The Molecular Pharmacology and Therapeutics of Angiogenesis

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Abstract

Angiogenesis is the formation of new vasculature on top of the pre-existing ones, which is critical during the species' life span. It is responsible for delivering oxygen and nutrients to metabolically active tissues, therefore playing an essential role in physiological conditions, such as wound healing and pathological circumstances, like malignancies. Although sharing similar characteristics, the blood vessels in pathological angiogenesis develop irregularly and disorganizedly. Therefore, the leakage of materials from blood vessels is familiar in abnormal angiogenesis, which may result in much higher interstitial pressure than internal pressure. Whether the angiogenesis is physiological or pathological, the proliferation of normal dormant endothelial cells is an essential requirement. Additionally, angiogenesis is also initiated by vascular endothelial growth factor A (VEGF-A) interacting with the vascular endothelial growth factor receptor 2 (VEGFR2) or the co-receptor neuropilin-1 (NRP-1). The Vegfa gene can be spliced in many ways, creating numerous VEGF-A isoforms, each with unique interactions with VEGFR2. Several drugs have been approved as the first- or second-line of angiogenesis, thereby preventing cancer progression. The widely used is monoclonal antibodies (bevacizumab) and receptor tyrosine kinase inhibitor (sorafenib and axitinib). However, they lack selectivity and, as a result, are less effective and have considerable adverse effects. As a natural drug, Paclitaxel has an anti-angiogenic effect in addition to its chemotherapeutic effect. However, different doses of paclitaxel have different anti-angiogenic mechanisms and different effects on different species.

Keywords: ***Angiogenesis; Endothelial cells; VEGF; VEGFR; NRP1; Bevacizumab; Paclitaxel.***

1. Introduction

The process by which existing vascular networks create new blood vessels receives strict regulation by the body [1]. This process, known as angiogenesis, is in charge of enabling vascularization to a previously avascular region, and delivering nutrients and oxygen to metabolically active tissues [2]. Angiogenesis has a significant influence on physiological processes that occur during both childhood and adulthood, with the most familiar function of wound healing [3]. There is no doubt that angiogenesis plays a significant role during menstrual cycle in women, as the uterine endometrium goes through regular development and disintegration cycles, which require massive vascular network [4]. It also provides sufficient vasculature to tissues. Therefore, without angiogenesis human my suffer myocardia infraction, stroke, and neurodegeneration .

In addition, it is hijacked in pathological circumstances including malignancies, inflammatory illnesses, and cardiovascular ailments [5]. In most cases of cancer, with the development of tumor size, the center of the tumor becomes increasingly hypoxic. So, they need a highly vascularized network as their driving force. Histological analysis of animal models and human premalignant non-invasive lesions has revealed that the induction of angiogenesis is already present in the early stage of cancer [6]. Experiments by Bergers *et al*. on solid and invasive cancers in mice found that angiogenesis was stable in these cancer cells [7].

While normal and pathological angiogenesis share many features, the proliferation of tumor vascular endothelial cells (ECs) is continuous and unresolved. It develops more rapidly in a disorganized manner, but its stability is poor and cell turnover is high. Therefore, the vessels are often morphologically poorly designed, unevenly distributed and leaky, allowing material to leak out and enter the surrounding interstitial environment due to losing ECs junctions and inadequate surrounding cell coverage (Figure 1) [8]. As the consequence of leakage, the interstitial pressure outside of the blood vessels is raised, which is going to be much harder to get drugs from the vasculature into area where it needs. In addition, the vessel lumens are more likely to be a heterogeneous mixture of ECs and dispersed tumor cells.

