REVIEW

Pro- and anti-angiogenic agents

Agents pro- et anti-angiogéniques

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Summary The vascular endothelium has been characterized in every organ system, and is described as a selective permeable barrier and as a dynamic and disseminated organ with endocrine function. These activities have been shown to result from the interactions of ligands with membrane-bound receptors as well as through specific junctional proteins and receptors that govern cell-cell interactions. The endothelial cells’ movement (e.g., angiogenesis) has been hypothesized to occur following the release of stimuli that could promote the formation of new blood vessels. Angiogenesis has also been reported to be the continued expansion of the vascular tree in avascular regions, as a result of the sprouting of endothelial cells from existing vessels. Most commonly, angiogenesis has been characterized during wound healing and tumour growth. Herein we summarize and discuss the latest results from fundamental laboratory research aimed at proving a link between the proliferation of cancer and angiogenesis, as well as the new rationale around novel pro- and anti-angiogenic molecules.

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Résumé L’endothélium vasculaire a été caractérisé dans tous les organes et est décrit comme une barrière perméable sélective constituée de cellules tapissant les parois de tous les organes ainsi que comme un système dynamique et disséminé à fonction endocrine. Ces activités ont été démontrées comme résultant d’interactions de ligands avec les récepteurs membranaires aussi bien qu’avec des protéines transmembranaires et des récepteurs gouvernant les interactions cellules-cellules. Le mouvement des cellules endothéliales (c’est-à-dire l’angiogénèse) a été supposé se produire suite à la libération de stimuli pouvant promouvoir la formation de nouveaux vaisseaux sanguins. L’angiogénèse a été également rapportée comme étant l’accroissement poursuivi de l’arbre vasculaire vers des zones avasculaires, suite à la formation de nouvelles ramifications de cellules endothéliales à partir de veines existantes. D’une manière générale, l’angiogénèse a été caractérisée lors de la cicatrisation et de l’invasion tumorale. Dans cette

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Abbreviations and definitions

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<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>ATE</td>
<td>Arterial thromboembolism is a sudden interruption of blood flow to an organ due to a blood clot adhering to the wall of an artery and blocks the flow of blood.</td>
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<tr>
<td>CAI</td>
<td>Carboxy amido-triazole is a novel calcium influx inhibitor with antiproliferative and antimitastatic activities.</td>
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<td>DITPA</td>
<td>Diiodothyropionic acid is a deiodinated form of triiodothyronine.</td>
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<td>DNA</td>
<td>Deoxyribonucleic acid constitutes the primary genetic material of all cellular organisms.</td>
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<td>ECs</td>
<td>Endothelial cells line the interior surface of blood vessels, forming an interface between circulating blood in the lumen and the rest of the vessel wall.</td>
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<td>EGF</td>
<td>Endothelial growth factor is a mitogenic polypeptide produced by many cell types and made in large amounts by some tumours.</td>
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<td>FGF</td>
<td>Fibroblast growth factors are heparin-binding proteins and key players in the processes of proliferation and differentiation of a wide variety of cells and tissues.</td>
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<tr>
<td>HA</td>
<td>Hyaluronic acid is a mucopolysaccharide that is found in spaces around tissues.</td>
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<td>LMWHs</td>
<td>Low molecular weight heparins is a mixture of heparins that have a lower mean MW and a decreased content of anti-2A than UFH.</td>
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<td>PGE2</td>
<td>Prostaglandin E2 is a lipid compound derived enzymatically from the arachidonic acid.</td>
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<td>T3</td>
<td>Triiodothyronine is a deiodinated form of thyroxine that occurs in peripheral tissues.</td>
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<tr>
<td>T4</td>
<td>Thyroxine is an iodine-containing hormone secreted by the thyroid gland.</td>
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<tr>
<td>Tetrac</td>
<td>Tetraiodothyroacetic acid is a metabolite of thyroxine.</td>
</tr>
<tr>
<td>TF</td>
<td>Tissue factor is a glycoprotein involved in blood coagulation.</td>
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<td>TFPI</td>
<td>Tissue factor pathway inhibitor is a plasma lipoprotein that regulates procoagulant effects of tissue factor.</td>
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<tr>
<td>TSP-1</td>
<td>Thrombospondin-1 is a glycoprotein that binds to connective tissues and serum proteins.</td>
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<tr>
<td>UFH</td>
<td>Unfractionated heparin is a mixture of sulfated glycosaminoglycans present in the liver and lungs, and possessing potent anticoagulant properties.</td>
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<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor is a signal protein produced by cells that is part of the system that restores the oxygen supply to tissues when blood circulation is inadequate.</td>
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VTE Venous thromboembolism is a condition in which a blood clot (thrombus) forms in a vein.

