Tumor Angiogenesis

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Abstract

Tumor angiogenesis is the growth of new blood vessels which is required for tumor growth and progression. Vascularization of the tumor occurs through a series of sequential steps before or during the multistep progression to neoplasia. Several events occur during the formation of new vessels including production of protease enzymes, unregulation of positive regulators of angiogenesis, and down regulation of negative regulators. In addition, tumor associated macrophage also influence angiogenesis by secreting enzymes, enzymes inhibitors and cytokines. Recent knowledge in tumor angiogenesis may have clinical implications in diagnosis and treatment. Quantification of microvessel density in tumor specimen correlates either metastasis or recurrence in many malignancies such as breast cancer and lung cancer. Therefore, assessment of tumor angiogenesis may serve as prognostic factors. Therapeutic applications include the development of new agents with antiangiogenic properties, vascular targeting drugs, antibody-based therapy, and gene therapy. Combination of antiangiogenic therapy with cytotoxic drugs may enhance antitumor activity. Moreover, the role of antiangiogenic therapy in adjuvant setting may provide and alternative approach to better cancer treatment in the near future.

Key word : Tumor Angiogenesis, Mechanism, Factors

Angiogenesis is a dynamic process which can be defined as the growth and development of new microvessels. It occurs during normal development (embryonic and postembryonic), during the menstrual cycle in the ovaries and endometrium, in wound healing, and a number of benign diseases e.g., chronic inflammations, diabetic retinopathy, psoriasis, osteoarthitis, and some immune reactions. It is controlled by many diverse, complex factors acting in concert in local environments. The growth of solid ne oplasms is always accompanied by neovascularization and is angiogenesis dependent(1).

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Moreover, tumor angiogenesis can facilitate metastatic spread[2]. Therefore, the essential role of angiogenesis in tumor progression and metastasis as well as and the existence of positive and negative regulated factors provide rationales for cancer treatment. Recent developments include methods to assess angiogenesis, study of mechanisms of angiogenesis, its prognostic implications as well as development of antiangiogenic drugs[3].

Mechanisms of tumor angiogenesis

Vascularization of the primary and its metastatic tumor is required for tumor growth and progression. Without a new blood supply, the tumor is unable to grow beyond 1-2 mm^3[4]. However, this state of restricted tumor size with the absence of angiogenesis does not necessarily to prevent tumor proliferation. Animal studies showed that tumor cells continue to proliferate at a high rate which is balanced by a high rate of tumor cell apoptosis, thus, no expansion of tumor mass occurs[5].

The generation of new blood vessels occur through a series of sequential steps. The process depends mainly on locomotion, proliferation, and tube formation by the capillary endothelial cells (Fig. 1). The vessels migrate into the tumor and vice-versa. Under physiologic conditions, the vascular endothelium is a quiescent tissue with a very low cell division rate. During angiogenesis, endothelial cells emerge from their quiescent state and proliferate rapidly. The endothelial basement membrane is degraded, new endothelial cells with vascular loops sprout, and recruitment of pericytes occur[6,7]. However, tumors impose modifications on a new capillary bed that differs from the angiogenesis induced by non-neoplastic cells. A capillary blood vessel in the tumor may contain more endothelial cells per lumen[8,9]. Tumor-induced vessels are often dilated and saccular[10]. A tumor may contain giant capillaries and arteriovenous shunt without intervening capillaries. Moreover, organization of vessels may differ from one tumor location to the next[10]. The tumor vessels are often leak and are abnormal in size and shape[11].

The population of tumor cells and capillary endothelial cells with the neoplasm constitute a highly integrated ecosystem. In this ecosystem, the mitotic index of the two cell populations depend on each other. Tumor cells apppear to stimulate endothelial-cell proliferation, and endothelial cells have an indirect effect over the rate of tumor growth[12].

