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TARGETING ANGIOGENESIS: AN OVERVIEW

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ABSTRACT

Blood vessels constitute the first organ in the embryo and form the largest network in the body, but sadly are often deadly. Angiogenesis is the process of generating new capillary blood vessels. Vasculogenesis is the term used for spontaneous blood-vessel formation, and intussusceptions is the term for new blood vessel formation by splitting off existing ones. Angiogenesis is a normal and vital process in growth and development, as well as in wound healing and in granulation tissue. It is also a fundamental step in the transition of tumors from a dormant state to a malignant one, leading to the use of angiogenesis inhibitors. Angiogenesis may be a target for combating diseases characterized by either poor vascularisation or abnormal vasculature. Application of specific compounds that may inhibit or induce the creation of new blood vessels in the body may help combat such diseases. In this review, we will present an overview of the knowledge gained in studies related to the identification and characterization of different inhibitors and regulators of angiogenesis and also to highlight briefly the pathological and physiological angiogenesis.

Keywords: Angiogenesis, vascularisation, regulators, inhibitors, growth factors, tumor angiogenesis.

INTRODUCTION

Blood vessels are assembled during embryonic development by vasculogenesis, in which a primitive vascular network is established from endothelial cell precursors called angioblast. The process of angiogenesis or neovascularisation has been thought to depend on the branching and extension of adjacent blood vessels. Recent work has demonstrated that angiogenesis can occur by recruitment of endothelial progenitor cells from the bone marrow. Angiogenesis is critical to chronic inflammation and fibrosis, to tumor growth and to vascularisation of ischemic tissue. Therefore great efforts have been made to explore therapeutic effects of agents that are proangiogenic (increase blood vessels when needed) and antiangiogenic (block pathologic angiogenesis). Small blood vessels consist of only endothelial cells whereas large blood vessels are surrounded by mural cells (pericytes in medium sized and smooth muscle cells in large vessels). Vessels can grow in several ways. Vasculogenesis refers to formation of blood vessels by endothelial progenitors. Angiogenesis and arteriogenesis refer to sprouting and subsequent stabilization of these sprouts by mural cells and collateral growth denotes the expensive growth of pre-existing vessels, forming collateral bridges between arterial networks. Both capillary angiogenesis and arterial growth are targets for therapy, as distal capillaries distribute the flow while proximal

arterioles provide bulk flow to the tissue. When vessel growth is deregulated, it has a major impact on our health and contributes to the pathogenesis of many disorders. The best known are cancer, psoriasis, arthritis, blindness, obesity, asthma , atherosclerosis, infectious disease, heart and brain ischemia, neurodegeneration, hypertension, pre-eclampsia, respiratory distress and osteoporosis[1,2].

DISCUSSION

Angiogenesis from endothelial precursor cells:

The formation of hematopoietic and vascular system is closely linked during embryonic development. The two systems share a common precursor, the hemangioblast, which can generate hematopoietic stem cells and angioblast, the cells that give rise to the vascular system. Angioblast proliferate, migrate to peripheral sites and differentiate into endothelial cells that form arteries, veins and lymphatics. They can also generate pericytes and smooth muscle cells of the vessel wall (periendothelial cells). It has now been established that angioblast like cells called EPC has been stored in the bone marrow of adults and can be recruited into the tissue to initiate angiogenesis. The nature of homing mechanism is uncertain. Marrow derived circulating angioblasts, or EPC in adults, express markers of hematopoietic stem cells as well as endothelial specific markers such as vascular endothelial- cadherin, E-selectin and the tie2 receptors. EPC participate in the replacement of lost endothelial cells, in the re-endothelization of vascular implants and in the neovascularisation of ischemic organs, cutaneous wounds and tumors. It has been proposed that the number of circulating EPC may influence vascular functions and determine the risk of cardiovascular disease[3].

Angiogenesis from pre-existing vessels:

In this type of angiogenesis, there is vasodilatation and increased permeability of existing vessels, degradation of extracellular matrix and migration of endothelial cells.

