eISBN: 978-1-68108-155-7
ISBN: 978-1-68108-156-4

eISSN: 2210-268X
ISSN: 2452-3240

# Anti-Angiogenesis Drug Discovery and Development

## Volume 3

**Editors:**

**Atta-ur-Rahman**

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# Anti-Angiogenesis Drug Discovery and Development

## *(Volume 3)*

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ISSN (Online): 2210-268X

ISSN (Print): 2452-3240

ISBN (Online): 978-1-68108-155-7

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# PREFACE

Angiogenesis is a normal process for healing and reproduction. It involves the growth of new capillary blood vessels from the pre-existing vessels. However, in disease states angiogenesis leads to abnormal blood vessel growth. This then becomes the underlying process for many deadly diseases, including cancers and retinopathy. Pharmaceutical R&D is currently focused on devising strategies to target biochemical and cellular events involved in the angiogenesis in search of treatment of various diseases. An important approach has been to inhibit the action of various enzymes and factors involved in the complex cascade of reactions leading to new blood vessel formation.

The 3rd volume of the book series "*Anti-Angiogenesis Drug Discovery and Development*" is a compilation of five excellent reviews, written by leading researchers in these fields. Each review is focused on a certain important aspect of angiogenesis and the modulation of the angiogenesis process through chemotherapeutic agents as possible treatments of various diseases.

Makhouli *et al*. have contributed a review on various clinical and pre-clinical trials on beta-blockers, such as catecholamine, for the treatment of retinopathy of prematurity (ROP). ROP is a disease of premature babies, which involves abnormal blood vessel formation in the retina. This growth can cause the retina to detach from the back of the eye, leading to complete blindness. The diseases range from being mild to severe and currently no treatment is available. The authors have summarized the results of various clinical and preclinical studies of oral propranolol treatment, and evaluated the efficacy and safety of such treatment at various stages of ROP. Propranolol is used in the treatment of tremors hypertension (high blood pressure), heart rhythm disorders, angina (chest pain), and in other heart or circulatory disorders. It is also used to prevent heart attack, and reduce the severity and frequency of migraine headaches. The use of propranolol in ROP is an important step forward, as well as an example of drug repurposing.

Integrins represent a family of adhesion receptor proteins involved in angiogenesis. The review contributed by Lazarovici *et al*. is focused on the development of antagonists (disintegrins) of various types of integrins for therapeutic purposes. Obtustatin and viperistatin, two members of the disintegrin protein family, have been used as templates for the synthesis of two linear and cyclic peptides, containing the KTS binding motif. More specifically, two synthetic cyclic linear peptides, vimocin and vidapin, have attracted major scientific interests as dual antagonists of α1/α2 integrins. *In vitro* and *in vivo* studies indicated their high potency in inhibiting the adhesion of integrins, thus slowing down the angiogenesis induced by vascular endothelial growth factors. The results of various studies, including

stability, potency, efficacy, computational modeling, etc. indicate the promising nature of vimocin and vidapin.

The last three reviews are focused on the therapeutic potential of various antiangiogenic drug candidates in the treatment of cancers, such as lung cancer, hepatocellular carcinoma, and glioblastoma. All three reviews start with the detail descriptions of the role of angiogenesis in tumor progression, and molecular events in angiogenic process, followed by the development of various anti-agiogenic compounds to inhibit the angiogenesis cascade as potential drugs. The results of animal studies and clinical trials present a mixed picture, which demands further studies in this field.

Ellis and Al Farsi review the current status of antiangiogenic therapy in the treatment of lung cancer, known for very high level of angiogenic activity, aggressive nature, and very low survival rate. Antiangiogenic drugs can offer a better therapeutic option for lung cancer, and thus vigorous research is being conducted on this class of potential drugs. The authors have exhaustively reviewed the results of clinical studies on various combinations of therapeutic agents *i.e*. antiangiogenic monoclonal antibody bevacizumab and various classes of anti-cancer drugs, on the improvement of survival, disease progression, tumor recurrence, and metastasis in NSCLC (Non-small cell lung cancer) patients. The results of these studies were found to be non-conclusive, though some improvement in survival rate has been noted. The authors present a strong case in favor of further studies in the identification of biomarkers, as well as additional translational research.

