by tumor cells, it is unlikely that a straightforward inhibition of angiogenic stimuli will be effective as an approach to anticancer therapy. Although many studies in animal models, as well as some human clinical trials, have proven that effective inhibition of a single step in the angiogenetic cascade would be able to somehow suppress angiogenesis, we are still far from a complete understanding of those mechanisms that could possibly lead to fulfill the expectation that an effective inhibitor of a single key step in this cascade would be able to completely suppress angiogenesis and therefore tumor growth.

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