Cancer Related Inflammation and Tumor Angiogenesis

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1. Introduction

Today, the relationship between inflammation and cancer has been widely accepted. Cancer-related inflammation (CRI) was even considered as 'the other half of the tumor'. Angiogenesis plays an important role in the evolution of both cancer and inflammatory diseases. It has been well established that inflammation is a defensive reaction of living tissue to injury which involves vascular response. The establishment of tumor also generates new blood vessel formation, mainly through hypoxia. In addition, the inflammatory cells infiltrating the tumor tissue, particularly tumor-associated macrophages (TAM), also contribute to tumor angiogenesis. Angiogenesis triggered by CRI has been considered as a potential target for cancer therapy.

As depicted in Fig. 1, the multistep development of cancer is thought to require six biological capabilities including: sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis [2]. Recently, cancer-related inflammation, a key component of tumor microenvironment, has been proposed to promote tumor progression and serve as the seventh hallmark of tumor (Fig. 1) [1].

Cancer inflammation has long been proposed as promoter of tumor growth. As early as in the 19th century, observations have been made that tumors often arose at sites of chronic inflammation, and that inflammatory cells were present in human tumors [3]. Although this idea has waned for a long time, a renaissance of the inflammation-cancer connection suggested by multiple lines of evidence has led to a currently accepted paradigm [3-5]. These lines of evidence categorized by Mantovani *et al.* [5] are listed below.

- Inflammatory diseases (e.g., inflammatory bowel disease) could increase the risk of developing different types of cancer including bladder, cervical, gastric, intestinal, esophageal, ovarian, prostate and thyroid tumors. Inflammatory cells, chemokines and cytokines are present in the microenvironment of all tumors in both experimental animal models and humans from the earliest stages of development. Signs of 'smoldering' inflammation are present even in tumors for which a firm causal relationship to infection has not been established (for example, breast tumors). Epidemiological studies have revealed that chronic inflammation predisposes to different types of cancer suggesting that underlying infections and inflammatory responses are linked [3].
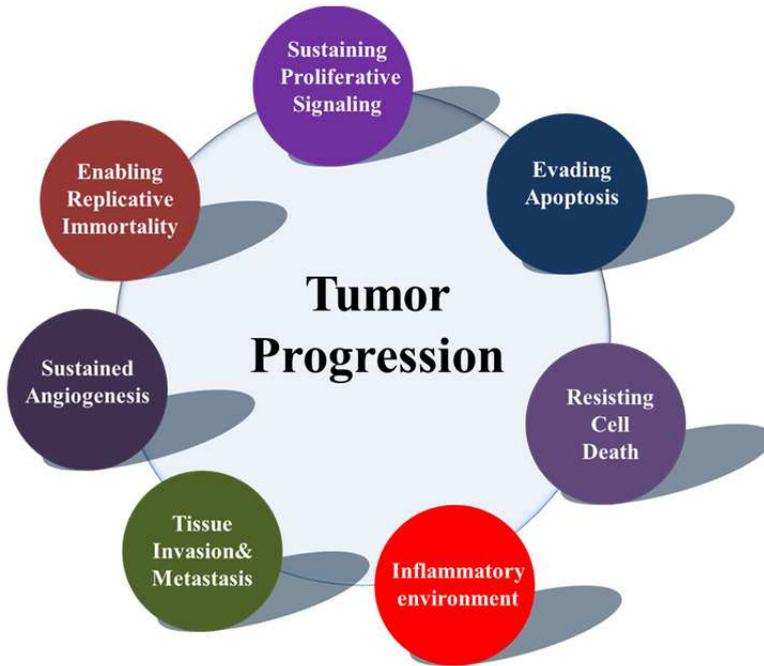
Fig. 1. Hallmarks of cancer [1].

- Signaling pathways involved in inflammation operate downstream of oncogenic mutations (such as mutations in the genes encoding RAS, MYC and RET).
- Adoptive transfer of inflammatory cells or overexpression of inflammatory cytokines promotes the development of tumors.
- Non-steroidal anti-inflammatory drugs (NSAIDs) reduce the risk of incidence of several tumors (e.g., colon and breast cancer) and mortality caused by these cancers. Protection offered by NSAIDs supports the idea that inflammation is a risk factor for certain cancers.
- The targeting of inflammatory mediators (e.g., TNF-α and IL-1β), key transcription factors involved in inflammation (e.g., NF-κB and STAT3) and tumor infiltration of inflammatory cells decreases the incidence and spread of various tumors.

2. Key factors and cells in cancer-related inflammation

In the tumor microenvironment, products of inflammatory cells influence almost every aspect of tumorigenesis and tumor progression [5]. Their effects on tumor angiogenesis will be discussed in more details in Part 3.

Two pathways have been schematically identified as the connection between initiation of cancer and inflammation, as intrinsic pathway and extrinsic pathway. In the intrinsic pathway, internal genetic events which cause neoplasia, at the same time, would trigger the expression of inflammation-related programs and then guide the construction of an

Factors	Biological activity
IL-1β	Elevated level in patient with leukoplakia and oral cancer [71]; Inducing chemical carcigenesis of fibrosarcomas, *in vivo* [72]; host derived or exogenous IL-1β enhancing tumor invasiveness and metastasis, *in vitro* and *in vivo* [73]
IL-6	Involved in tumor progression, such as in multiple myeloma, plasmacytomas, intestinal cancer, *in vitro* and *in vivo*, mainly through NF-κB and STAT3 [74]; Elevated IL-6 level be correlated with HCC, *in vivo* experiment and in clinical [75, 76]; Elevated level in patient with leukoplakia and oral cancer [71]
IL-23	Elevated in intestinal polyps and colorectal carcinoma [77]
IFN-γ	Enhancing the antitumor activity of alveolar macrophages, *in vitro* [78]
TNF-α	Elevated level in patient with leukoplakia and oral cancer [71]
LXs	Inhibiting the tumor growth of transplanted H22 in mice, *in vitro* and *in vivo* [79]; inhibiting hepatocyte growth factor-induced invasion of human hepatoma cells, in vitro [80]

Table 1. Effect of variety of inflammatory mediators on tumor progression.

inflammatory microenvironment inside tumor tissue. RET oncogene in papillary carcinoma of the thyroid is a typical example for this intrinsic pathway [6]. It should be noticed that although those oncogenes might be representative of different pro-inflammatory molecular classes and actions, they will share the capacity to orchestrate all CRI circuits [1].