Hepatocellular carcinoma (HCC) is the most malignant tumor with a very high mortality rate. Available treatment options are few, and far from ideal. HCC is known for the high level of neo-angiogenesis. A series of angiogenic factors have been identified in HCC as possible targets for anti-angiogenic drug discovery. Recently, several anti-agiogenic agents were developed, and extensive animal studies and clinical trials have been conducted. The review, contributed by Sun *et al.*, describes the salient features of various clinical and pre-clinical studies, as well as limitation, challenges, and recent advances in the possible treatment of HCC by anti-angiogenic agents, and their combinations with classical anti-HCC drugs.

The last review of this volume, contributed by Martinho *et al*., focuses on the treatment of glioblastoma multiforme (GBM) by an FDA approved anti-angiogenic monoclonal antibody, bevacizumab. The results of clinical trials on bevacizumab in combination with radiotherapy and the anti-cancer drug temozolomide (TMZ) have shown improved survival rate in GBM patients. However, data about the quality of life, and functional improvements are conflicting. Reports of adverse effects of bevacizumab further complicate the situation. This requires a better understanding of the biology and mechanism of the angiogenic process involved the in progression of GBM, and improved clinical study design.

We wish to express our gratitude to all the eminent contributors for the timely submission of their articles which have resulted in this excellent 3rd volume of the eBook series volume in this important field. It is the result of efficient coordination and excellent management of the entire team of Bentham Science Publishers. We would like to appreciate the joint efforts of Mr. Omer Shafi (Assistant Manager Publications), Mr. Shehzad Naqvi (Manager Publications) and team leader Mr. Mahmood Alam (Director Publications). We sincerely hope that this volume will receive wide appreciation from the readers.

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CHAPTER 1

# Beta-Blocker Therapy for Retinopathy of Prematurity

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**Abstract:** Vascular endothelial growth factor (VEGF) plays a critical role in the pathogenesis of the vaso-proliferative phase of retinopathy of prematurity (ROP), being up-regulated by the adrenergic system and down-regulated by beta-blockers. Beta-blockers are anti-angiogenic agents and as such they decelerate the growth of skin hemangiomas and decrease the risks associated with life-threatening hemangiomas. Numerous previous studies investigated the effect of catechol amines on the angiogenesis of various cells in culture or on relevant animal models of angiogenesis. As of April 2015, two prospective randomized trials recently evaluated oral propranolol treatment for established ROP. Mahout *et al.* studied 20 premature infants with ROP (10-oral propranolol, 10–placebo) and showed a 50% reduction of need for invasive interventions. Filippo *et al.* treated 52 infants with ROP using oral propranolol and achieved 48% and 58% risk reduction of progression to stage 3 and stage 3 plus ROP, respectively, and a 52% reduction in the need for laser or bevacizumab therapy. To avoid systemic adverse effects of oral propranolol, rabbits and mice with oxygen-induced retinopathy (OIR) recently received propranolol eye drops, producing retinal concentrations similar to those measured after oral administration but with significantly lower plasma concentrations. Propranolol eye drops promoted OIR recovery in mice. Although the systemic (oral) or topical (eye drops) routes for beta-blocker therapy for ROP appear promising, questions have emerged regarding effectiveness, safety, tolerability and timing of treatment. Additionally, should beta-blockers prove safe and

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effective for ROP in premature infants, then the optimal therapeutic approach should be determined: Rescue Therapy for established ROP *versus* pre-emptive prophylactic treatment in premature infants at high risk for developing ROP, mainly those born prior to 28 weeks' gestation. In this chapter we review the literature as to the discovery of the key role of catechol amines on angiogenesis and their effects *via* VEGF as well as the approaches taken to implement anti-angiogenic therapy for ROP in animals and humans.

**Keywords:** Angiogenesis, Beta adrenergic receptors, Bevacizumab, Cancer, Catechol amines, Cell culture, Epinephrine, Neo-vascularization, Oxygen induced retinopathy, Propranolol, Retinopathy of prematurity, Vascular endothelial growth factor.

## 1. WHAT IS ALREADY KNOWN ON THIS TOPIC

The management of retinopathy of prematurity is currently expectant with invasive interventions (laser photocoagulation of ischaemic retina for threshold ROP or intravitreal injection of bevacizumab) being performed as "rescue" therapies. Nowadays, non-invasive medical therapies for earlier stages of ROP are being considered.