Introduction

The term ‘angiogenesis’ has been used to describe the growth of new blood vessels. This process has been seen naturally in the body both in healthy and diseased states. In the healthy body, angiogenesis can readily be seen in wound healing where blood flow is restored to the injured tissue. In simplistic terms, it has been reported that the healthy body can control that complex chemical reaction through ‘on’ switch (angiogenesis stimulators) or ‘off’ switch (angiogenesis inhibitors) mechanisms.

In 1971, Dr. Judah Folkman (considered by many as the ‘’father of angiogenesis’’) hypothesized that angiogenesis was a factor enabling malignant tumour growth in cancer [1]. In 1989, Dr. Jean Plouët and Dr. Napoleon Ferrara made ground-breaking discoveries when they identified and isolated the protein vasculotropin, later named VEGF, the component that was shown to play a crucial role in vessel development [2,3]. By 1996, the late Dr. Jeffery Isner (a pioneer in gene therapy) reported the first of many clinical trials conducted concerning angiogenesis, using VEGF in treating 156 patients with critical limb ischemia [4]. Since then, angiogenesis has been considered as intrinsically responsible for the branch of existing cells, allowing the expansion of the vascular tree in avascular regions, as a result of the sprouting of ECs from existing vessels [5].

It has been characterized most commonly during wound healing and tumour metastasis. Fundamental research has elucidated that proteins on the EC surface act together to mediate cell-extracellular matrix interactions. Among these, the integrin family of receptors has been shown to serve both a tethering and an information transfer function in order to facilitate cell movement [6]. Cell replication and programmed cell death have also been shown to occur through integrin-ligand binding [7].

In haemostatic condition, ECs were found to facilitate transit of plasma and cellular constituents throughout the vasculature [8]. Nevertheless, in inflammation and thrombocytosis (overexpression of platelets) conditions, perturbations (such as overexpression of a ligand) have been reported to disrupt these activities and induce ECs to shift to a prothrombotic microenvironment that was shown to favour malignant angiogenesis and the development of epithelial cancers (lung, kidney, breast) [9]. In any case, once the equilibrium of regulating stimuli has been broken, the angiogenic response has been characterized as the degradation of the vessel wall by some enzymes, and then the migration of ECs to form capillary sprouts [10].
A scientific consensus has therefore been defined in the terms that pharmaceutical research needed to provide new molecules that could promote physiological angiogenesis in healthy, aging patients. Also, the observations that tumour growth beyond a few millimetres required recruitment and formation of a new microcirculation, added to the consensus that blood vessel-directed therapy (e.g., anti-angiogenesis drugs) might be effective in treating cancer [11].

**Angiogenesis**

**Pro-angiogenesis**

Findings by Isner et al. have been consistent with the notion that VEGF could function as an endogenous regulator of endothelial integrity in artery wall [12]. Also acid and basic FGF have been characterized as the best and most potent angiogenesis factors [13]. EGF has been determined, in its turn, to be the growth factor that stimulates division, growth, and differentiation of the epithelium. As well, some proteins that bind specific DNA sequences such as angiotensin II have been reported to control cellular growth and/or differentiation [14]. The mechanism involved in transduction of the signals of these growth factors in ECs has been reported to involve a series of protein-protein interactions that ultimately activate other proteins, several kinases, or nuclear factors [15]. However, these proteins have been isolated as macromolecules with high molecular weights; they did not represent suitable drugs to be used in order to regulate angiogenesis.