As in the extrinsic pathway, inflammatory conditions would just help to facilitate tumor development. Chronic inflammation acts as a trigger to increase cancer risk or progression. Chronic inflammatory conditions associated with cancer development include chronic infections (e.g., Helicobacter pylori for gastric cancer and mucosal lymphoma; papilloma virus and hepatitis viruses for cervical and liver carcinoma, respectively), autoimmune diseases (e.g., inflammatory bowel disease for colon cancer) and inflammatory conditions of uncertain origin (e.g. prostatitis for prostate cancer) [2].

There is also close connection between inflammation and metastasis. A successful establishment of a metastatic lesion depends on both intrinsic properties of the tumor cells and factors derived from the tumor microenvironment that often contains secretory products of immune cells such as IL-1, IL-6, TNF and RANKL. All of these are known to augment tumor cells' ability to metastasize by affecting several steps in the cells' dissemination and implantation at secondary sites [7].

Notably, vascular endothelial growth factor-A (VEGF-A), one of the most important stimulators in tumor angiogenesis, is also an inflammatory factor inducing strong macrophages chemotaxis in tumor [8].

Besides the cytokines and chemokines mentioned above, in recent years, short noncoding RNAs termed microRNAs (miRNAs) have been described as a novel class of molecular promoters of neoplastic progression that control gene expression on the post-transcriptional level [9]. Some of the miRNAs play a crucial role both in inflammation and cancer. For example, miR-21 has been found to be deregulated in most types of cancers and therefore was classified as an onco-miR. Meanwhile, miR-21 also plays roles in chronic inflammatory diseases including cardiac and pulmonary fibrosis as well as myocardial infarction [10]. In contrast, miR-146a acts as a molecular brake on inflammation, myeloid cell proliferation, as well as oncogenic transformation [11].

3. Introduction about tumor angiogenesis

Although induction of new blood vessels by solid tumors had been first recognized by Virchow nearly 150 years ago [12], tumor angiogenesis is frequently linked to the name of Dr. Judah Folkman who founded this field nearly 40 years ago. Folkman proposed that the growth of all solid tumors is dependent on angiogenesis and suggested that suppression of tumor blood vessel growth would offer a new option for cancer therapy [13] .

Angiogenesis, the sprouting of new blood vessels from pre-existing endothelium, is an important component of various biological processes including embryonic vascular development, organ regeneration, wound healing, and recovery from myocardial ischemia or peptic ulcer. However, it is also a part of many pathologies that depend on neovascularization, such as diabetic retinopathy, rheumatoid arthritis and tumor growth [14]. The expansion of cancer requires the formation of new blood vessels due to oxygen and nutrients that can obtained by diffusion. Notably, the newly formed tumor vessels also provide a gateway for tumor cells to enter circulation and metastasize to distant organs [15]. Tumor vessels are characterized by lack of maturation, absence of smooth muscle cells, missing adrenergic innervation and lymphatic drainage, discontinuous endothelial lining, and sinusoidal vessel plexuses [16]. Tumor vasculature differs in many aspects from the vasculature of normal organs. The vessel diameter varies significantly in most tumors as compared with vessels in normal tissues. It is still unclear whether the vascular architecture of an individual tumor is tumor type-specific.

Our knowledge of the mechanisms underlying angiogenesis has increased dramatically in the past decades. Angiogenesis is a complex multistep process involving close orchestration of endothelial cells, soluble factors, and extracellular matrix. Usually, the vascular endothelium is a quiescent tissue with a very low turnover rate. However, in response to angiogenic factors, endothelial cells emerge from quiescence and become motile and proliferative. The initiation or termination of angiogenesis is tightly controlled by the net balance between positive and negative regulators. Positive factors include EC mitogenic factors such as fibroblast growth factor-1 and -2 (FGF-1, -2), transforming growth factor-*a* (TGF-*a*), VEGF-A and some non-mitogenic factors such as cytokines, CXC chemokines, and angiopoietins. Inhibitors of angiogenesis include the internal peptide fragments of extracellular matrix proteins (for instance, angiostatin and endostatin) [14, 17].

The complex steps in new vessel formation have been intensively investigated in recent years. The main steps are: (1) tipping the angiogenic balance, (2) destabilization of pre-existing blood vessels basement membrane by protease, (2) cell adhesion, (3) migration of EC toward the angiogenic stimulus, (4) proliferation, (5) formation of a capillary tubes, (6) loop formation by connection of individual sprouts, (7) vessel wall maturation (alignment of pericytes and smooth muscle), (7) formation of new basement membrane [14, 15, 18]. The ability of tumors to stimulate neovascularization is determined by their "angiogenic switch," of which the on/off is dictated by the inflammatory or hypoxic microenvironment inside tumor [15].

4. Inflammatory cells and cytokines in tumor angiogenesis

The above two processes, angiogenesis and inflammation, are closely linked in the following ways: (i) they are coupled in some chronic inflammatory diseases including Crohn disease, diabetes, psoriasis, rheumatoid arthritis, osteoarthritis, obesity, ocular diseases as well as

cancer; (ii) inflammatory cells interact with endothelial cells, fibroblasts and ECM in the inflamed loci; and (iii) the same molecular events trigger both inflammation and angiogenesis (Table 2) [19].

Factors	Biological activity
VEGF	Inducing confluent microvascular ECs to invade collagen gels and form capillary like structures, *in vitro* [81]
	Angiogenic properties in the chick chorioallantoic membrane, the rabbit cornea and numerous mice xenograft models, *in vivo*[21]
	Elevated VEGF levels and its correlation with increased risk of metastasis and overall poor prognosis in different cancers, reviewed by Ferrara [82]
IL	IL-1β increasing EC outgrowth independently of VEGF, *in vivo* [83]
	IL-6 inducing vascular EC proliferation, tube formation and VEGF expression, *in vitro* [84]
Eicosanoids	12(S)-HETE and 15-HETEs as mediators of insulin and EGF-stimulated mammary epithelial cell proliferation and as synergistic effectors of bFGF- and PDGF-regulated growth of vascular endothelial cells, *in vitro* [15]
LXs	Synthetic analog of ATL inhibiting VEGF- and LTD4-stimulated angiogenesis, *in vitro* and *in vivo* [37, 85]; inhibiting actin cytoskeleton reorganization of EC stimulated with VEGF, *in vitro* [86]
	LXA$_4$ inhibiting proinflammatory cytokine responses; attenuating LTD4 and VEGF-stimulated proliferation and tube formation, *in vitro* [35]
Chemokines	CXCL8, induced by Ras to enhance VEGF-A and then acting on ECs to promote vessel formation, *in vitro* [39]
	CXCL12 promoting GSC-initiated angiogenesis by stimulating VEGF production, *in vitro* and *in vivo* [87]

Table 2. Effect of variety of inflammatory mediators on angiogenesis.