## 2. WHAT THIS CHAPTER ADDS

Oral propranolol is apparently safe when given as systemic therapy to premature infants with retinopathy of prematurity. Propranolol could reduce by about half the need for invasive interventions for retinopathy of prematurity (laser photocoagulation and intravitreal injection of bevacizumab). Studies are underway now for evaluation of earlier initiation of propranolol therapy in premature infants who are potential candidates for development of ROP. In addition, topical eye propranolol drops are compared to systemic oral propranolol therapy.

## 3. INTRODUCTION

Retinopathy of prematurity (ROP) occurs mainly in premature infants born before 28 weeks' gestation. Extreme prematurity and relative hyperoxia are the main risk factors for ROP while aberrant angiogenesis of the retinal vasculature is the

common pathogenetic pathway of ROP. So far, the main therapeutic modalities for ROP include destruction of retinal areas with aberrant angiogenesis by laser photocoagulation or by medications with anti-angiogenic activity.

Stimulation of beta adrenergic receptors (β-AR) by either epinephrine or norepinephrine (NE), down regulates neo-vascularization [1]. Noteworthy are the key role of VEGF in retinal neovascularization and the successful therapeutic trials with VEGF blockers. Hence, improving animal models of oxygen-induced retinopathy (OIR) is equally important for investigating anti-angiogenic therapies. Rezzola *et al.* [2] developed a novel murine retina angiogenesis assay where retinal fragments from adult mice were embedded in a fibrin gel in the presence of human recombinant VEGF. Starting from the 3rd-4th day of incubation, endothelial cell sprouts invaded the fibrin gel. The effect of VEGF was dose-dependent, with maximal stimulation being observed after 7 days. As to potential toxicity of bevacizumab, Kaempf *et al.* [3] found bevacizumab to be well tolerated by ganglion and photoreceptor cells.

In a mouse model of OIR, Dal Monte *et al.* [4] have recently shown that subcutaneous (sc) administration of the non-selective β-AR blocker propranolol ameliorates angiogenic processes in the retina when its effects are evaluated at postnatal day (PD) 17. After propranolol administration on the eye, mice were first tested for retinal concentrations of propranolol as compared with those measured after sc or per os administration. The results showed that 2% topical propranolol has efficiency (in terms of final propranolol concentration in the retina) comparable to that of 20 mg/kg propranolol sc or per os which is significantly higher than those observed with doses and administration routes that are currently used with children [4].

Propranolol ophthalmic solutions reduced VEGF and IGF-1 up-regulation in response to hypoxia. As a result of its inhibitory effects on hypoxia-induced proangiogenic factors, propranolol significantly lessened retinal neovascularization in the superficial but not in the deep vascular plexus. An evaluation of retinal neovascularization at PD21 showed that propranolol was still effective in inhibiting OIR. These findings support the hypothesis that β-AR blockade can efficiently counteract OIR and suggest that topical eye application

**CHAPTER 2**

# Disintegrin-Based, Synthetic Cyclic KTS Peptides as Novel Dual Antagonists of α1β1/α2β1 Integrins with Antiangiogenic Activity

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**Abstract:** Snake venoms comprise disintegrins, proteins which target integrin receptor-dependent cell adhesion by endothelial cells. The disintegrins Obtustatin and Viperistatin, were used as lead compounds for the synthesis of linear and cyclic peptides containing the KTS binding motif. The most active linear peptide pointed to the importance of Cys19 and Cys29, and the presence of Arg24 for biological activity, and was used as the basic linear sequence for the synthesis of cyclic peptides. The most potent peptides, named Vimocin and Vidapin, showed a high potency ($IC_{50}$ = 0.17 nM) and intermediate efficacy (20% and 40%) in inhibiting adhesion of α1/α2 integrin-overexpressing cells to collagen. Vimocin was more active in inhibiting wound healing and corneal micropocket vascularization, whereas Vidapin was more potent in reducing endothelial cell migration in the Matrigel tube assay. Both compounds similarly inhibited proliferation of endothelial cells and angiogenesis induced by vascular endothelial growth factor or glioma tumor cells in the chorioallantoic membrane angiogenic assay. These peptides were well tolerated by mice after intravenous

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injection. They showed stability in human serum between 10–30 hours. The *in vitro* and *in vivo* potency of these cyclic peptides is consistent with the computational modeling indicating conformational similarities to the parental molecules. Vidapin significantly increased the survival of mice injected with B16 melanoma cells up to 73 days, whereas the median survival time of animals in this tumor experimental model is 40 days. These findings propose that Vimocin and Vidapin can serve as dual α1β1/α2β1 integrin antagonists in angiogenesis and cancer therapy.