Several biochemical events occur sequentially during the formation of new vessels. They include production of protease enzymes such as urokinase and collagenase by both tumor and endothelial cells and changes in the basement membrane

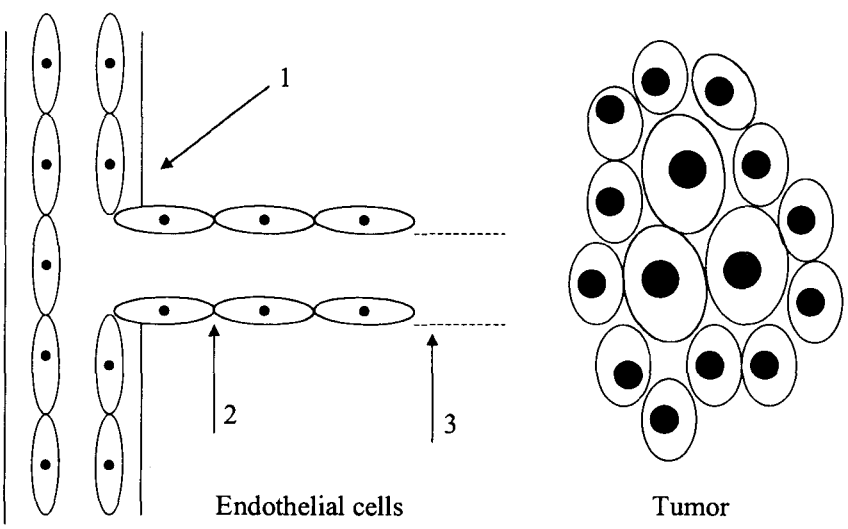

Fig. 1. Illustration of the sequential steps of tumor angiogenesis
1. Extracellular matrix breakdown
2. Endothelial cell migration and proliferation
3. Tube formation

components (sulfated glycosaminoglycan, fibronectin, laminin collagen type IV, and E-selectin). Proteolytic activity is critical for migration and invasion of endothelial cells into the neighboring tissue [13,14]. The vessels leak fibrinogen and plasminogen, and tissue factor is activated, producing hypercoagulability and extravascular fibrin deposition. Fibrin leaks from the new vessels, undergo local degradation to form a scaffold to guide endothelial tube formation.

Certain cell adhesion molecules such as E-selection in up-regulated in tumor endothelium and is involved in capillary morphogenesis[15]. Certain integrin such as αVß3[16] and αVß5[17] which are essential for the endothelial cell's viability are induced by the newly proliferating and migrating endothelial cells.

During early stages of tumor development, it obtains nutrients, oxygen and exchange of wastes by simple diffusion which becomes inadequate as it grows larger. Neovascularization permits rapid tumor growth and survival by providing nutrient, oxygen, growth factors and removal of catabolites from tumor and endothelial cells. Moreover, the vascularized tumor is not only perfuse (perfusion effect), but receives stimuli from endothelial cells (paracrine effect). Endothelial cells release growth factors such as bFGF, VEGF, PDGF[18], and cytokines such as IL-1, IL-6[19], IL-8[20], and GM-CSF[21] that stimulate tumor cells.

The angiogenic switch which activates tumor angiogenesis involves the upregulation of positive regulators of angiogenesis and must be accompained by downregulation of negative regulating factors. Recently, many angiogenic factors have been discovered, sequenced, and cloned (Table 1). Several proteins inhibit endothelial cell proliferation and may contribute to restriction of endothelial cell growth. Interestingly, thrombospondin, an antiangiogenic factor is under the control of p53 tumor suppressor gene[22]. p53 is a nuclear protein involved in cell-cycle regulation, DNA repair, and apoptosis. Mutation of p53 gene enable cells to survive under hypoxic conditions, and to switch on the angiogenic phenotype by enhancing VEGF production[23] and down-regulation of thrombospondin. Regardless of how these suppressors of angiogenesis operate, tumors must override them in order to switch to the angiogenic phenotype. Some antian-

Table 1. Angiogenic factors.