The major steps are listed below:

- Vasodilatation in response to nitric oxide and VEGF induced increased permeability of the pre-existing vessels.
- Proteolytic degradation of the basement membrane of the parent vessels by metalloproteinase and disruption of cell to cell contact between endothelial cells of the vessels by plasminogen activator.
- Migration of endothelial cells towards the angiogenic stimuli.
- Proliferation of endothelial cells, just behind the leading front of the migrating cells.
- Maturation of the endothelial cells, which includes inhibition of growth and remodelling into capillary tubes.
- Recruitment of periendothelial cells to support the endothelial tubes and form the mature vessels[4].

Angiogenesis inhibitor

An angiogenesis inhibitor is a substance that inhibits angiogenesis. Some angiogenesis inhibitors are a normal part of the body's control, some are administered as drugs, and some come from diet. Angiogenesis inhibitors were once thought to have potential as a "silver bullet" treatment applicable to many types of cancer, but this has not been the case in practice. Nonetheless, inhibitors are used to treat cancer, macular degeneration in the eye, and other diseases that involve a proliferation of blood vessels.

Matrix-Derived Inhibitors of Angiogenesis

Arresten: The main component of vascular basement membranes is type IV collagen, forming a mesh-like structure with other macromolecules, such as laminin, heparan sulfate proteoglycans, fibronectin, and entactin. Arresten is a recently identified endogenous inhibitor of angiogenesis. It is a 26-kDa molecule derived from the noncollagenous

(NC1) domain of the α1 chain of type IV collagen. Arresten selectively inhibits endothelial cell tube formation. It also inhibits the formation of new blood vessels in Matrigel plug assay in mice. Arresten inhibits endothelial cell proliferation and migration. Arresten does not have a significant effect on the proliferation of several cancer cell lines even at very high doses. Arresten affects metastasis leading to significant reduction of pulmonary nodules in arresten-treated mice and inhibition of large and small renal cell carcinoma tumor growth. The existing evidence suggests that arresten might function via α1β1 integrin and block the binding of α1β1 integrin to the type I collagen. In this regard, α1 integrin neutralizing antibodies can suppress angiogenesis associated with tumor growth. The β1 integrin is also involved in angiogenesis. Ablation or blocking of the interactions with integrin α1β1 inhibits indicating that integrin α1β1 acts as a proangiogenic factor. Arresten might also function via binding to heparan sulfate proteoglycan and previous studies have shown that heparan sulfate proteoglycan binds to the α1 NC1 domain of type IV collagen[5,6,7].

Canstatin: Canstatin is a 24-kDa fragment of the α2 chain of type IV collagen. Recombinant canstatin significantly inhibits endothelial cell migration and tube formation in a dose-dependent manner. Canstatin inhibits serum-stimulated human endothelial cell proliferation and induces apoptosis with no inhibitory effect on proliferation or apoptosis of nonendothelial cells. Canstatin also suppresses growth of tumors in human xenograft mouse models, with histology revealing decreased CD31-positive vasculature. It inhibits the phosphorylation of Akt, focal adhesion kinase (FAK), mammalian target of rapamycin (mTOR), eukaryotic initiation factor 4E-binding protein-1 (4E-BP1), and ribosomal S6 kinase in cultured human umbilical vein endothelial cells (HUVEC). It also induces Fas ligand (FasL) expression, activates procaspase-8 and -9 cleavage, reduces mitochondrial membrane potential, and increases cell death[8.9.10].

Endorepellin: Perlecan is basement membrane heparan sulfate proteoglycan that plays key roles in vascular growth. The COOH-terminal end of perlecan, called endorepellin or perlecan domain V, potently inhibits several aspects of angiogenesis: endothelial cell migration, collagen-induced endothelial tube morphogenesis, and blood vessel growth in the chicken chorioallantoic membrane assay and in mouse Matrigel plug assays. Endorepellin binds to endothelial cells as well as to squamous cell carcinoma cells and breast carcinoma cells via high-affinity receptors. Interestingly, endorepellin binds endostatin, another matrix-derived inhibitor of angiogenesis, and counteracts its antiangiogenic effects[11].