Modern research techniques in drug design have led to the identification of the pro-angiogenesis effect of T₄ (the thyroid hormone) [16] and its metabolites T₂ [17] and DITPA [18] (Fig. 1). Since then, several model systems have been used to validate that concept. Together with the positive results from the animal studies, these results led to these small molecules being considered as potent natural drugs [19] whose action was found to be initiated at the integrin α₁β₃ receptor. T₄ has subsequently been conjugated to materials in nanoparticulate size that have been much more reactive than other materials made up of larger particles (e.g., polymers) [20]. This new formulation of the synthetic drug (Fig. 1) has since then been revealed to be pro-angiogenic in several angiogenesis models and could represent a simple drug candidate potently deprived of side effects that could stimulate physiological angiogenesis [21].

**Anti-angiogenesis**

Several mechanisms are thought to mediate the development of drug resistance, among which the ability of cancer cells to efflux cytotoxic chemicals, to metabolize drugs, or to repair damaged molecules, are the most described. Impaired cellular ability to undergo apoptosis or senescence are also recognized as key players in cancer resistance to therapy [22—25]. Thus angiogenesis inhibitors were attractive from a clinical standpoint for a feature shared in common by cancer chemotherapeutics in general: an activity principally directed, if not limited to nonquiescent, actively proliferating ECs. Of most relevance have been the observations that TSP-1 was a highly potent inhibitor of angiogenesis in vivo and was able to block capillary EC proliferation, migration, and tube formation induced by angiogenic factors [26]. CAI was found to be an inhibitor of non-voltage-gated calcium channels including ionophore channels [27]. Modulation of calcium influx by CAI and subsequently some receptor-activated transmembrane signalling pathways, has been shown to result in cytostatic inhibition of proliferation and invasive behaviour of malignant cells in vitro and in vivo [28]

These macromolecules have molecular weights highly above what has been desired for drug design. In parallel to the research on the thyroid hormone T₄, one metabolite of this hormone, tetrac (Fig. 1), has nevertheless made the difference with other potential small molecule drugs [29]. This compound, metabolized by the body, has shown since then unprecedented non-toxic inhibition of neo-angiogenesis in vitro and in vivo. Also, this potential new drug has been revealed to suppress the proliferation of cancer cell lines that already have reached the resistant state to numerous anti-cancer drugs such as doxorubicin in vivo [30].

The discovery that tetrac induced the blockade of neo-angiogenesis by interacting with the same cell-surface integrin as T₄ has boosted the development of a new formulation for tetrac with enhanced activity [31]. The nanoparticulate tetrac (Fig. 1) has revealed its potent activity against the medullary carcinoma of the thyroid and against human renal carcinoma [32—35].

The use of nanoparticles for the delivery of small molecules is thought to ameliorate drug diffusion, stability, and targeting. Drug-loaded nanoparticles can also be linked to monoclonal antibodies directed against cancer-specific antigens and in theory at least, this approach should enhance specific targeting and reduce toxicity [36]. However, due to their relatively large size, nanoparticles cannot cross the blood brain barrier and this represents a serious limitation to their use.