Vascular endothelial growth factors (VPF/VEGF)
Vascular endothelial growth factor A (VEGF A)
Vascular endothelial growth factor B (VEGF B)
Vascular endothelial growth factor C (VEGF C)
Fibroblast growth factor
Acidic fibroblast growth factor (FGF-1)
basic bibroblast growth factor (FGF-2)
Fibroblast growth factor 3 (FGF-3, int-2)
Kaposi sarcoma fibroblast growth factor
Angiogenin
Transforming growth factor alpha (TFG-a)
Transforming growth factor beta (TFG-b)
Tumor necrosis factor alpha (TNF- a)
Platelet-derived endothelial cell growth factor (PDECGF)
Hepatocyte growth factor (scatter factor)
Interleukin 8 (IL-8)
Proliferin
Substance P
Granulocyte colony-stimulating factor (G-CSF)
placental growth factor
platelet-activating factor
Non-peptides
Lactate
Hyaluronic acid fragment
Erucamide
Nicotinamide
Prostaglandin E1 and E2 (PG-E1, PG-E2)
Tripeptide glycine-histidine-lysine (complexed to copper)
1-butyryl glycerol
Adenosine

Table 2. Antiangiogenic factors (fragments of larger proteins).

Tissue inhibitors of metalloproteinases (TIMP)
16-kd fragment of prolactin
Basic fibroblast growth factor (bFGF) soluble receptor
Transforming growth factor beta (TFG-b)
Interferon alpha, beta, gamma
Placental proliferin-related protein
Murine epidermal growth factor (fragment 33-42)
Interleukin 1 (IL-1)
Interleukin 12 (IL-12)
Proliferin related protien
2-methoxyextrdiol
Retinoic acid
Tumor necrosis factor-alpha (high concentration)
Prolactin
Angiostain (38 kd of plasminogen)
Fibronectin
Murine EGF
Platelet factor 4 (fragment)
SPARC fragment
Thrombopondin 1 (fragment)

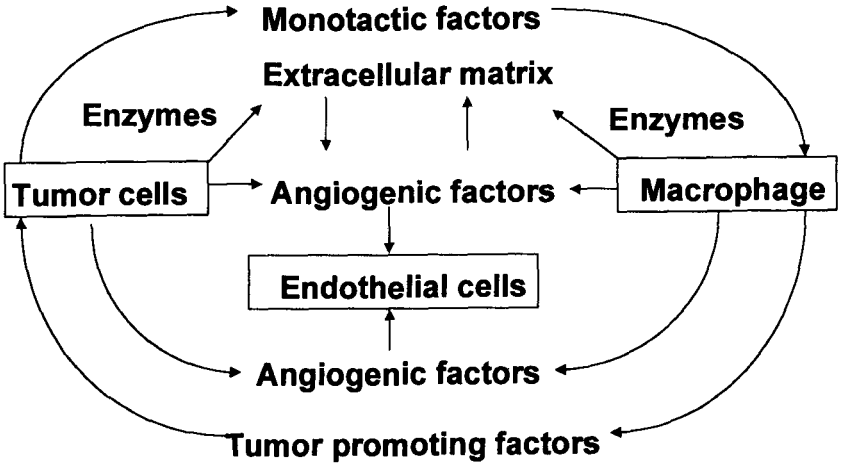

Fig. 2. Tumor-associated macrophage and tumor angiogenesis.

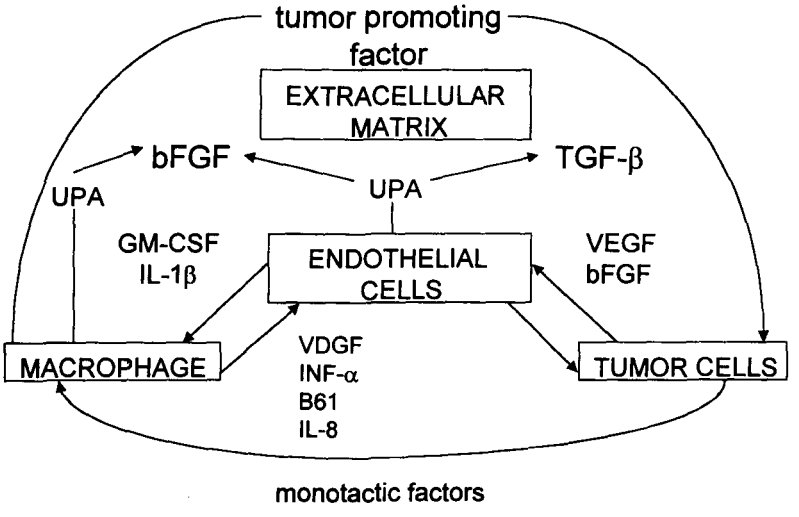

Fig. 3. Angiogenic cytokine networks in tumor angiogenesis.