Endostatin: Endostatin is an endogenous collagen XVIII–derived angiogenesis inhibitor identified and purified from murine hemangioendothelioma cell line and later characterized in mice. It corresponds to a 20-kDa fragment derived from the COOH-terminal NC1 domain of type XVIII collagen[12]. Recombinant endostatin efficiently blocks angiogenesis and suppresses primary tumor growth and metastasis in experimental animal models without any apparent side effects, toxicity, or development of drug resistance. Recent studies have reported that endostatin interferes with FGF-2-induced signal transduction, blocking endothelial cell motility inducing apoptosis, causing G1 arrest of endothelial cells through inhibition of cyclin D1, blocking VEGF-mediated signaling and blocking tumor necrosis factor. Recently, it is shown that endostatin down-regulates many signaling pathways in human microvascular endothelium associated with proangiogenic activity and at the same time up-regulating many antiangiogenic genes[13].

Endostatin binds to the α5β1 integrin and inhibits the migration of endothelial cells by blocking signaling pathways via Ras and Raf. Moreover, a recent study shows that endostatin action is dependent on expression of E-selectin on the endothelial cells, although a direct binding of endostatin to E-selectin was not observed. Therefore, more work needs to be done to sort out the exact mechanism of action associated with endostatin. Endostatin inhibits the activation and activity of certain matrix metalloproteinases (MMP; i.e., MMP-2, -9, and -13 and MT1-MMP) and it binds directly to at least MMP-2 and -9[14,15].

Endostatin-like fragment from type XV collagen: Based on a homology search with endostatin, a 22-kDa fragment of collagen XV was found with 70% homology to endostatin. It inhibits the migration of endothelial cells but has no effect on proliferation. Systemic administration of endostatin-like fragment from type XV collagen (EFC-XV) suppresses the growth of tumors in a xenograft renal carcinoma model. It seems that the functions of endostatin and EFC-XV somewhat overlap, double knockout mice show no additional defects compared with the single knockout mice[16,17].

Anastellin—a fibronectin fragment: Incubation of soluble fibronectin with a small fibronectin-derived fragment, called anastellin, results in a polymeric form of fibronectin that is strongly antimetastatic in tumor-bearing mice. Both anastellin and polymeric fibronectin reduce tumor growth in mice, and the tumors are less vascularized. Anastellin is unable to inhibit Matrigel plug angiogenesis in mice that lack plasma fibronectin, but it is fully active in mice that are null for vitronectin, which like fibronectin is a major endothelial cell adhesion protein[18].

Fibulins: Proteolytic digestion of basement membrane preparations by elastases and cathepsins releases fragments, which possess antiangiogenic activity. The report recently shows that fibulin 5 promotes wound healing in vivo. Considering that wound healing is dependent on angiogenesis, this report opposes the notion that fibulin 5 is an inhibitor of angiogenesis. Future studies will hopefully shed more light on this opposing action of fibulin 5[19].