The role of inflammatory cells and cytokines in tumor angiogenesis is discussed in details in the following.

4.1 VEGF and tumor angiogenesis

VEGF-A, one of the most essential stimulators in tumor angiogenesis, was first reported by Senger, Dvorak and co-workers back in 1983 [20]. Collectively, the evidence from over 2 decades of experimental work together with the recent clinical results firmly put VEGF as the central mediator in promoting angiogenesis via a direct effect on ECs and mainly through its binding to VEGFR-2 [21]. Another major effect of VEGF-A in the angiogenesis, cancer and metastases process is the ability to increase vascular permeability. It has been postulated that VEGF increases permeability by increasing the vesico-vascular organelles, fenestrations and trans-cellular gaps [22, 23]. In cancer, under the influence of VEGF, metastases to the peritoneal cavity leads to vascularization and hyper-permeability leads to malignant ascites formation and death.

VEGF-A is up-regulated by transcription factor hypoxia inducible factor alpha (HIF1-α) in response to various stimuli including hypoxia, cytokines, growth factors and nitric oxide. As well documented, HIF1-α is central to oxygen homeostasis during embryonic development

and postnatal life in both physiological and pathophysiological processes such as tumor growth, ischemia and tissue repair. It could respond to reduced oxygen tensions and control the expression of many genes involved in metabolism, angiogenesis, tumorigenesis, and metastasis [24]. The activity and amount of HIF-1α are regulated through proteasomal degradation by hydroxylation of its proline residues. Under hypoxia, a condition commonly occurring in growing solid tumors, the enzymatic activity of hydroxylases is limited. As a result, HIF1-α subunit is stabilized. This leads to formation of the dimer that enters the nucleus and binds to promoters of target genes, thereby inducing transcription of VEGF-A and other angiogenic factors [25].

4.2 Eicosanoids and tumor angiogenesis

Metabolism of archidonic acid (AA) through cyclooxygenase (COX), lipoxygenase (LOX), or P450 epoxygenase pathways leads to the formation of various eicosanoids that have potent biologic effects on a wide spectrum of physiological and pathological processes, including inflammation, fever, arthritis, and cancer. In the past decade, eicosanoids have emerged as key regulators of cancer progression. Studies using molecular and pharmacological approaches have found that enzymes involved in the eicosanoid production are overexpressed in cancer cells, enhance their angiogenic potential and simulate tumor growth *in vivo* [15].

Human prostate carcinoma (PCa) is a typical example to illustrate the influence of eicosanoids on tumor angiogenesis. It was indicated that the extent of angiogenesis is associated with PCa progression and the level of vascularization positively correlates with tumor stages [15]. There are several reports describing an increase in COX-2 expression in PCa tumors as compared with normal epithelial tissues [15]. Liu et al. examined the relationship between COX-2 expression and VEGF-A production under cobalt chloride $(CoCl_2)$-stimulated hypoxia in three human PCa cell lines. This study performed in a human metastatic prostate cancer cell line determined that VEGF-A induction by $CoCl_2$-induced hypoxia is maintained by a concomitant and persistent increase of COX-2 expression and sustained elevation of PGE_2 synthesis. This finding suggested that COX-2 activity, reflected by PGE_2 production, is involved in hypoxia-induced VEGF-A expression, which, in turn, modulates prostatic tumor angiogenesis [26]. They further tested the effect of COX-2 inhibitor, NS398, *in vivo*. NS398 efficiently inhibited growth of tumors from PC-3 cells in mice by decreasing angiogenesis and VEGF-A expression [27].

The pro-angiogenic effects of COX-2 are mediated primarily by three products of AA metabolism: thromboxane A_2 (TXA_2), prostaglandin E_2 (PGE_2), and prostaglandin I_2 (PGI_2). Downstream pro-angiogenic actions of these eicosanoid products include: (1) production of VEGF-A [28]; (2) promotion of vascular sprouting, migration, and tube formation [15]; (3) enhanced EC survival via Bcl-2 expression and Akt signaling [29]; (4) activation of epidermal growth factor receptor-mediated angiogenesis [30].

LOX is another lipid peroxidase dioxygenase family responsible for eicosanoids production. Overexpression of 12-LOX and 15-LOX in prostate cancer cells stimulates tumor angiogenesis and growth. For example, both EC migration and Matrigel implantation assays indicated that stable expression of 12-LOX in PC-3 cells increased their angiogenic potential compared with neomycin control [15]. These findings suggest that increased expression of 12-LOX in human PCa cells stimulates growth of prostate tumors by enhancing their angiogenicity. Similar observations regarding the role of 12-LOX in tumor angiogenesis were also made in breast cancer [31]. The product of 12-LOX, 12(S)-HETE, has been found

to exert various effects on endothelial cells [15]. It was demonstrated that, when co-incubated with microvascular ECs, Lewis lung carcinoma cells or B16 melanoma (B16a) cells can synthesize 12(S)-HETE in sufficient amounts to induce EC retraction [32]. The fact that tumor cell–induced EC retraction could not be blocked by COX inhibitors, but by a specific LOX inhibitor, BHPP, provided further proof that LOX enzyme plays an important role in tumor angiogenesis [32]. Some studies indicated that 12(S)-HETE act as a mitogen for microvascular ECs. The expression of 15-LOX-1 in PC-3 tumors cells was also found to stimulate tumor angiogenesis and growth [33]. Besides 12-LOX and 15-LOX, 5-LOX was also shown to promote tumor development by potentiating the pro-angiogenic response [34].