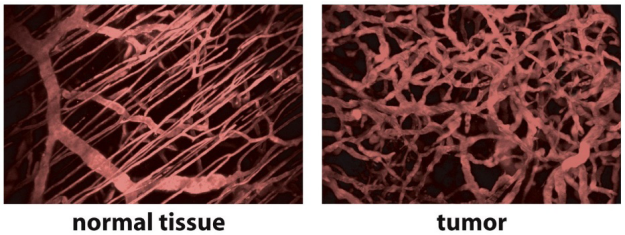

Figure 1. The morphology of normal and tumor

angiogenesis. The image on the left shows normal vascular tissue, which is highly controlled and organized. The small capillaries are branching out from the large vessels orderly. On the right, however, is the vasculature feeding around the tumor, where there are no regulated and organizational features. Figure is derived from Weinberg RA [9].

2. Mechanisms Causing Angiogenesis

Whether the angiogenesis is physiological or pathological, one of the necessary conditions is the proliferation of normal dormant endothelial cells (ECs) [10]. In addition, together with the proliferation of ECs, the permeability of blood vessels and the pressure of tissue fluid increase, making the blood vessels near the tumor more chaotic and disorganized. Therefore, inhibition of ECs proliferation is beneficial in preventing angiogenesis and tumor formation. This has been demonstrated in mice, namely in a transgenic mouse model of islet cell carcinogenesis treated with endothelial cell inhibitors, where angiogenic transformation of mouse tumors is inhibited and tumor expansion is disturbed [7]. Carmeliet in his review also showed that some anti-angiogenic drugs could inhibit endothelial cell proliferation and vessel development, they would also cause regression of existing vessels by increasing endothelial cell mortality [3].

Angiogenesis is influenced by a number of pro-angiogenic factors. Vascular endothelial growth factor A (VEGF-A) is an antiparallel homologue connected by a chain of disulfide linkages that is one of the most potent regulators of angiogenesis in health and sickness [2]. As a family member of VEGF, VEGF-A is released by a variety of cells, including endothelial cells and roughly half of tumor cells. In addition, inflammatory stimuli and ischemia can also induce the secretion of VEGF-A [11]. There are several isoforms of VEGF-A, which is determined by the alternative splicing of the Vegfa gene and each has distinct property. The most common types of splicing are the exon 8, and exon 6a and 7. Splicing the exon 8 at its proximal and distal ends generates VEGF-A isoforms with entirely opposite pharmacological properties. Although the two isoforms differ only 6 amino acids, the VEGF165a is a pro-angiogenic factor but the VEGF165b is an anti-angiogenic factor [2]. The splicing of exon 6a and 7 produces a smaller effect, which the VEGF-A isoforms have different bioavailability [12].

The receptor tyrosine kinase (RTK) vascular endothelial growth factor receptors (VEGFR), specifically VEGFR2, is the receptor for VEGF. VEGFR2 activation starts by VEGF-A binding to the IgG-like domains D2 and D3, leading to VEGFR2 dimerization. Following that, VEGFR2 undergoes a conformational shift that causes intracellular tyrosine residues to be trans- or auto-phosphorylated, resulting in the recruitment of adaptor proteins and initiate a cascade of signaling events [13]. One of the main signalling pathways that occurs below is the phosphorylation of tyrosine residue Y1175 in response to the interaction of VEGF-A and VEGFR2, resulting in the recruitment of the adaptor protein PLCγ, followed by the hydrolysis of PIP2 and IP3 and the activation of receptors such as Raf, MEK and others, ultimately promoting angiogenesis, vascular permeability and cell migration, adhesion and survival. Experiments performed by Laura Kilpatrick et al. demonstrated that VEGF-A interacts with VEGFR2 to promote the proliferation of HUVECs [14].

Angiogenesis is also promoted by the co-receptor transmembrane glycoprotein neuropilin-1 receptor (NRP-1). The effect of NRP-1 to vascular system was demonstrated by the experiment that some problems in vascular development in mice after knocking out the Nrp1 gene [15]. Several studies found that NRP-1 is upregulated in tumor cell with VEGFR2 to mediate the development and metastasis of cancer cells. As a consequence, NRP-1 was regarded as the co-receptor of some RTKs like VEGFR2 by selectively improving the binding of VEGF-A isoforms to VEGFR2. In the presence of neuropilin-1, VEGF-A binds to the b1 domain of NRP-1 directly and binds to VEGFR2 in cis or trans direction depending on whether NRP-1 is on the same cell as VEGFR2, improving VEGFR2 signaling outcome. This is demonstrated by antibodies directed against the NRP-1 b1/b2 structural area preventing VEGF-A-induced angiogenesis and endothelial cell proliferation in the cornea [16].