Thrombospondins: Thrombospondin-1 (TSP-1) was the first protein to be recognized as a naturally occurring inhibitor of angiogenesis. It is a large multifunctional ECM glycoprotein that regulates various biological events, like cell adhesion, angiogenesis, cell proliferation and survival, transforming growth factor-β (TGF-β) activation, and protease activation. Some studies suggest that TSP-1 may possess dual activity (proangiogenic and antiangiogenic) depending on proteases that generate fragments of TSP-1. It has been shown to inhibit tumor growth and metastasis, thus making it a potent inhibitor of in vivo neovascularization and tumorigenesis. Overexpression of TSP-1 in mice suppresses wound healing and tumorigenesis, whereas the lack of functional TSP-1 results in increased vascularization of selected tissues. Expression of TSP-1 has been inversely correlated with malignant progression in breast and lung carcinomas and melanomas[20,21]. TSP-2 also shows antiangiogenic activity. Injection of TSP-2-transfected squamous cell carcinoma cells into the dermis of nude mice resulted in inhibition of tumor growth that was even stronger than the inhibition observed with TSP-1-transfected cells. The combined overexpression of TSP-1 and TSP-2 completely prevented tumor formation. Daily injections of TSP-2 resulted in a significant inhibition of the growth of human squamous cell carcinomas in vivo and reduced tumor vascularization. Possible mechanisms for this antiangiogenic activity are inhibition of VEGF-induced endothelial cell migration, tube formation, and increased endothelial cell–specific apoptosis[22].

Tumstatin: The entire 28-kDa fragment of α3 chain of NC1 domain of type IV collagen was

named tumstatin. In vivo overexpression of tumstatin domains by tumor cells inhibits their invasive properties in a mouse melanoma model. Tumstatin inhibits formation of new blood vessels in Matrigel plug assays and suppresses tumor growth of human renal cell carcinoma and prostate carcinoma in mouse xenograft models. This is associated with in vivo endothelial cell–specific apoptosis. The antiangiogenic and proapoptotic activity of tumstatin is specific for endothelial cells. Maeshima et al. show that tumstatin functions as an endothelial cell–specific inhibitor of protein synthesis[23]. MMPs are capable of degrading type IV collagen and liberating fragments containing tumstatin. MMP-9 is the most effective in cleaving tumstatin-containing fragments from type IV collagen, but MMP-2, -3, and -13 also can release tumstatin. MMP-9-deficient mice have decreased levels of tumstatin in their blood, and the tumors in MMP-9-null mice grow faster than the tumors in wild-type mice[24].

Non-Matrix-Derived Inhibitors of Angiogenesis

Angiostatin: Angiostatin is a cryptic fragment of plasminogen that possesses antiangiogenic properties, a property not shared by the parent molecule (plasminogen). Angiostatin was originally purified from serum and urine of mice bearing s.c. Lewis lung carcinoma, where the growth of metastases was inhibited by tumor-generated angiostatin. Angiostatin also inhibits migration and tube formation associated with proliferating endothelial cells[25,26].

Cleaved antithrombin III and prothrombin kringle-2: Circulating clotting factors in the blood seem to play an important role in angiogenesis. It inhibits angiogenesis and tumor growth[27].

Chondromodulin-I: Although cartilage contains many angiogenic factors during ossification, in adults it is an avascular tissue. The 25-kDa cartilage-specific NC1 matrix protein chondromodulin-I is a strong inhibitor of angiogenesis. Local administration of recombinant human chondromodulin-I almost completely blocks vascular invasion and tumor growth in vivo. Furthermore, it inhibits the growth of colon adenocarcinoma in vivo, implying therapeutic potential for other solid tumors.[28]

Soluble Fms-like tyrosine kinase 1: The soluble version of VEGFR-1 [soluble Fms-like tyrosine kinase 1 (sFlt-1)] was identified by Kendall et al. sFlt-1 has a strong affinity for VEGF and placental growth factor. In this regard, several reports have suggested that sFlt-1 can serve as an antitumor agent by inhibiting VEGF. The relevance for sFlt-1 at the normal physiologic concentration in the regulation of cancer progression is not understood[29].

Interferons: IFNs, pleiotropic cytokines that regulate antiviral, antitumor, apoptotic, and cellular immune responses, were the first endogenous antiangiogenic regulators identified. IFNs inhibit angiogenesis induced by tumor cells in mice. IFN-α or IFN-β has biological activity against squamous cell carcinomas and inhibits angiogenesis in tumor-bearing nude mice. IFN-α and IFN-β treatment also inhibits angiogenesis by down-regulation of bFGF expression but other studies suggest that the action of IFN-α is not mediated by bFGF or VEGF[30,31].