giogenic proteins are internal fragment of larger molecules, most of which themselves lack antiangiogenic activity (e.g. prolactin[24] and fibronectin [25]) or more potent products of antiangiogenic parent molecules (e.g. platelet factor 4[26]). Several are the proteolytic product of larger molecules (Table 2).

Recent evidence suggests that tumor-associated macrophage can influence angiogenesis (Fig. 2). It can release angiogenic cytokines directly[27, 28] or indirectly by secreting extracellular matrix-degrading enzymes[29] which release angiogenic factors that have been sequestered by the matrix into active molecules (Fig. 3). Furthermore, macrophages can inhibit neovascularization by the secretion of enzyme inhibitors[30], and the production of cytokines which alter enzyme secretion by endothelial cells[31,32] (Table 3). Tumor cells also produce many monotactic factors that lead to the attraction of macrophages into the tumor area[33-35] (Table 4). Once in position, tumor-associated macrophage may then express an angiogenic phenotype, stimu-

Table 3. Angiogenic cytokines and extracellular matrix modulators producted by tumor-associated macrophape.

Angiogenic cytokines	Extracellular matrix modulating factors
VEGF	Enzymes and inhibittors
bFGF	collagenase
EGF	tPA
TNF- a	PAI-1
TP	Cytokines
WGF/SF	bFGF
IGF-1	TGF- b
IL-8	TNF-a
	Angiotropin
	PDGF
	IL-6

Table 4. Monotactic factors produced by tumor cells.

Monocyte chemotactic protein-1 (MCP-1)
Monocyte chemotactic protein-2 (MCP-2)
Monocyte chemotactic protein-3 (MCP-3)
Granulocyte-macrophage colony-stimulating factor (GM-CSF)
Granulocyte colony-stimulating factor (G-CSF)
Monocyte colony-stimulting factor (M-CSF)
Vascular endothelial growth factor (VEGF)

lating vascularization and enchancing local tumor growth. Therefore, macrophage may be an appealing target for future therapeutic strategies in cancer treatment.

Metastasis and Tumor Angiogenesis

Angiogenesis is necessary at the beginning and the end of the metastatic cascade. For a tumor cell to metastasize, they are many steps: It must gain access to the vasculature in the primary tumor, survive the circulation, arrest in the microvasculature of the target organ, exit from this vasculature, grow in the target organ, and induce angiogenesis[36]. Tumor cells are rarely shed into the circulation before a primary tumor is vascularized but they can appear in the circulation continuously after neovasculaziation of the primary tumor. The number of tumor cells shed from the primary tumor correlated with the density of tumor blood vessels and decreased angiogenesis is associated with a decrease rate of metastasis. Tumor cells can enter the circulation by penetrating through proliferating capillaries that have fragmented, leaky basement membranes. Angiogenic factors produced by the tumors such as bFGF and VEGF induce increased production of plasminogen activators and collagenases in proliferating endothelial cells, thus further contributing to degradation of the basement membrane and facilitating the entry of tumors cells. Tumor angiogenesis allows invasion and metastasis and also needs to occur at distant sites to allow growth of metastases[37]. A primary tumor containing a high proportion of angiogenic malignant cells is more likely to generate metastases that the are already angiogenic when they arrive at the target tissue. This evidence suggests the metastatic process is angiogenesis dependent and provides a rationale for using anti-angiogenic therapy for prevention and treatment of tumor metastasis.