**Keywords:** Adhesion, Antiangiogenic, Anticancer, Conformation, Cyclic, Disintegrin, Disulfide bond, Endothelial cell, Integrin, KTS motif, Lead compound, Linear, Migration, Molecular dynamic simulations, Molecular modeling, Partial antagonist, Peptides, Peptidomimetic, Proliferation, Stability.

## 1. ANGIOGENESIS

Angiogenesis, the generation of new capillaries, is a complex, still tightly regulated process involving multiple cells, growth factors and extracellular matrix modeling. Angiogenesis occurs by endothelial cells (EC) proliferation and migration leading to capillaries sprouting from the existing microvasculature. Newly formed blood vessels carry oxygen, hormones, nutrients and electrolytes and secrete a variety of growth factors with paracrine and juxtacrine activity on endothelial cells, fibroblasts and pericytes forming the new vasculature [1]. Once these new capillaries have formed and matured, the endothelial cells lining the newly formed blood vessels stop proliferating and become quiescent. Judah Folkman recognized that angiogenesis is crucial for the supported growth of solid tumors [2]. Solid tumors that lack sufficient vascularization become necrotic [3] and/or apoptotic [4] and fail to grow beyond a limited size of 2 $mm^3$, and turning off the "angiogeneic switch" is a potential anti-cancer therapy [5]. The tumors that undergo neovascularization enter a phase of rapid growth and exhibit increased metastatic potential [6] since the leaky blood vessels which invade and surround the tumor provide a way for cancer cells to enter the circulation and to metastasize to distinct organs. Thus, many if not all organs may involve cancers in which angiogenesis is an important component. Inhibition of the early steps of angiogenesis has been recognized as an appealing approach for the treatment of human cancers [7]. Angiogenesis inhibition is a promising therapeutic approach,

with very mild side effects, because throughout the body the vasculature is substantially quiescent with very low turnover rate. Therefore, the search for natural antiangiogenic factors and synthetic chemicals may lead to the development of specific antiangiogenic drugs for the supporting therapy of cancer.

## 2. A1B1 AND A2B1 INTEGRINS ARE TARGETS IN ANGIOGENESIS

Integrins are a family of cell surface receptors which mediate adhesion to multiple ligands and mediate cell-cell and cell-extracellular matrix (ECM) interactions as well as cell signaling. These heterodimeric receptors comprise non-covalently linked distinct subunits, α and β, usually containing a large extracellular binding domain, a transmembrane region, and a short cytoplasmic domain. Upon ligand binding, integrins cluster and recruit *via* their cytoplasmic domains cytoskeletal, adaptor and signaling proteins, thus eventually forming focal adhesions. Focal adhesions anchor the cell to the subjacent extracellular matrix (ECM) ligands, but also convey signals into the cell [8]. From the focal adhesion sites signal pathways diverge and regulate adhesion, migration, proliferation, and survival [9]. Integrins play an important role in the angiogenic process, in physiological as well as in pathological blood vessel formation [10, 11]. Two major collagen binding integrins are α1β1 and α2β1 [8]. The expression of α1β1, α2β1, as well as α4β1, α5β1, α6β1, α6β4, α9β1, αvβ3 and αvβ5 endothelial cell integrins is strongly induced by pro-angiogenic growth factors or chemokines [12 - 14]. The α1β1 integrin is abundantly expressed on human endothelium [15] and on lymphocytes which infiltrate the tumor tissue in several solid cancers [16]. Senger *et al*. identified critical cross talk functions for the α1β1 and α2β1 integrins not only in EC migration but also in supporting signal transduction by the major angiogenic factor, VEGF [17]. Integrin α2β1 also indirectly regulates angiogenesis by promoting platelet deposition within tumors and damaged tissues [18, 19]. The α1β1 and α2β1 integrins directly contribute to mouse mammary carcinoma cell invasion by regulating matrix metalloproteinase-3 (MMP-3) which is involved in extracellular matrix proteolysis [20], a prerequisite for EC migration during the angiogenic process. A key role for α2β1 in angiogenesis was shown by using small molecule, inhibitors of collagen-induced angiogenesis [21]. The importance of α1β1 and α2β1 integrins in tumor angiogenesis was unambiguously