Interleukins: ILs is a family of leukocyte-derived proteins with broad-ranging effects on multiple physiologic properties, including angiogenesis**.** ILs bearing a NH2-terminal Glu-Leu-Arg (ERL) motif, such as IL-8, tends to display proangiogenic properties, whereas those lacking this motif have been found to inhibit angiogenesis. IL-1, mainly secretable IL-1β, is involved in inflammation, tumor growth, and metastasis. IL-4 inhibits bFGF-induced angiogenesis. IL-12 can inhibit angiogenesis. IL-18 inhibits FGF-stimulated endothelial cell proliferation in vitro[32,33].

2-Methoxyestradiol: 2-Methoxyestradiol (2-ME), an endogenous estradiol metabolite, is an inhibitor of angiogenesis, with direct effect on cancer cells. The endogenous physiologic role for 2-ME as an inhibitor of angiogenesis is not yet understood[34].

Pigment epithelium-derived factor: Because pigment epithelium-derived factor (PEDF), a noninhibitory member of the serpin superfamily, was identified to be responsible for the avascularity of ocular compartments in 1999, it is the most potent inhibitor of angiogenesis in the mammalian eye and is involved in the pathogenesis of angiogenic eye diseases, such as proliferative diabetic retinopathy. It also has neurotrophic activity both in retina and in the central nervous system[35,36]. Its antiangiogenic activity is selective, in that PEDF targets only new vessel growth but spares existing ones, and it is reversible. Recent study highlights two beneficial effects of PEDF treatment on tumor growth and expansion. One is the suppression of tumor angiogenesis. Overexpression of PEDF was found to significantly inhibit melanoma growth. In addition, PEDF may serve as a multifunctional antitumor agent in neuroblastomas, inhibiting angiogenesis while promoting the numbers of Schwann cells and differentiated tumor cells that in turn produce PEDF[37].

Prolactin fragment: The intact prolactin (23 kDa) is enzymatically cleaved in several different tissues to generate a 16-kDa (16K PRL) and 8-kDa fragment. Although the intact prolactin has activities consistent with proangiogenesis, the generation of the 16K PRL fragment exposes a cryptic antiangiogenic activity[38].

Platelet factor-4: PF-4 is a protein released from platelet α-granules during platelet aggregation that has been shown to have antiangiogenic properties both in vitro and in vivo. PF-4 inhibits angiogenesis by associating directly with FGF-2, inhibiting its dimerization and blocking FGF-2 binding to endothelial cells[39].

Tissue inhibitors of matrix metalloproteinases: Tissue inhibitors of matrix metalloproteinases (TIMP) suppress MMP activity and ECM turnover. In addition to their MMP inhibitory activity, TIMPs have pluripotent effects on cell growth, apoptosis, and differentiation. TIMP-2 inhibits angiogenic factor–induced endothelial cell proliferation in vitro and angiogenesis in vivo, independent of MMP inhibition.[40]

Vasostatin: Vasostatin, a NH2-terminal domain of human calreticulin inclusive of amino acids 1,180, is a potent angiogenesis inhibitor. It selectively inhibits endothelial cell proliferation and angiogenesis in response to stimulation from growth factors and suppresses tumor growth[41].

Troponin I: Troponin I (Tn I) is a novel cartilage-derived angiogenesis inhibitor, which inhibits endothelial cell proliferation and angiogenesis in both in vivo and in vitro model systems. Tn I also inhibits metastasis of a wide variety of tumors in vivo. Tn I is a subunit of the troponin complex, which along with tropomyosin is responsible for the calcium-dependent regulation of striated muscle contraction[42].