Oncogenes and Tumor Suppressor Genes

A number of oncogenes and tumor suppressor genes play a key role in the multistep process of tumor angiogenesis. Specific steps in the process of tumor antiogenesis are regulated by several oncogenes (Table 5). Some angiogenic factors belong to the growth factor class of oncogenes (int 2, hst, FGF-5, erb A) and can simultaneously stimulate tumor growth and maintain angiogenesis. Tumor cells stimulate vascular endothelial cells indirectly through recruitment of inflammatory cells that release angiogenic cytokines and extracellular matrix degrading enzymes. Oncogenes such as ras promote protease production by tumor cells which facilitates vessel growth by supplementing the matrix-degrading function of activated endothelial cells[38]. Upon activation, endothelial cells express proto-oncogenes of the transcription factor class [39] (c-ets-1, e-rel, fos, jun, v-myc) which in turn activates the expression of other genes including growth factor and growth factor receptor classes (i.e., erb B) of cellular proto-oncogenes. Activation of growth factor and their receptors lead to a cascade of signal transduction involving cytoplasmic kinase classe of oncogenes (fps, mos, src). Together, this network of proto-oncogenes provides endothelial cells the means to receive and respond to angiogenic stimuli. Moreover, ras is also required to initiate signalling for endothelial cell migration, and essential for persistence of the migratory phenotype[40].

Table 5. Oncogenes and tumor angiogenesis.

Class	Oncogenes	Tumor angiogenesis
Growth factor	Int2 (FGF-3)	Overexpressed in Kaposi Sarcoma, enhances angiogensis in breast cancer
	hst (FTF-4), FGF-5	growth factor overexpressed in tumor
	erbA	cells
Growth factor receptor	Tek	recruitment of smoothe muscle cells and pericytes into the growing vessels, mutation of the receptoe leads to abnormal vessel formation
	met	stimulate angiogenesis in endothelial cells
	erb B	mutation causes angiosarcoma
Membrane-associated	ras	induces hemagiomas in mouse skin enhances endothelial cell motility increase production of metalloproteinases and latent proteases immortalizes cultured endothelial cell increased number of vessels in organ culture of prostate produces multifocal hemangiomas in
Cytoplasmic kinase	fps	transgenic mice induces Kaposi sarcoma like lesion
	mos	immortalize endothelial cells hypoxia-induced VEGF
	src	regulate genes involved in matrix
Transcription factors	ets	degradation endothelial invasion, expression of adhesive protein regulates the expression of adhesion
	rel	proteins stimulates VEGF in papilloma
	fos	inhibits thrombospondin
	jun, v-myc	

A number of cells expressing tumor suppressor genes switch their phenotype from anti-angiogenic to angiogenic when the tumor suppressor gene is inactivated[41]. Cells that lose a tumor suppressor gene become more angiogenic by decreased secretion of angiogenic of inhibitors. Loss of wild-type p53 tumor suppressor gene causes a decrease in the secretion of anti-angiogenic inhibitor thrombospondin by human fibroblasts, and breast cancer cells. As a result, the cells switch from an anti-angiogenic to an angiogenic phenotype[22, 42]. Thrombospondin secretion from glioblastoma cells is stimulated by an unidentified gene on chromosome 10[43]. Wild-type p53 can depress VEGF production and increases inhibitory thrombospondin[44]. Transfection of the von Hippel Lindau tumor suppressor gene into a human renal cell carcinoma line suppresses VEGF production[45]. These data suggests that the loss of angiogenic inhibitors mediated by a loss of a tumor suppressor gene may be essential in order for the developing tumor cells to become angiogenic. In order to become angiogenic, tumor cells that lose a tumor suppressor gene secrete decreased amounts of angiogenic inhibitors, whereas, tumor cells with an activated oncogene increase their secretion of angiogenic stimulators.