CHAPTER 3

# Current Status of Anti-Angiogenic Therapy in the Clinical Management of Lung Cancer

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**Abstract:** Increased angiogenic activity occurs frequently in lung cancer and results in biologically more aggressive disease. There has been intense research into therapeutic agents that inhibit angiogenesis and may improve treatment options for patients with lung cancer. Bevacizumab, a monoclonal antibody directed against serum VEGF, in combination with carboplatin and paclitaxel chemotherapy, has been shown to improve survival for NSCLC patients. Meta-analysis of trials of bevacizumab in combination with platinum-based chemotherapy for NSCLC, show a 10% reduction in the risk of death (HR 0.90, 95%CI 0.81 – 0.99). However, therapy with bevacizumab is limited to NSCLC patients with non-squamous histology, good performance status, no brain metastases and the absence of bleeding or thrombotic disorders. Similar efficacy has been seen also with carboplatin, pemetrexed plus maintenance pemetrexed chemotherapy. In the second-line setting, the addition of ramucirumab to docetaxel chemotherapy, resulted in a modest improvement in survival, adding a second anti-angiogenic treatment option for patients with NSCLC.

A large number of trials in NSCLC have been conducted evaluating oral anti-angiogenic compounds, both in first-line therapy in combination with chemotherapy, or upon disease progression, either as combination, or single agent therapy. Some level of activity has been observed with most agents. No clear improvements in overall survival have been observed, although a subgroup analysis of a trial evaluating the addition of

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nintedanib to docetaxel showed improved survival limited to patients with adenocarcinoma. However, these findings require validation. All of the oral anti-angiogenic agents result in added toxicities. Some agents have resulted in an increased risk of death, limiting their development. Currently, there is no established role for anti-angiogenic therapy in SCLC, although there is some promise for sunitinib as maintenance therapy following platinum and etoposide chemotherapy.

Despite the large number of anti-angiogenic agents evaluated in clinical trials, there is evidence supporting a limited number of agents as treatment options for patients with lung cancer. To date no biomarkers have been identified. It is unclear whether treatment effects in a subpopulation, are lost among a larger unselected population of patients. There is a need for additional translational research to identify predictive biomarkers for anti-angiogenic therapy.

**Keywords:** Angiogenesis, Anti-angiogenic therapy, Chemotherapy, Fibroblast derived growth factor, Monoclonal antibodies, Non small cell lung cancer, Overall survival, Platelet derived growth factor, Progression free survival, Randomized clinical trials, Small cell lung cancer, Tyrosine kinase inhibitors, Vascular endothelial growth factor.

## 1. ANGIOGENESIS IN THE PATHOGENESIS OF LUNG CANCER

Since its introduction in 1971, the concept of angiogenesis in cancer has gained considerable interest from researchers. Dr. Judah Folkman postulated that tumors need to recruit their own blood supply in order to grow beyond a certain size [1]. This process, named tumor angiogenesis, involves the production of soluble growth factors by the tumor itself. Our understanding of these processes has continued to evolve over time. Angiogenesis is observed in many different cancers including lung cancer [2]. This complex process that depends on many regulatory factors, happens throughout the body. Activating and inhibiting factors regulate angiogenesis and their level of may influence the aggressiveness of cancer [3]. The list of growth factors that induce tumor angiogenesis is long. Understanding their roles resulted in the development and evaluation of multiple therapeutic agents as potential cancer treatments, either alone, or in combination with systemic chemotherapy. Anti-angiogenic therapy provides several benefits including normalizing the abnormal vasculature in cancer, improving delivery of chemotherapy and enhancing its anti-vascular effect and preventing the rapid

repopulation after the systemic treatment [4].