More recent evidence has emerged the role of Lipoxins (LXs) and other lipid mediators, including the resolvins and neuroprotectins whose biosynthesis is linked in space and time to the resolution phase of an inflammatory response [35]. LXs have previously been shown to modulate responses of ECs including stimulation of prostacyclin production by human umbilical vein endothelial cell (HUVEC) [36]. Using HUVEC, Bake and his colleges demonstrated that LXA_4 inhibited VEGF-A-stimulated inflammatory responses including IL-6, TNF-α, IFN-β and IL-8 secretion, as well as endothelial ICAM-1 expression, and up-regulated an inflammatory inhibitor, IL-10. Consistent with these anti-inflammatory and pro-resolution responses to LXA_4, they found that LXA_4 inhibited leukotriene D4 and VEGF-A-stimulated proliferation and angiogenesis, as determined by tube formation of HUVEC. It was believed that the underlying molecular mechanisms is associated with the decrease of VEGF-A-stimulated VEGF receptor-2 (VEGFR-2) phosphorylation and downstream signaling events including activation of phospholipase C-γ, ERK1/2, and Akt [35]. Effects of LXA_4 on ECs may be of particular relevance given the biosynthesis of this agent within the inflamed vasculature. In human enterocytes and leukocytes, LXA_4 and its analogs inhibited the release of the cytokine IL-8 and IL-6, which has been recently reported to induce angiogenic activity in a carcinoma cell line. And, in an *in vivo* model, LXA_4 and its synthetic analogs stimulated the production of IL-4, a cytokine with anti-angiogenic properties. Furthermore, the proteolytic activity necessary to digest the basement membrane, a crucial step in the angiogenic process, can be regulated by LXs at nanomolar concentration through preventing the synthesis of metalloproteinases (MMP) and increasing the tissue inhibitor of metalloproteinase (TIMP-1) protein. Collectively, these data indicate that LXs regulate EC responses *in vitro* and *in vivo* which are relevant for tumor-angiogenesis [14].

Aspirin-triggered-15-epi-lipoxins (ATL) is one analogue of LXA_4. It is well known that aspirin's therapeutic mechanism of its anti-inflammatory action is through acetylation of COX-2 and inhibition of COX-2-derived eicosanoids. Furthermore, acetylated COX-2 could also induce the biosynthesis of ATL in different types of cell, including ECs [34]. ATL are generated *in vivo* during cell–cell interactions, that can involve, for example, EC–neutrophils, and display potent inhibitory actions in several key events in inflammation [14]. It is noteworthy that the modulation of EC proliferation and VEGF receptor signal transduction reported by Bake closely parallels the bioactions of the synthetic ATL which has been reported to inhibit VEGF-stimulated proliferation of HUVEC with a maximal effect of 50% at 10 nM , suggesting similar efficacy to LXA_4 [37].

4.3 Chemokines family and tumor angiogenesis

Chemokines govern directed chemotaxis in nearby responsive cells during immune responses and inflammatory reactions by signaling through corresponding G_i protein-coupled receptors of the CXC chemokine receptor (CXCR) and CC chemokine receptor

(CCR) family. In recently years, chemokine family, including ligands and receptors, has become the focus in anti-tumor research field.

CXCL8, one of glutamic acid-leucine-arginine (ELR+) chemokine, is up-regulated in several types of cancers, including pancreatic, lung, melanoma, breast, prostate and ovarian cancers [38]. In human cervical epithelioid carcinoma HeLa cell, CXCL8 was also induced by Ras, which has been shown to enhance VEGF-A and then act on endothelial cells to promote vessel formation [39]. Conversely, inhibition of CXCL8 led to an increase in tumor necrosis consistent with a defect in tumor vasculature and paracrine mechanism of action.

Activation of CXCR by ELR+ CXC chemokines would elicit a localized immune response, which could facilitate angiogenesis [4]. CXCR2 is proved to be a common receptor shared by most ELR+ CXC chemokines. Activation of this receptor expressed in ECs had been shown to inhibit endothelial apoptosis, and induce migration and tube formation in ECs, processes linked to angiogenesis [40]. In a study on syngeneic murine Lewis lung cancer ectopic and orthotopic tumor model systems in CXCR2(+/+) and CXCR2(-/-) C57BL/6 mice, morphometric analysis of the primary tumors in CXCR2(-/-) mice demonstrated increased necrosis and reduced vascular density. These findings were further confirmed in CXCR2(+/+) mice using specific neutralizing antibody to CXCR2. The results of these studies support the notion that CXCR2 mediates the angiogenic activity of ELR(+) CXC chemokines in a preclinical model of lung cancer [41]. Similar effect of ELR(+) CXC chemokines and CXCR2 on tumor-associated angiogenesis was also shown in pancreatic cancer [42]. *In vitro*, ELR+ CXC chemokines in supernatants from multiple pancreatic cancer cell lines had significantly higher level compared with an immortalized human pancreatic ductal epithelial cell line. Furthermore, both recombinant ELR+ CXC chemokines and co-culturing with BxPC-3 significantly enhanced proliferation, invasion, and tube formation of HUVEC. These biological effects were significantly inhibited by treatment with a neutralizing antibody against CXCR2. *In vivo*, anti-CXCR2 antibody significantly reduced tumor volume as well as proliferation index and Factor VIII microvessel density.

CXCL12, ligand of CXCR4 receptor, also possesses angiogenic properties and is involved in the outgrowth and metastasis of CXCR4-expressing tumors and in certain inflammatory autoimmune disorders, such as rheumatoid arthritis [43].

4.4 Inflammation-related miRNA and tumor angiogenesis

In recent years, light has been shed on the connection between inflammation-related miRNA and tumor progression [9]. Two biologically active miRNAs, miR-126 and its complement miR-126*, have been reported to impair cancer progression through signaling pathways that control tumor cell proliferation, migration, invasion, and survival. Conversely, they may have a supportive role in the progression of cancer as well, which might be mediated by the promotion of blood vessel growth and inflammation. This effect of miR-126 and miR-126* on vascular functions could be explained by the fact that they are encoded by the intron 7 of the epidermal growth factor-like domain 7 (egfl7) gene. The endothelial cell-derived secreted protein EGFL7 has been suggested to control vascular tubulogenesis. Knock-out studies in zebrafish and mice suggested a major role of miR-126 in angiogenesis and vascular integrity, which was mediated by the repression of inhibitors of VEGF-A-induced proliferation in ECs.

4.5 Inflammatory cells and tumor angiogenesis

Tumor-associated macrophages (TAM) are prominent in the stromal compartment of virtually all types of malignancy. These highly versatile cells respond to the presence of

stimuli in different parts of tumors with the release of a distinct repertoire of growth factors, cytokines, chemokines, and enzymes [44]. Plasticity and diversity have long been known as hallmarks of the monocyte-macrophage differentiation pathway under inflammatory conditions [45]. Inflammation-induced angiogenesis is accompanied by macrophage infiltration. M2-type macrophages support angiogenesis and lymphangiogenesis by releasing pro-angiogenic growth factors such as IL-8, VEGF-A, VEGF-C and EGF [46, 47]. In a pancreatic cancer model, IL-4 induced high expression of cathepsin in TAM that then mediated tumor growth, angiogenesis and invasion *in vivo* [48]. TAM have also been demonstrated as the main cells producing semaphorin 4D within the tumor stroma. The latter is critical for tumor angiogenesis and vessel maturation [49]. There is a significant correlation between the number of infiltrating macrophages and the microvascular density or tumor tumor progression levels in glioblastomas and melanoma [50, 51].