Assessment of Tumor Angiogenesis

Tumor angiogenesis can be assessed histologically by microvessel staining with monoclonal antibodies that detect vascular endothelium (ie) antibodies to cell adhesion molecules CD31[46] and CD34 and to factor VIII associated antigen by a standard immunoperoxidase staining technique. Tumor neovascularization could occur anywhere within a tumor but most frequently appears at the growing edges of the tumors known as "hot spot". Microvessel density can be determined by light microscopy in areas of the most intense neovascularization or "hot spot"[47]. Counting these hot spots and grading vascular density shows a correlation between increasing vascular density and distant metastasis. It

also provides independent prognostic information for many cancers. Microvessel density predicts metastatic risk because areas of high microvessel density increase the vascular surface area; thus facilitating escape of tumor cells into the circulation. Assessment of vascular density may be facilitated by computed image analysis and Chalkley counting.

To discover and test the efficacy of new anti-angiogenics, it is imperative to have appropirate *in vivo* models of angiogenesis. Some commonly used *in vivo* assays are hamster cheek pouches, rabbit ear chambers, dorsal skin and subcutaneous air sacs in rodents, semi-transparent vascularized membranous tissues, chick chorioallantoic and yolk sac membranes, iris and avascular corneas, sponge implant models (cannulated sponge model, disc angiogenesis system), rat freeze-injured skin graft assay, matrigel plugs and alogeinate-tumor pellets, conventional tumor models and transfectants, and human severe combined immunodeficinecy (SCID) mouse chimera. Despite the availabity of many model systems of angiogenesis, the most consistent limitation has been the availability of simple, reliable, reproducible, quantitative assays of the angiogenic response.

Clinical Implication of Tumor Angiogenesis

Angiogenesis research may be translated into clinical use for both diagnostic and therapeutic applications.

Quantification of microvescel density in a primary tumor specimen at the intial diagnosis of cancer may predict the risk of metastases or recurrence. A positive correlation between tumor angiogenesis and risk of metastasis, tumor recurrence, and death has been reported for a variety of tumors including breast cancer,[48] head and neck cancer [49], malignant melanoma[50], prostate cancer[51], ovarian cancer[52] bladder carcinoma[53], brain tumors[54], non small-cell lung cancer[55], testicular germ cell tumor[56], rectal carcinoma[57], and multiple myeloma[58].

Determination of angiogenic factors in blood, body fluid, urine or tissue of cancer patients also provides prognostic information of tumor progression and can guide therapy. Serum levels of basic fibroblast growth factor (bFGF) increase in patients with renal cell carcinoma[59] and provides a prognostic test for brain cancer[60]. Elevated levels of bFGF was found in urine of more than 37 per cent of patients with a wide spectrum of cancers[61]. The bFGF levels in CSF of children with brain tumors correlated with microvessel density in histologic sections and provided a prognostic indicator of risk of mortality[60]. Increased tissue levels of bFGF in renal cell carcinoma corelated with the risk of death[59]. Bladder cancer with a high level of VEGF was more invasive and metastatic than bladder cancer expressing low levels[62].

There are two types of therapy involving angiogenesis. One type of angiogenic therapy applies to returning the foci of proliferating microvessels to their normal resting stage and to prevent their regrowth. The second approach deals with vascular targeting and aims to specifically destroy the existing vasculature and induce tumor necrosis. Angiogenic therapy and vascular targeting has a number of advantages over tumor directed treatment. Endothelial cells are directly accessible to intravenous injection and the occlusion of individual capillaries leads to the death of thousands of tumor cells. Destruction of only a minority of endothelial cells with a capillary leads denudation of the endothelial lining and the formation of an occlusive

Table 6. Anti-angiogenic factors and drugs.

Compound
Preclinical
OLX-514
Ct-2584
Metastat
Angiostatin
FR-111142
VEGF inhibition
GM 1474
Vitaxin
Sol FLT-1 VEGF receptor
FEC-26644 and FCE 26950
αvß3 antagonist
Inbibitors of receptor tyrosine kinases
B-0829
Phase I
Interleukin-12
BB 2516 (marimastat)
Tecoglan
Polysulfated polysaccharide from seaweed cell walls
CA I
CI-994
CM 101
Phase I/II
AGM-1470
Phase II
Platelet factor 4
Thalidomide

thrombus within the vessels which may cause ischemic tumor necrosis. A vascular targeting agent should work for most types of solid tumors. More importantly, endothelial cells are genetically stable and therefore should not mutate to become resistant to the therapy as tumor cells often do. Combrestatin A-4 is a newly developed vascular targeting agent that selectively works against proliferating endothelial cell *in vitro* and induces vascular shutdown of tumors *in-vivo*(63).