Regulators of angiogenesis

Angiopoietins: The angiopoietins, Ang1 and Ang2, are required for the formation of mature blood vessels, as demonstrated by mouse knock out studies. Ang1 and Ang2 are protein growth factors which act by binding their receptors, Tie-1 and Tie-2; while this is somewhat controversial, it seems that cell signals are transmitted mostly by Tie-2; though some papers show physiologic signaling via Tie-1 as well. These receptors are tyrosine kinases. Thus, they can initiate cell signaling when ligand binding causes a dimerization that initiates phosphorylation on key tyrosines[43].

Fibroblast growth factor: The fibroblast growth factor (FGF) family with its prototype

members FGF-1 (acidic FGF) and FGF-2 (basic FGF) consists to date of at least 22 known members. Most are single-chain peptides of 16-18 kDa and display high affinity to heparin and heparan sulfate. The FGF-receptor family is composed of seven members get activated through autophosphorylation induced by a mechanism of FGF-mediated receptor dimerization. Receptor activation gives rise to a signal transduction cascade that leads to gene activation and diverse biological responses, including cell differentiation, proliferation, and matrix dissolution, thus initiating a process of mitogenic activity critical for the growth of endothelial cells, fibroblasts, and smooth muscle cells. Expression of basic FGF and its receptor FGF-2 has been identified in normal oral keratinocyte, as well as in oral cancer. Furthermore, the levels of bFGF found in these tumors were similar to or lower than those found in the adjacent normal mucosa. Elevated levels of bFGF in the serum and urine of SCCHN patients and have correlated this with poor prognosis[44].

VEGF: It belongs to the VEGF family, which currently consists of six members: VEGF-A (or VEGF), PlGF,VEGF-B, VEGF-C, VEGF-D, and of virus VEGF.(VEGF-E) It has been demonstrated to be a major contributor to angiogenesis, increasing the number of capillaries in a given network[45]. Upregulation of VEGF is a major component of the physiological response to exercise and its role in angiogenesis is suspected to be a possible treatment in vascular injuries. In vitro studies clearly demonstrate that VEGF is a potent stimulator of angiogenesis because, in the presence of this growth factor, plated endothelial cells will proliferate and migrate, eventually forming tube structures resembling capillaries. VEGF causes a massive signaling cascade in endothelial cells. Mechanically, VEGF is upregulated with muscle contractions as a result of increased blood flow to affected areas. The increased flow also causes a large increase in the mRNA production of VEGF receptors 1 and 2. The increase in receptor production means muscle contractions could cause upregulation of the signaling cascade relating to angiogenesis. As part of the angiogenic signaling cascade, NO is widely considered to be a major contributor to the angiogenic response because inhibition of NO significantly reduces the effects of angiogenic growth factors. However, inhibition of NO during exercise does not inhibit angiogenesis, indicating there are other factors involved in the angiogenic response[46,47].

MMP: Another major contributor to angiogenesis is matrix metalloproteinase (MMP). MMPs help degrade the proteins that keep the vessel walls solid. This proteolysis allows the endothelial cells to escape into the interstitial matrix as seen in sprouting angiogenesis. Inhibition of MMPs prevents the formation of new capillaries. These enzymes are highly regulated during the vessel formation process because destruction of the extracellular matrix would decrease the integrity of the microvasculature[48].

DII4: Delta-like ligand 4 (DII4) is a recently discovered protein with an important negative regulatory effect on angiogenesis. Dll4 is a transmembrane ligand, for the notch family of receptors[49].

Angiogenesis in inflammation

The Hallmark of the acute inflammation is increased vascular permeability leading to escape of a protein rich – fluid into extravascular spaces. There is a discrete order of events in physiologically acute inflammation and repair. However, these events become disorganized during chronic unresolved inflammation and carcinogenesis. This local microenvironment has led to the suggestion that tumors are ‘wounds that do not heal’. The constant disruption of homeostasis by proliferating epithelial cells produces a chronic inflammatory reaction, which is an abortive attempt to re-establish

homeostasis through tissue remodeling. However, the classic players in acute inflammation (granulocytes, macrophages, endothelial cells and fibroblasts) that ordinarily lead to the resolution of a wound through an orderly series of events instead react paradoxically to the presence of dysfunctional epithelial cells by promoting their survival and replication. This process includes inflammatory angiogenesis[50].