**Cancer**

**Cancer and inflammation**

In response to various growth factors, hormones, or cytokines, phospholipase A₂ has been described to metabolize phospholipids from the membrane, and the resulting product, the arachidonic acid, to be biochemically transformed by several enzymes such as cyclooxygenase, lipoxygenase, or P-450 epoxygenase into eicosanoids. In the literature, strong experimental results have been reported on these mediators of the inflammation pathway. They were found to be expressed and then overexpressed all over the development of cancer. Further, these biomolecules have been linked with a causative effect to cancer invasion [37]. Despite the fact that most of the eicosanoids have been found to be expressed in the first stage of the disease, their overexpression during the development of cancer has recently been documented. For example, PGE₂ (Fig. 1), one of these eicosanoids, has been shown to stimulate angiogenesis pathways by binding to specific cell receptors [38]. From the products of the isoforms of the cyclooxygenase and lipoxygenase enzymes, the 15-LOX-2 enzyme has been
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Figure 1  Molecules biosynthesized by the human body that have been reproduced by chemical synthesis: the thyroid hormone (1) and its deionated analogs T3 (2), DITPA (3), Tetrac (4); their innovative formulations: T4-nano (5), Tetrac-nano (6) which show how hormones could have been grafted on a nanoparticle bead; Heparin (7), Hyaluronic acid (8) and PGE2 (9).

Molécules produites par le corps humain pouvant être reproduites par voie chimique.
reported to bear a beneficial effect against cancer invasion when expressed [39]. Moreover, several lines of evidence have suggested aspirin as the inhibitor of choice of the cyclooxygenase enzymes in colorectal cancer [40]. Indeed this drug showed beneficial effects in many trials [41,42].

Cancer and angiogenesis

It has been widely accepted that for a neoplasm to grow and/or metastasize, a subgroup of cancerous cells must undergo a switch to an angiogenic phenotype that would result in the formation of a capillary network around the tumour body (Fig. 2). Truly, natural inhibitor stimuli could face a deregulation that has been found to shift the equilibrium towards the overexpression of negative stimuli, which would favour cancer invasion [43]. In this matter, there has been no shortage of suspects to study. Such endogenous negative effects have been hypothesized to occur by breaking the barriers to trans endothelial migration of the Trojan cells bearing angiogenic cytokines. The down regulation of matrix proteins, such as metalloproteinase inhibitors, which have been known to inhibit the matrix degradation required for angiogenesis to occur, has also been involved [44]. Other matrix proteins, such as heparin sulphate, have also been studied for their ability to freeze the release or activity of otherwise bound angiogenic proteins [45]. The growing complexity of the potent deregulation of these extracellular factors has been extended more recently to intracellular mechanisms that regulate life or death of cellular elements required for blood vessel growth. Moreover, cell surface integrins have been studied for being a key intermediate in the angiogenesis signalling pathway [46].

Cancer and heparins

UFH (Fig. 1) and its improved version LMWHs have been reported with poly-pharmacological activity at various levels [47]. Earlier studies have focused on the plasma anti-Xa and anti-IIa pharmacodynamics of the different LMWH. These diverse pharmacological actions include stimulation of the release of the vascular TFPI, inhibition of inflammation (via NF kappaB), inhibition of key matrix degrading enzymes, inhibition of platelet-cancer cell adhesion, and other mechanisms. There has been much evidence supporting the notion that LMWH could play a key role in thrombosis, in hypercoagulation, cancer, angiogenesis, and inflammatory disorders [48]. In that regard, many cancer patients reportedly have a hypercoagulable state, with recurrent thrombosis due to the impact of cancer cells and chemotherapy or radiotherapy on the coagulation cascade [49].

There have also been clinical reports suggesting survival benefits from UFH and LMWH that go beyond their anti-haemostatic effects [50]. Moreover, it’s been proven that its anticoagulant effect prevented further complication that the primary tumour growth ensured [51]. Heparin has also been shown to exert an inhibitory effect on cell adhesion by interference on CD 44, a cell receptor enhanced in a variety of malignant tumours [52]. HA (Fig. 1) has been described as the principal binder of this receptor and as a possible link between the tumour cell and the host tissue. Heparin has thus been hypothesized to competitively interfere with CD44-HA interactions, thus reducing the occurrences of cell adhesion.