Currently, there are three therapeutic approaches being developed at preclinical and clinical levels. Pharmacologically anti-angiogenic drugs are currently being developed against biochemical targets to stop neovasculature (Table 6), vascular targeting drugs aim to destroy the existing tumor vasculature, and immunotherapeutically specific antibody based therapy for vascular targeting. Gene therapy approach using tumor necrosis factor-alpha (TNF-a) gene regulated by promotors activated in tumor endothelium has also been recently developed. An alternative to TNF-a is to target prodrug activating enzymes within vasculature. Antisense phosphorothioate oligonucleotides has also been developed at a preclinical level as an angiogenesis inhibitor.

Optimal antiangiogenic therapy requires longer continous cycles than do conventional cytotoxic agents. Regression of a rapidly growing capillary bed is a slower process compared to the lysis of tumor cells. Combination of antiangiogenic therapy with cytotoxic drugs may be more effective than either drugs alone. It may be more effectively used in long term maintenance therapy and in an adjuvant setting. It is likely that antiangiogenic therapy as well as vascular targeting will become new alternative therapeutic approaches to cancer treatment in the near future.

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การสร้างเส้นเลือดของโรคมะเร็ง

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การสร้างเส้นเลือดของโรคมะเร็งเป็นการสร้างเส้นเลือดใหม่ ซึ่งจำเป็นสำหรับการเจริญเติบโตและลุกลามของโรค ขบวนการของการสร้างเส้นเลือดของก้อนมะเร็งเกิดเป็นลำดับขั้นตอนก่อนหรือระหว่างการเกิดโรคมะเร็งหลายขั้นตอน เหตุการณ์ต่าง ๆ ที่เกิดขึ้นระหว่างการสร้างเส้นเลือดใหม่ประกอบด้วยการสร้างน้ำย่อยโปรตีนเอส การเพิ่มตัวกระตุ้นหรือยับยั้งการสร้างเส้นเลือด ยิ่งไปกว่านั้นเซลล์เม็ดเลือดขาวแมคโครฟาซมีบทบาทต่อการสร้างเส้นเลือดโดยหลั่งน้ำย่อย สารยับยั้งน้ำย่อยและไซโตไคน์ ความรู้ในปัจจุบันเกี่ยวกับการสร้างเม็ดเลือดของมะเร็งอาจประยุกต์ใช้ทางคลินิกได้ทั้งในการวินิจฉัยและรักษาโรค การตรวจนับปริมาณเส้นเลือดในก้อนมะเร็งมีความสัมพันธ์กับการแพร่กระจายหรือการกลับเป็นซ้ำของโรคในมะเร็งหลายชนิด เช่น มะเร็งเต้านม และมะเร็งปอด ดังนั้นการตรวจนับเส้นเลือดอาจใช้เป็นปัจจัยพยากรณ์โรคได้ การประยุกต์ใช้ทางคลินิกในการรักษาประกอบด้วยการพัฒนายาใหม่ที่ยับยั้งการสร้างเส้นเลือดยาที่ทำลายเส้นเลือด การรักษาด้วยแอนติบอดี้ และยีนบำบัด การใช้การรักษาด้วยการยับยั้งการสร้างเส้นเลือดร่วมกับยาเคมีบำบัดอาจเพิ่มฤทธิ์ทำลายมะเร็ง ยิ่งไปกว่านั้นบทบาทของการรักษาแบบยับยั้งการสร้างเส้นเลือด เพื่อป้องกันการกลับเป็นซ้ำของโรคหลังการรักษาอาจเป็นทางเลือก ในการบำบัดรักษาโรคมะเร็งที่ดีขึ้นในอนาคตอันใกล้นี้

คำสำคัญ : การสร้างเส้นเลือดของโรคมะเร็ง, กลไก, ปัจจัยเกี่ยวข้อง

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