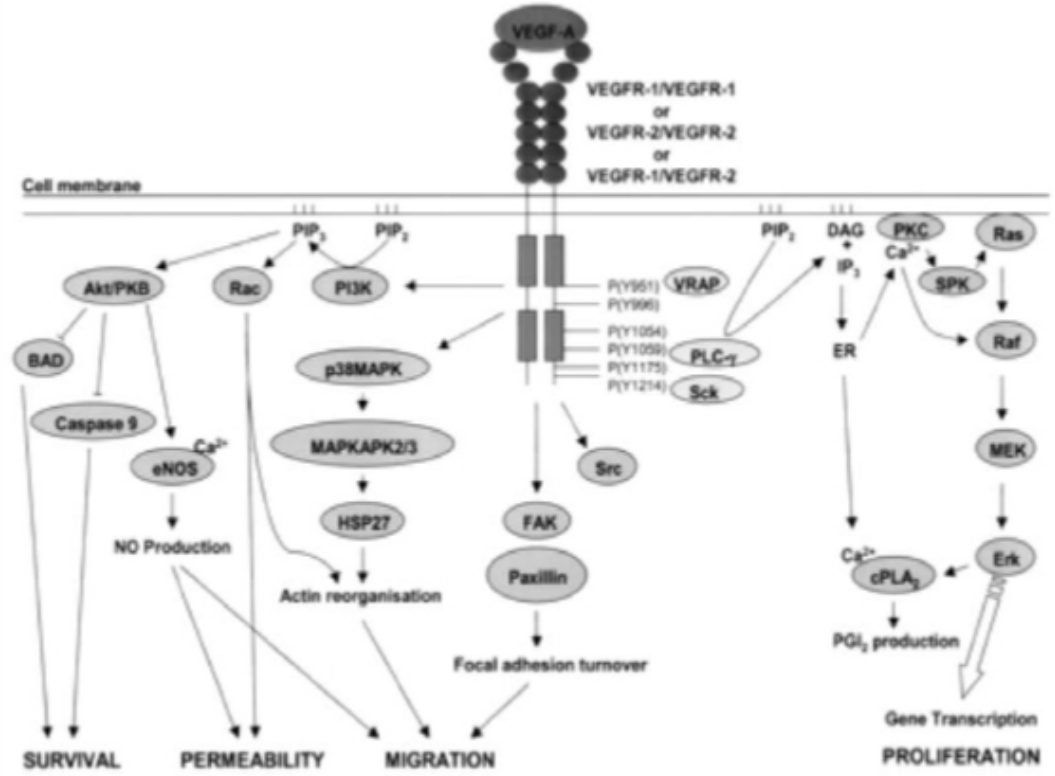


**Fig. (1).** Vascular endothelial growth factor and angiogenesis [reprinted with permission from A Hoeben, *et al.* Parmacological Reviews 2004; 56: 549-580].

The most important factors associated with angiogenesis include vascular endothelial growth factor (VEGF) [5, 6], platelet derived growth factor (PDGF) [7] and fibroblast derived growth factor (FGF) [8]. Vascular endothelial cells depend for their survival on serum VEGF (VEGF-A) that stimulates proliferation and migration, inhibits apoptosis and modulates the endothelial permeability [9]. Binding of VEGF to the VEGF receptor (VEGFR-2) triggers a cascade of molecular events that involves phosphoinositide 3-kinase (PI3-kinase) pathway and drives tumor angiogenesis, cell survival and proliferation pathways (Fig. **1**) [9 - 11]. Tumors that demonstrate VEGF-A overexpression are associated with a shorter survival [12]. Given this important role in tumor progression, the VEGF/VEGF-R pathway has been studied extensively as a target for anti-

CHAPTER 4

# Angiogenesis in Hepatocellular Carcinoma (HCC) and its Potential Applications in the Development of Anti-HCC Drugs

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**Abstract:** Hepatocellular carcinoma (HCC) is a primary tumor of the liver and one of the most malignant of tumors, with a mortality rate ranked second worldwide. Conventional therapeutics such as liver transplants and surgical resection, are of importance in HCC treatments. However, those treatments are limited and have a high frequency of tumor recurrence and metastasis. Currently, there is only one FDA approved anti-HCC drug - Sorafenib, which is well-tolerated. Thus, there is a great need to develop more effective HCC-targeting drugs. Angiogenesis, with the involvement of various angiogenic and anti-angiogenic factors, is fundamental for tumor growth, invasion and metastasis. HCC is a tumor type having a high level of neo-angiogenesis, and widely varying vascularity during tumor development. The accumulated experimental and clinical data indicate that HCC tumor progression is

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strongly associated with angiogenesis, with poor HCC prognosis related to the increase in micro-vascular density. A series of angiogenic factors have been investigated in HCC and show anti-HCC potential. Presently, there are certain anti-angiogenic therapeutics that have been evaluated in animal models and clinical trials. In this chapter, we will review the advances of HCC angiogenesis, angiogenic factors, the applications of anti-angiogenic agents, and also discuss the limitation, challenge and other potential strategies in searching for anti-HCC drugs via targeting angiogenesis.