EC activation is manifested through chemokine production and up-regulation of surface adhesion molecules that facilitate adhesion of leukocytes that, in turn, cause more pronounced inflammation [52]., Leukocytes including TAM not only activate ECs but also promote and strengthen the entire process of tumor angiogenesis.

5. Oxidative stress in tumor angiogenesis

Dysregulation of redox status is a typical feature of many types of cancer [25]. It is widely accepted that the imbalance between the generation and clearance of reactive oxygen/nitrogen species (ROS/RNS) aids the development of the tumor mainly by inducing genomic instability. However, recent research has provided multiple evidences that ROS and other free radicals, such as nitric oxide, often produced at elevated levels within tumor tissue, may function as signaling molecules that initiate and/or modulate different regulatory pathways involved in tumorigenesis and metastasis [53]. High levels of ROS induce cell death, apoptosis and senescence; however, at the same time, low levels of ROS are important mediators in signaling pathways regulating growth and survival of endothelial and other cells [25, 54].

The role of ROS in angiogenesis is well established. ROS were demonstrated to trigger the secretion of the most potent angiogenic factor – VEGF-A, in many cell types and induce proliferation, migration, cytoskeletal reorganization and tubular morphogenesis in ECs in vitro [55-57]. Increased intracellular levels of ROS were demonstrated in different settings to stabilize HIF1-α, a key upstream regulatory of VEGF-A expression, not only under tumor hypoxia but also under normoxic conditions [58]. For example, up-regulation of HIF-1α in response to stimulation with angiotensin II (Ang II) and thrombin was shown to be dependent on the elevation of H_2O_2 levels and cells with compromised antioxidant capacity in normoxia [59].

6. Current treatments of inflammation-stimulated tumor angiogenesis

Folkman's original hypothesis has opened a new era in today's biomedical research and changed the face of cancer medicine [60]. Modulation of angiogenesis for disease therapy was proposed nearly 40 years ago. As a result, many protein-based and chemical anti-angiogenic drugs have been developed for treating human malignancies.

In fact, since the VEGF-A discovery and characterization and subsequent determination of its receptors/pathways involved, enormous effort has been put into developing enormous

effort has been put into developing anti-VEGF agents.. In 2004, after 3-decades preclinical validation, bevacizumab, a humanized anti-VEGF-A neutralizing antibody, was approved by the US FDA for the clinical use to treat metastatic colorectal cancer in human patients [61]. This antibody was the first specific angiogenic inhibitor for use in clinical oncology. And following this initial success, bevacizumab has been expanded as one of the key component of the first-line therapeutic choices against various human cancers [60]. Clinical trials have reported positive response from patients treated with bevacizumab as a single agent or in combination with cytotoxic agents [62, 63].

Beside bevacizumab, various other types of molecules have been developed to target the VEGF pathway. These include proteins that bind VEGF such as VEGF trap, VEGF receptor antibody IMC-1121B or antagonists such as vatalinib, inhibitors of receptor tyrosine kinase such as sunitinib, sorafenib, and ZD6474 [64, 65]. There are also vaccines based on xenogeneic or non-xenogeneic homologous molecules targeting VEGF-A or VEGFR [66].

Unfortunately, patients with various types of tumors have different response to anti-angiogenic therapy. While a small fraction of most common solid tumors such as colorectal, lung and breast cancers respond, some cancer types show intrinsic refractoriness [60]. But, we should not forget that the effectiveness of almost all therapeutic modalities is influenced by the micro-architecture and the gradients of essential nutrients around vessels [16]. This has been the driving force in the fields of anti-angiogenic drug development in tumor therapy.

For prostate cancer, it has been shown that androgen regulates the expression of VEGF-A and that androgen withdrawal regresses prostate tumors, partly, by restraining their blood supply. Since prostate cancer eventually progresses to androgen independence, other mechanisms must take over at later stages of tumor [15]. *In vivo*, COX-2 inhibitor, NS398, efficiently inhibited growth of PC-3 tumors in mice and decreased angiogenesis. The same study showed that VEGF-A expression was also significantly down-regulated in the NS398-treated tumors [27]. Various well-documented clinical and experimental studies have also confirmed the effect of NSAIDs in the prevention of certain types [14]. The mechanism of aspirin acts to reduce the incidence and risk of these cancers is not clear but some articles indicated that it is result from the reduction of angiogenesis [67, 68]. Epidemiologic studies show that individuals taking nonselective COX inhibitor or NSAIDs, including aspirin, have a significant reduction in CRC mortality, compared with those who did not these drugs [69].

In vivo, anti-CXCR2 antibody significantly reduced tumor volume as well as proliferation index and Factor VIII microvessel density [42]. Thus, CXCR2 should be considered as a novel anti-angiogenic target in pancreatic cancer.

ROS scavenging by antioxidants was recently demonstrated to inhibit angiogenesis in a model of myocardial infarction in rats [11]. Current clinical anti-angiogenic approaches in oncology exploit VEGF-A-VEGFR-2 axis, with the application of VEGF-A neutralizing antibodies (bevacizumab) and small-molecule VEGFR-2 tyrosine kinase inhibitors (sorafenib, sunitinib) [21]. However, this treatment do not provide a cure but only moderately prolongs patients' lives [21, 70]. Recent progress in the understanding of redox modulation of regulation and signaling of VEGF-A may create possibilities to develop more universal anti-angiogenic drugs by targeting ROS.

Intensive research resulted in the development of several FDA-approved drugs on angiogenesis in tumor. However, most of the clinical trials of single anti-angiogenic agents in combination with traditional anticancer treatment yielded disappointing results. Thus,

targeting multiple pathways regulating angiogenesis, such as inflammation, has been considered a promising target for therapeutic interventions. These clinically related issues need to be further addressed at molecular levels to understand the underlying mechanisms. Elucidation of molecular mechanisms linking cancer and inflammation may provide new targets for inhibition of angiogenesis and tumor progression.