3. Therapeutic Agents of Angiogenesis

According to the angiogenesis mechanism stated above, VEGF can be used as a target for cancer therapy by inhibiting angiogenesis (Table 1). The humanized pan-anti-VEGFA monoclonal antibody bevacizumab has now been found to prevent angiogenesis and therefore inhibit tumor progression. Tumor requires oxygen and nutrients to maintain its rapid growth rate. In the presence of hypoxia, a great amount of VEGF-A is secreted to bind to VEGFR and NRP-1 to initiate angiogenesis by promoting proliferation of endothelial cells. Bevacizumab acts to recognize and neutralize all biologically active VEGF-A isoforms, thereby preventing the ligand from binding to its receptors, VEGFR1 and 2. Once the interaction is blocked, some downstream signalling pathways are also limited, ultimately leading to compromised both endothelial cell proliferation and angiogenesis [17].

The effect of bevacizumab can be evidenced in animal

models. Lin Y. S *et al.* demonstrated that weekly injection of 2-3 mg/kg bevacizumab was adequate to decrease vascular endothelial growth factor activity in cynomolgus monkeys [18]. In one experiment done by Varey *et al.*, VEGF-A-transfected LS174t colon cancer cells were injected into nude mice, which were divided into two groups and treated with the same dose of bevacizumab and saline. This experiment showed that bevacizumab effectively inhibited tumor enlargement in the mice [12]. Furthermore, bevacizumab is used primarily in patients that are already suffered from quite advanced stage diseases. Consequently, bevacizumab was approved by FDA and is now used as first- and second-line therapy in the clinical management of metastatic colorectal cancer, first-line therapy for metastatic non-small cell lung cancer (NSCLC) and metastatic renal cell cancer [17]. However, their primary efficacy aside, bevacizumab is expensive, and are associated with serious side effects such as hypertension and cardiovascular disease [17]. The choice of an appropriate drug that is inexpensive, mild and yet effective is therefore crucial.

Apart from the monoclonal antibody, we should not neglect to the receptor tyrosine kinase inhibitors (RTKIs), a small molecule that target VEGF receptors. One example is sorafenib. As a multi-targeted RTKI, it would target several different receptors and signaling pathways, including VEGFRs. Sorafenib is shown to exert anti-proliferative and anti-angiogenic effect and is consequently approved for the treatment of advanced renal cell carcinoma (RCC) and hepatocellular carcinoma (HCC) [19]. This could be demonstrated by the clinical trial for patients with RCC, where there is a significant improvement in the progression of free survival compared to placebo. However, patients treated with sorafenib always appear adverse reactions not related to anti-VEGFR2. The reason is that it targets other receptors, such as c-kit, B-Raf and PDGFR [20]. Another is axitinib, which is the second-generation small molecular inhibitor, with more selectivity for VEGFR2 and improved potency. Therefore, this drug, which is currently approved for second-line treatment for renal cell carcinoma which is refractory (not responding) to other multi-targeted RTKIs, is more selective for VEGFR2 than sorafenib [19].

Researchers have turned their attention to natural medicines, of which paclitaxel is one that is well known to the public. Derived from the bark of Taxus revifolia, paclitaxel is a natural compound used in the treatment of cancer with a specific mechanism of action. Unlike chemotherapeutic drugs that prevent the polymerization of microtubule protein structures into microtubules, such as colchicine and vincristine, paclitaxel promotes the polymerization and stabilization of microtubules in living cells. As a result, chromosomes are unable to reach the poles of the cell, which impedes the mitotic process and thus cell proliferation [21]. The mechanism of paclitaxel suggests that it can be effective in treating cancer through targeting tumor cells with cytotoxicity. Data from several clinical studies suggest that paclitaxel can be used as a first- or second-line drug and has been widely used to treat cancers such as ovarian and non-small cell lung cancer with few side effects [22]. In addition to being naturally available, paclitaxel can be manufactured synthetically by a variety of methods, thereby significantly reducing costs [19], meaning that paclitaxel may be a better option among anti-endothelial acting drugs.