Leukocytes are cells of defense. Their main function is to protect our body against invading microorganisms. Activated endothelial cells promote leukocyte recruitment at inflammatory sites; new blood vessel formation, sustains chronic inflammation, and lymphatic vessels transport antigens and antigen-presenting cells to lymph nodes, where they stimulate naive T and B lymphocytes to elicit an antigen-specific immune response. In contrast, leukocytes and lymphocytes are far less efficient in protecting us from cancer, the "enemy from within." Worse, cancer can exploit inflammation to its advantage. Monocytes and macrophages produce tumor promoting factors which stimulate angiogenesis, lymphangiogenesis and tumor cell invasion. Recent reports indicate that angiopoietin Tie and the ephrin Eph ligand receptor systems regulate leukocyte trafficking across the endothelium, thereby revealing connections among the regulation of vascular homeostasis, angiogenesis, and leukocyte trafficking[51].

Tumor angiogenesis and Metastasis

Tumor growth is often a multi-step process that starts with the loss of control of cell proliferation. The cancerous cell then begins to divide rapidly, resulting in a microscopically small, spheroid tumor, an in situ carcinoma. As the tumor mass grows, the cells will find themselves further and further away from the nearest capillary. Finally, the tumor stops growing and reaches a steady state, in which the number of proliferating cells counterbalances the number of dying cells. The restriction in size is caused by the lack of nutrients and oxygen. In situ carcinomas may remain dormant and undetected for many years, and metastases are rarely associated. Yet, several months or years later, an in situ tumor may switch to the angiogenic phenotype, induce the formation of new capillaries, and start to invade the surrounding tissue. The "angiogenic switch" depends on a net balance of positive and negative angiogenic factors in the tumor. Thus, the angiogenic phenotype may result from the production of growth factors, such as FGF-2 and VEGF, by tumor cells and/or the down-regulation of negative modulators, like TSP-1, in tissues with a quiescent vasculature. In both normal and pathological angiogenesis, hypoxia is the main force initiating the angiogenic process. Also, several oncogenes such as v-ras, K-ras, v-raf, src, fos and v-yes induce the up-regulation of angiogenic factors like VEGF and increase the production of cytokines and proteolytic enzymes. In contrast, the tumor suppressor p53 has been found to cause degradation of HIF-1a, inhibition of VEGF production, and stimulation of the inhibitor TSP-1.The final step in the progression of a tumor is metastasis. Neovascularization of a primary tumor increases the possibility that cancer cells will enter the blood stream and spread to other organs and is also necessary for the growth of metastases in distant organs. Most of the micrometastases have a high death rate and are not vascularized until they switch to the angiogenic phenotype[52,53,54].

Physiological angiogenesis

Besides during embryogenesis, angiogenesis is also activated in the female reproductive system during ovulation, corpus luteum formation, and embryo implantation. During these processes, angiogenesis is mediated mainly by VEGF. Neovascularization also plays a critical role in successful wound healing that is probably

regulated by growth factors such as FGF-2 and VEGF. Macrophages may contribute to the healing process by releasing these angiogenic factors. Angiogenesis is generally associated with aerobic exercise and endurance exercise. While arteriogenesis produces network changes that allow for a large increase in the amount of total flow in a network, angiogenesis causes changes that allow for greater nutrient delivery over a long period of time[55].

Diseases caused by abnormal or excessive angiogenesis

Numerous organs: Cancer (activation of oncogenes and loss of tumor suppressor genes), infectious disease (pathogens express angiogenic genes, induce angiogenic programs or transforms Ecs), autoimmune disorders (activation of mast cells or other leukocytes).