7. Abbreviation

Arachidonic acid, AA;
Aspirin-triggered-15-epi-lipoxins, ATL;
Cancer-related inflammation, CRI;
Cyclooxygenase, COX;
CXC chemokine receptor, CXCR;
CC chemokine receptor, CCR;
Endothelial cell, EC;
Fibroblast growth factor, FGF;
Human umbilical vein endothelial cells, HUVEC;
Lipoxygenase, LOX;
Lipoxin, LX
Metalloproteinases, MMP;
Short noncoding RNAs termed microRNAs, miRNAs;
Non-steroidal anti-inflammatory drugs, NSAIDs;
Prostate carcinoma, PCa;
Prostaglandin E_2, PGE_2;
Prostaglandin I_2, PGI_2;
Tumor-associated macrophages, TAM;
Thromboxane A_2, TXA_2;
Tissue inhibititor of metalloproteinase , TIMP-1;
Transforming growth factor-*a*, TGF-*a*;
Vascular endothelial growth factor-A, VEGF-A;

8. References

[1] Colotta, F., et al., *Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability.* Carcinogenesis, 2009. 30(7): p. 1073-81.

[2] Hanahan, D. and R.A. Weinberg, *Hallmarks of cancer: the next generation.* Cell, 2011. 144(5): p. 646-74.

[3] Balkwill, F. and A. Mantovani, *Inflammation and cancer: back to Virchow?* Lancet, 2001. 357(9255): p. 539-45.

[4] Coussens, L.M. and Z. Werb, *Inflammation and cancer.* Nature, 2002. 420(6917): p. 860-7.

[5] Mantovani, A., et al., *Cancer-related inflammation.* Nature, 2008. 454(7203): p. 436-44.

[6] Borrello, M.G., et al., *Induction of a proinflammatory program in normal human thyrocytes by the RET/PTC1 oncogene.* Proc Natl Acad Sci U S A, 2005. 102(41): p. 14825-30.

[7] Mantovani, A., *Cancer: Inflaming metastasis.* Nature, 2009. 457(7225): p. 36-7.

[8] Cursiefen, C., et al., *VEGF-A stimulates lymphangiogenesis and hemangiogenesis in inflammatory neovascularization via macrophage recruitment.* J Clin Invest, 2004. 113(7): p. 1040-50.

[9] Porta, C., et al., *Tolerance and M2 (alternative) macrophage polarization are related processes orchestrated by p50 nuclear factor kappaB.* Proc Natl Acad Sci U S A, 2009. 106(35): p. 14978-83.

[10] Kumarswamy, R., I. Volkmann, and T. Thum, *Regulation and function of miRNA-21 in health and disease.* RNA Biol, 2011. 8(5).

[11] Boldin, M.P., et al., *miR-146a is a significant brake on autoimmunity, myeloproliferation, and cancer in mice.* J Exp Med, 2011. 208(6): p. 1189-201.

[12] Papoutsi, M., et al., *Development of an arterial tree in C6 gliomas but not in A375 melanomas.* Histochem Cell Biol, 2002. 118(3): p. 241-9.

[13] Folkman, J., *Tumor angiogenesis: therapeutic implications.* N Engl J Med, 1971. 285(21): p. 1182-6.

[14] Fierro, I.M., *Angiogenesis and lipoxins.* Prostaglandins Leukot Essent Fatty Acids, 2005. 73(3-4): p. 271-5.

[15] Kelm, J.M., et al., *Method for generation of homogeneous multicellular tumor spheroids applicable to a wide variety of cell types.* Biotechnol Bioeng, 2003. 83(2): p. 173-80.

[16] Konerding, M.A., et al., *Evidence for characteristic vascular patterns in solid tumours: quantitative studies using corrosion casts.* Br J Cancer, 1999. 80(5-6): p. 724-32.

[17] Folkman, J., *Role of angiogenesis in tumor growth and metastasis.* Semin Oncol, 2002. 29(6 Suppl 16): p. 15-8.

[18] Hall, K. and S. Ran, *Regulation of tumor angiogenesis by the local environment.* Front Biosci, 2010. 15: p. 195-212.

[19] Ono, M., *Molecular links between tumor angiogenesis and inflammation: inflammatory stimuli of macrophages and cancer cells as targets for therapeutic strategy.* Cancer Sci, 2008. 99(8): p. 1501-6.

[20] Senger, D.R., et al., *Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid.* Science, 1983. 219(4587): p. 983-5.

[21] Pourgholami, M.H. and D.L. Morris, *Inhibitors of vascular endothelial growth factor in cancer.* Cardiovasc Hematol Agents Med Chem, 2008. 6(4): p. 343-7.

[22] Dvorak, H.F., *Rous-Whipple Award Lecture. How tumors make bad blood vessels and stroma.* Am J Pathol, 2003. 162(6): p. 1747-57.

[23] Bates, D.O. and S.J. Harper, *Regulation of vascular permeability by vascular endothelial growth factors.* Vascul Pharmacol, 2002. 39(4-5): p. 225-37.

[24] Zhu, X.Y., et al., *Disparate effects of simvastatin on angiogenesis during hypoxia and inflammation.* Life Sci, 2008. 83(23-24): p. 801-9.

[25] Tertil, M., A. Jozkowicz, and J. Dulak, *Oxidative stress in tumor angiogenesis- therapeutic targets.* Curr Pharm Des, 2010. 16(35): p. 3877-94.

[26] Liu, X.H., et al., *Upregulation of vascular endothelial growth factor by cobalt chloride-simulated hypoxia is mediated by persistent induction of cyclooxygenase-2 in a metastatic human prostate cancer cell line.* Clin Exp Metastasis, 1999. 17(8): p. 687-94.

[27] Liu, X.H., et al., *Inhibition of cyclooxygenase-2 suppresses angiogenesis and the growth of prostate cancer in vivo.* J Urol, 2000. 164(3 Pt 1): p. 820-5.

[28] Inoue, H., et al., *Regulation by PGE2 of the production of interleukin-6, macrophage colony stimulating factor, and vascular endothelial growth factor in human synovial fibroblasts.* Br J Pharmacol, 2002. 136(2): p. 287-95.

[29] Nor, J.E., et al., *Up-Regulation of Bcl-2 in microvascular endothelial cells enhances intratumoral angiogenesis and accelerates tumor growth.* Cancer Res, 2001. 61(5): p. 2183-8.

[30] Pai, R., et al., *Prostaglandin E2 transactivates EGF receptor: a novel mechanism for promoting colon cancer growth and gastrointestinal hypertrophy.* Nat Med, 2002. 8(3): p. 289-93.

[31] Connolly, J.M. and D.P. Rose, *Enhanced angiogenesis and growth of 12-lipoxygenase gene-transfected MCF-7 human breast cancer cells in athymic nude mice.* Cancer Lett, 1998. 132(1-2): p. 107-12.