The mechanism of chemotherapy drugs is to inhibit the progression of cancer by stopping the division of cancer cells and inducing apoptosis. However, some studies have suggested that paclitaxel may have an anti-angiogenic effect, that is, inhibiting the proliferation of endothelial cells. Although initially a small body of evidence suggested that chemotherapeutic anticancer drugs, such as doxorubicin, 5-fluorouracil and vincristine, may limit the proliferation of endothelial cells and thus limit angiogenesis [10]. Nowadays a large number of experiments to verify this additional antiangiogenic function of paclitaxel, but with various mechanism. The paclitaxel could have a cytostatic effect at low concentration, and a cytotoxic effect at higher concentration. While the cytotoxic effect is to induce apoptosis of endothelial cells through increasing pro-apoptotic protein, the cytostatic effect is to inhibit proliferation without inducing apoptosis [23]. Furthermore, long-term action of paclitaxel may restrict expression of VEGF by tumor cells, thereby ultimately influencing angiogenesis indirectly. [24]. The effect of paclitaxel is also species-selective, which is illustrated by the experiment done by Wang et al. [10]. In that assay, they used ultra-low dose paclitaxel to act on endothelial cells of human and mice in vitro separately and found only the proliferation of human ECs was inhibited. Only if they increased the concentration of paclitaxel did the proliferation of mice ECs being blocked.

4. Conclusion

In conclusion, the objective of this review is to gain a better understand of the pharmacology of angiogenesis and the therapeutic agents of it, the summary of Therapeutic Agents of Angiogenesis is shown in table 1. In fact, angiogenesis is present during the life cycle of human being and animals. In normal situation, angiogenesis safeguards the continuation of life, however, solid tumor growth also requires angiogenesis. Several mechanisms are known to be activate angiogenesis. VEGF-A is an

important mediator of angiogenesis, which is secreted by a numerous kind of cells or in hypoxia condition and binds to vascular endothelial growth factor receptors (VEGFR), notably VEGFR2, to initiate a cascade of signaling events. This promotes angiogenesis, vascular permeability, and cell migration, adhesion, and survival. Thus, VEGF-A can be used as a target for cancer therapy by inhibiting angiogenesis. Some monoclonal antibiotics, such as bevacizumab, and RTKIs, such as sorafenib, have now been found to inhibit tumor progression. Nevertheless, they do not cure patients fully without causing side effects. Researchers find unexpected effect of paclitaxel in treating angiogenesis. Paclitaxel may have cytostatic and cytotoxic effect depending on its concentration, and it can also inhibit VEGF expression. Therefore, caution is needed in concentrations for different effects. In the future we will investigate more detailed mechanisms and treatments related to angiogenesis, and only through a thorough understanding of this angiogenic mechanism will we be able to target them more effectively throughout the drug development process.

Table 1. Summary of Therapeutic Agents of Angiogenesis

Medicines	Pharmacological Effect	Diseases for which the drug is approved for treatment
Bevacizumab	Binds VEGF-A isoforms preventing its interaction with VEGFR1 and 2 [17]	Metastatic colorectal cancer (mCRC) Metastatic non-small cell lung cancer (mNSCLC) Metastatic renal cell cancer (mRCC)
Sorafenib	Target several different receptors and signaling pathways, including VEGFRs [19]	Advanced renal cell carcinoma (aRCC) Hepatocellular carcinoma (HCC)
Axitinib	More selectivity for VEGFR2 [19]	Advanced renal cell carcinoma (aRCC)
Paclitaxel	Inhibiting the proliferation of endothelial cells [10] Restrict expression of VEGF by tumor cells by long-term use [24]	

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