Cancer and the coagulation system

Chemotherapeutic agents are associated with thrombogenic mechanisms, including the release of procoagulants and cytokines from tumour cell; production of toxic agents that act directly on the endothelium, a natural anticoagulant [53]; and reductions in levels of natural anticoagulants [54]. In so doing, cancer therapies initiate the coagulation cascade, promoting thrombosis and tumour growth and producing unfavourable outcomes [55]. Current evidence suggests that the tissue factor/factor VII pathway could mediate the most abundant pro-coagulant stimulus in malignancy via the increase in thrombin generation. Thus, TF has also been hypothesized to be a key component in tumour neo-angiogenesis [56].

The coagulation system and angiogenesis

By studying the signal transduction pathway of ligand binding, activation of signalling cascades, signal transduction, and gene activation, TF has been reported to influence angiogenesis by both coagulation dependent and independent pathways [57]. Yet, inhibitors of TF have yet been demonstrated to modulate the processes of in vitro endothelial tube formation [58]. TF has also been involved in the indirect ease of pro-angiogenic effect by downstream generation of thrombin, which itself has been shown to promote angiogenesis [59,60].

Thrombosis associated with bevacizumab treatment

Cancer patients are susceptible to significant risk of thrombosis, which is further increased by the use of anti-angiogenic therapy such as bevacizumab and chemotherapy. Results from several studies showed an increase in both ATE and VTE incidence in bevacizumab treatment groups [61—63]. Overall, the incidence of both ATE and VTE in bevacizumab treatment groups is serious enough that it must be recognized and precautions should be taken.

Discussion

Angiogenesis, being responsible of the transcription of important protumorigenic factors, including VEGF, has been selected as a preferred target for new anti-cancer treatments. The first clinically available inhibitor of the VEGF pathway was bevacizumab. Unfortunately, second generation inhibitors more likely targeted multiple targets rather than one selectively [64]. Since then, clinical use unravelled the potential of these molecules to stimulate in the mean time undesirable cardiovascular effects. So far, hypertension, arterial or venous thrombotic events (including strokes and myocardial infarctions) and heart failure have been associated with the intake of these compounds [62,63,65]. Anti-angiogenesis agents do not represent the solution to cancer; nevertheless, the output from the
Conclusion

In physiological states, such as wound healing, neovascularization has been shown to be strictly limited and finely tuned by a balance of stimulatory and angiogenic factors. Nevertheless, these controls have also been shown to fail and result in formation of a pathologic capillary network during the development of many diseases including cancer. However, the hypothesis that tumour growth is angiogenesis dependent is only consistent with the observation that angiogenesis is necessary but not sufficient for continued tumour growth. In fact, while the absence of angiogenesis will severely limit tumour growth, the onset of angiogenesis activity in a tumour has been revealed to allow, but not to guarantee continued expansion of the tumour population.

Haemostatic complications have been the second most common cause of mortality in cancer patients, particularly in those with pancreatic, gastrointestinal, or lung cancer, and ten per cent of newly diagnosed myeloma patients treated with any type of chemotherapy have developed deep venous thrombosis. The impact of cancer cells and chemotherapy on the activation of the coagulation cascade has been found responsible for a pro-thrombotic state found in many cancer patients. Various mechanisms may be related to the activation of the coagulation or fibrinolytic systems in cancer and have been involved in tumour development, progression, and metastasis. Activation of coagulation could have both systemic and local consequences that could result in the regulation of inflammatory cell infiltration and induction of angiogenesis. The tumour-generated aggregation of many biological mediators has been found to result in the formation of a physical barrier protecting the tumour from exogenous anti-cancer agents. Thus, pro-angiogenesis drugs should be used to stimulate physiological angiogenesis in aging, healthy patients; as drug-resistance and drug-delivery issues are to be overcome on existing drugs, more research is needed to find target specific anti-angiogenesis agents, which should be used to prevent and treat pathological angiogenesis.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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