Blood vessels: vascular malformations (Tie-2 mutation), DiGeorge syndrome (low VEGF and neuropilin-1 expression), HHT (mutations of endoglin or ALK-1), cavernous haemangioma (loss of Cx37 and Cx40), artherosclerosis, transplant arteriopathy.

Adipose tissue: Obesity (angiogenesis induced by fatty diet, weight loss by angiogenesis inhibitors.

Skin: Psoriasis, warts, allergic dermitis, scar keloids, pyogenic granulomas, blistering disease, Kaposi sarcoma in AIDS.

Eye: Persistent hyperplastic vitreous syndrome (loss of Ang-2 or VEGF, diabetic retinopathy, retinopathy of prematurity, choroidal neovascularisation (TIMP-3 mutation).

Lung: Primary pulmonary hypertension (germline BMPR-2 mutation, somatic EC mutations), asthma, nasal polyp.

Intestine: Inflammatory bowel and periodontal disease, ascites, peritoneal adhesions.

Reproductive system: Endometriosis, uterine bleeding, ovarian cyst, ovarian hyperstimulation.

Bone and joints: Arthritis, synovitis, osteomyelitis, osteophyte formation[56-59].

Diseases caused by insufficient angiogenesis or vessel regression

Nervous system: Alzheimer disease (vasoconstriction, microvascular degeneration and cerebral angiopathy due to EC toxicity by amyloid), Amyotrophic lateral sclerosis, diabetic neuropathy (impaired perfusion and neuroprotection, causing motoneuron or axon degeneration due to insufficient VEGF production), stroke (correlation of survival with angiogenesis in brain, stroke due to arteriopathy, notch-3 mutations).

Blood vessels: Atherosclerosis (impaired collateral vessel development), hypertension (microvessel rarefaction due to impaired vasodilatation or angiogenesis), diabetes (impaired collateral growth and angiogenesis in ischemic limbs), restenosis (impaired re-endothelization after arterial injury at old age).

Gastrointestinal: Gastric or oral ulcerations (delayed healing due to angiogenesis inhibitors by pathogens), crohn disease (due to mucosal ischemia).

Skin: Hair loss (due to angiogenesis inhibitors), skin purpura, telangiectasia and venous lake formation (age dependent reduction of vessel number maturation due to EC telomere shortening).

Reproductive system: Pre-eclampsis (EC dysfunction resulting in organ failure, thrombosis and hypertension due to deprivation of VEGF by soluble Flt-1, menorrhagia (fragility of SMC –poor vessels due to low Ang-1 production.

Lung: New natal respiratory distress (insufficient lung maturation and surfactant production in premature mice due to reduced HIF-2alpha and VEGF production), pulmonary fibrosis, emphysema (Alveolar EC apoptosis upon VEGF inhibition).

Kidney: Nephropathy (Age related vessels loss due to TSP-1 production).

Bone: Osteoporosis, impaired bone fracture healing (impaired formation due to age

dependent decline of VEGF- driven angiogenesis, angiogenesis inhibitors prevents fracture healing)[60-63].

CONCLUSION

Angiogenesis is the process of generating new capillary blood vessels and is among the key events in various tissue destructive pathologic processes, such as tumor growth, metastasis, arthritis, etc., as well as in physiologic processes, like organ growth and development, wound healing, and reproduction. A hypothesis that tumor growth is angiogenesis dependent was first proposed by Folkman. Angiogenesis is thought to depend on a delicate balance between endogenous stimulators and inhibitors. Stimulators of angiogenesis include growth factors, such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and platelet-derived growth factor, and hypoxic conditions that activate hypoxia-inducible factor-1, which itself can up-regulate angiogenic proteins, as well as angiogenic oncogenes, such as Ras, and tumor suppressors, such as p53. Endogenous inhibitors of angiogenesis include various antiangiogenic peptides, hormone metabolites, and apoptosis modulators. As our understanding

of mechanism of angiogenesis increases, this will have a significant impact on several divergent areas in biology, including growth and development, wound healing, neoplasia, and tissue reconstruction.

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