[32] Honn, K.V., et al., *Tumor cell-derived 12(S)-hydroxyeicosatetraenoic acid induces microvascular endothelial cell retraction.* Cancer Res, 1994. 54(2): p. 565-74.

[33] Kelavkar, U.P., et al., *Overexpression of 15-lipoxygenase-1 in PC-3 human prostate cancer cells increases tumorigenesis.* Carcinogenesis, 2001. 22(11): p. 1765-73.

[34] Romano, M., et al., *5-lipoxygenase regulates malignant mesothelial cell survival: involvement of vascular endothelial growth factor.* Faseb J, 2001. 15(13): p. 2326-36.

[35] Baker, N., et al., *Lipoxin a4: anti-inflammatory and anti-angiogenic impact on endothelial cells.* J Immunol, 2009. 182(6): p. 3819-26.

[36] Brezinski, M.E., et al., *Lipoxins stimulate prostacyclin generation by human endothelial cells.* FEBS Lett, 1989. 245(1-2): p. 167-72.

[37] Fierro, I.M., J.L. Kutok, and C.N. Serhan, *Novel lipid mediator regulators of endothelial cell proliferation and migration: aspirin-triggered-15R-lipoxin A(4) and lipoxin A(4).* J Pharmacol Exp Ther, 2002. 300(2): p. 385-92.

[38] O'Hayer, K.M., D.C. Brady, and C.M. Counter, *ELR+ CXC chemokines and oncogenic Ras-mediated tumorigenesis.* Carcinogenesis, 2009. 30(11): p. 1841-7.

[39] Rak, J., et al., *Oncogenes and tumor angiogenesis: differential modes of vascular endothelial growth factor up-regulation in ras-transformed epithelial cells and fibroblasts.* Cancer Res, 2000. 60(2): p. 490-8.

[40] Li, A., et al., *IL-8 directly enhanced endothelial cell survival, proliferation, and matrix metalloproteinases production and regulated angiogenesis.* J Immunol, 2003. 170(6): p. 3369-76.

[41] Keane, M.P., et al., *Depletion of CXCR2 inhibits tumor growth and angiogenesis in a murine model of lung cancer.* J Immunol, 2004. 172(5): p. 2853-60.

[42] Matsuo, Y., et al., *CXC-chemokine/CXCR2 biological axis promotes angiogenesis in vitro and in vivo in pancreatic cancer.* Int J Cancer, 2009. 125(5): p. 1027-37.

[43] Liekens, S., D. Schols, and S. Hatse, *CXCL12-CXCR4 axis in angiogenesis, metastasis and stem cell mobilization.* Curr Pharm Des, 2010. 16(35): p. 3903-20.

[44] Lewis, C.E. and J.W. Pollard, *Distinct role of macrophages in different tumor microenvironments.* Cancer Res, 2006. 66(2): p. 605-12.

[45] Biswas, S.K. and A. Mantovani, *Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm.* Nat Immunol, 2010. 11(10): p. 889-96.

[46] Mantovani, A., et al., *Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes.* Trends Immunol, 2002. 23(11): p. 549-55.

[47] Schmidt, T. and P. Carmeliet, *Blood-vessel formation: Bridges that guide and unite.* Nature, 2010. 465(7299): p. 697-9.

[48] Gocheva, V., et al., *IL-4 induces cathepsin protease activity in tumor-associated macrophages to promote cancer growth and invasion.* Genes Dev, 2010. 24(3): p. 241-55.

[49] Sierra, J.R., et al., *Tumor angiogenesis and progression are enhanced by Sema4D produced by tumor-associated macrophages.* J Exp Med, 2008. 205(7): p. 1673-85.

[50] Nishie, A., et al., *Macrophage infiltration and heme oxygenase-1 expression correlate with angiogenesis in human gliomas.* Clin Cancer Res, 1999. 5(5): p. 1107-13.

[51] Torisu, H., et al., *Macrophage infiltration correlates with tumor stage and angiogenesis in human malignant melanoma: possible involvement of TNFalpha and IL-1alpha.* Int J Cancer, 2000. 85(2): p. 182-8.

[52] Pober, J.S. and W.C. Sessa, *Evolving functions of endothelial cells in inflammation.* Nat Rev Immunol, 2007. 7(10): p. 803-15.

[53] Trachootham, D., et al., *Redox regulation of cell survival.* Antioxid Redox Signal, 2008. 10(8): p. 1343-74.

[54] Storz, P., *Reactive oxygen species in tumor progression.* Front Biosci, 2005. 10: p. 1881-96.

[55] Birk, D.M., et al., *Current insights on the biology and clinical aspects of VEGF regulation.* Vasc Endovascular Surg, 2008. 42(6): p. 517-30.

[56] Luczak, K., et al., *Low concentration of oxidant and nitric oxide donors stimulate proliferation of human endothelial cells in vitro.* Cell Biol Int, 2004. 28(6): p. 483-6.

[57] Yasuda, M., et al., *Stimulation of in vitro angiogenesis by hydrogen peroxide and the relation with ETS-1 in endothelial cells.* Life Sci, 1999. 64(4): p. 249-58.

[58] Chandel, N.S., et al., *Mitochondrial reactive oxygen species trigger hypoxia-induced transcription.* Proc Natl Acad Sci U S A, 1998. 95(20): p. 11715-20.

[59] Gerald, D., et al., *JunD reduces tumor angiogenesis by protecting cells from oxidative stress.* Cell, 2004. 118(6): p. 781-94.

[60] Cao, Y., *Angiogenesis: What can it offer for future medicine?* Exp Cell Res, 2010. 316(8): p. 1304-8.

[61] Hurwitz, H., et al., *Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer.* N Engl J Med, 2004. 350(23): p. 2335-42.

[62] Desjardins, A., et al., *Bevacizumab and daily temozolomide for recurrent glioblastoma.* Cancer, 2011.

[63] Cannistra, S.A., et al., *Phase II study of bevacizumab in patients with platinum-resistant ovarian cancer or peritoneal serous cancer.* J Clin Oncol, 2007. 25(33): p. 5180-6.

[64] Morabito, A., et al., *Tyrosine kinase inhibitors of vascular endothelial growth factor receptors in clinical trials: current status and future directions.* Oncologist, 2006. 11(7): p. 753-64.

[65] Hanrahan, E.O. and J.V. Heymach, *Vascular endothelial growth factor receptor tyrosine kinase inhibitors vandetanib (ZD6474) and AZD2171 in lung cancer.* Clin Cancer Res, 2007. 13(15 Pt 2): p. s4617-22.

[66] Pan, J., et al., *Anti-angiogenic active immunotherapy: a new approach to cancer treatment.* Cancer Immunol Immunother, 2008. 57(8): p. 1105-14.

[67] Wang, D. and R.N. Dubois, *Cyclooxygenase-2: a potential target in breast cancer.* Semin Oncol, 2004. 31(1 Suppl 3): p. 64-73.

[68] Shtivelband, M.I., et al., *Aspirin and salicylate inhibit colon cancer medium- and VEGF-induced endothelial tube formation: correlation with suppression of cyclooxygenase-2 expression.* J Thromb Haemost, 2003. 1(10): p. 2225-33.

[69] Koehne, C.H. and R.N. Dubois, *COX-2 inhibition and colorectal cancer.* Semin Oncol, 2004. 31(2 Suppl 7): p. 12-21.

[70] Backer, M.V., C.V. Hamby, and J.M. Backer, *Inhibition of vascular endothelial growth factor receptor signaling in angiogenic tumor vasculature.* Adv Genet, 2009. 67: p. 1-27.

[71] Brailo, V., et al., *Salivary and serum interleukin 1 beta, interleukin 6 and tumor necrosis factor alpha in patients with leukoplakia and oral cancer.* Med Oral Patol Oral Cir Bucal, 2011.

[72] Krelin, Y., et al., *Interleukin-1beta-driven inflammation promotes the development and invasiveness of chemical carcinogen-induced tumors.* Cancer Res, 2007. 67(3): p. 1062-71.

[73] Apte, R.N., et al., *The involvement of IL-1 in tumorigenesis, tumor invasiveness, metastasis and tumor-host interactions.* Cancer Metastasis Rev, 2006. 25(3): p. 387-408.

[74] Naugler, W.E. and M. Karin, *The wolf in sheep's clothing: the role of interleukin-6 in immunity, inflammation and cancer.* Trends Mol Med, 2008. 14(3): p. 109-19.

[75] Soresi, M., et al., *Interleukin-6 and its soluble receptor in patients with liver cirrhosis and hepatocellular carcinoma.* World J Gastroenterol, 2006. 12(16): p. 2563-8.

[76] Malaguarnera, M., et al., *[Role of interleukin 6 in hepatocellular carcinoma].* Bull Cancer, 1996. 83(5): p. 379-84.

[77] Lan, F., et al., *IL-23/IL-23R: potential mediator of intestinal tumor progression from adenomatous polyps to colorectal carcinoma.* Int J Colorectal Dis, 2011.

[78] Zhou, F., et al., *[Study of the antitumor activity of alveolar macrophages after transfected human INF-gamma gene].* Zhongguo Fei Ai Za Zhi, 2011. 14(5): p. 452-5.

[79] Chen, Y., et al., *Lipoxin A4 and its analogue suppress the tumor growth of transplanted H22 in mice: the role of antiangiogenesis.* Mol Cancer Ther, 2010. 9(8): p. 2164-74.

[80] Zhou, X.Y., et al., *Lipoxin A(4) inhibited hepatocyte growth factor-induced invasion of human hepatoma cells.* Hepatol Res, 2009. 39(9): p. 921-30.

[81] Pepper, M.S., et al., *Potent synergism between vascular endothelial growth factor and basic fibroblast growth factor in the induction of angiogenesis in vitro.* Biochem Biophys Res Commun, 1992. 189(2): p. 824-31.

[82] Ferrara, N., *Vascular endothelial growth factor: basic science and clinical progress.* Endocr Rev, 2004. 25(4): p. 581-611.

[83] Lavalette, S., et al., *Interleukin-1beta inhibition prevents choroidal neovascularization and does not exacerbate photoreceptor degeneration.* Am J Pathol, 2011. 178(5): p. 2416-23.

[84] Zhu, B.H., et al., *(-)-Epigallocatechin-3-gallate inhibits VEGF expression induced by IL-6 via Stat3 in gastric cancer.* World J Gastroenterol, 2011. 17(18): p. 2315-25.

[85] Cezar-de-Mello, P.F., et al., *ATL-1, an analogue of aspirin-triggered lipoxin A4, is a potent inhibitor of several steps in angiogenesis induced by vascular endothelial growth factor.* Br J Pharmacol, 2008. 153(5): p. 956-65.

[86] Cezar-de-Mello, P.F., et al., *Aspirin-triggered Lipoxin A4 inhibition of VEGF-induced endothelial cell migration involves actin polymerization and focal adhesion assembly.* Oncogene, 2006. 25(1): p. 122-9.

[87] Ping, Y.F., et al., *The chemokine CXCL12 and its receptor CXCR4 promote glioma stem cell-mediated VEGF production and tumour angiogenesis via PI3K/AKT signalling.* J Pathol, 2011. 224(3): p. 344-54.
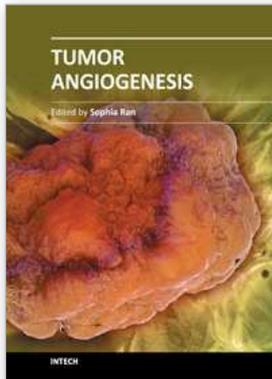
Tumor Angiogenesis

Edited by Dr. Sophia Ran

Tumor angiogenesis is the main process responsible for the formation of new blood vessels that promote tumor growth and metastasis. This process is driven by potent pro-angiogenic factors that are predominant in the tumor environment and are produced by both malignant cells and the host cells recruited to the tumor site. Tumor environment is characterized by the imbalance between pro-angiogenic and anti-angiogenic factors, which drives the construction of numerous but structurally defective vessels. These poorly perfused and abnormal vessels significantly contribute to the tumor pathology not only by supporting the expansion of the tumor mass but also by promoting chronic inflammation, enhancing thrombosis, impeding drug delivery, and disseminating tumor cells. These problems associated with tumor vasculature continue to attract great attention of scientists and clinicians interested in advancing the understanding of tumor biology and development of new drugs. This book complies a series of reviews that cover a broad spectrum of current topics related to the pathology of tumor blood vessels including mechanisms inducing new vessels, identification of new targets for inhibition of tumor angiogenesis, and potential clinical use of known and novel anti-angiogenic therapies. The book provides an update on tumor angiogenesis that could be useful for oncologists, cancer researchers and biologists with interests in vascular and endothelial cell behavior in the context of cancer.

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