**Keywords:** Angiogenesis, Anti-angiogenesis, Hepatocellular Carcinoma, Sorafenib, Targeting Drugs.

## 1. INTRODUCTION

With the occurrence of more than one million new cases each year, hepatocellular carcinoma (HCC) became the fifth most common tumor worldwide, and due to its high mortality, is a major global health problem [1]. HCC accounts for 85% - 90% of the primary liver cancers [2], with a 5-year overall survival rate in advanced HCC being less than 10% [3]. According to statistics, HCC caused 696,000 deaths worldwide in 2008 [4]. The occurrence of cases has distinct geographical variation, with the vast majority of cases (85%) occurring in developing countries, especially in Asia and sub-Saharan Africa. Lower rates are in Australia, Northern Europe, and the US [5]. China alone accounts for more than 50% of all cases worldwide [2] even though a decline in the incidence rate has been observed in Hong Kong and Shanghai. The main reason for this phenomenon is the high prevalence of hepatitis B virus (HBV) infection there. Most HCC cases (approximately 80%) are related to an infection of chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) [6], in which most patients also have cirrhosis [6]. Chronic liver injury at the cirrhosis stage promotes steato-hepatitis [2]. In addition, cirrhosis is present in the majority of HCC patients [7, 8].

In the prevalent areas, most HBV cases are transmitted through vertical and early horizontal transmission. Therefore, HBV vaccination given to newborns may dramatically reduce HBV infection in early life and decrease HCC cases in the future. However, the number of HCC cases increases in Europe and North America as the incidence of HCV infection and alcoholic cirrhosis rises [9, 10]. HCC mortality has nearly doubled during the period from 1975 to 1995 [9, 11]. In the coming years, a steady increase is predicted for the incidence of HCC because

of an increasing prevalence of non-alcoholic steato-hepatitis caused by obesity and metabolic syndrome [12].

Traditional HCC treatments, including resection, liver transplantation, percutaneous ethanol injection (PEI), radiofrequency ablation (RF) and transcatheter arterial chemoembolization (TACE), are only possible for HCC patients who are diagnosed with small tumor burdens [13]. As for conventional chemotherapy, there is limited efficacy for HCC patients, and currently there is no standard treatment for patients with non-resectable HCC [13, 14]. In addition, HCC prognosis is poor [15]. Patients with HCC and accompanied liver cirrhosis have a 2-year survival rate of 82%. Most patients with advanced cirrhosis have less than 32% of their 2-year survival rate [15, 16]. Therefore, there is an urgent need to develop a systemic and effective intervention therapy, especially for patients with advanced HCC. This is due to HCC being asymptomatic in the early stages and limited surveillance results in this disease eventually being diagnosed at an advanced stage. Innovative strategies, such as targeting the non-transformed, less resistant, tumor-supporting endothelial cells, are needed [17].

Currently, there are only a limited number of drugs as potential options for HCC treatment. There are several clinical trials under investigation [18]. To date, Sorafenib, a small-molecule kinase inhibitor, is the only FDA-approved drug for patients with advanced HCC. It has only modest effectiveness at prolonging patients' overall survival (OS) for 2-3 months [19]. The mechanism modulated by Sorafenib is not well known. Also, there is no unique biomarker to predict the effectiveness of Sorafenib. The tolerance and resistance seen in some HCC patients further limits the clinical efficacy of Sorafenib.

For both prevention and treatment, the strategies in recent years seemingly shift from broad disease-based treatments to specific therapies targeting aberrant cellular signaling pathways [18]. HCC is a tumor type with high levels of neo-angiogenesis and vascularity [20]. Angiogenesis is of importance for HCC progression and metastasis. Notably, the vascular vessels originate from hepatic arteries, but not the portal vein [20]. Arteriogenesis is defined as the growth of functional collateral arteries covered with smooth muscle cells from pre-existing arteries [21]. The increased arteriogenesis was seen in HCC by testing arterial

CHAPTER 5

# Bevacizumab for Glioblastoma Treatment: Reviewing Biological and Clinical Hypothesis for its Success and Failures

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**Abstract:** Glioblastoma multiforme (GBM) is the most aggressive malignant primary brain tumor in adults with a very poor prognosis. The standard treatment for newly diagnosed glioblastoma is surgical debulking followed by radiotherapy and temozolomide (TMZ) with additional maintenance with TMZ. However, this regimen offers modest benefits with a median survival of less than 15 months, with an inevitable recurrence.

GBM is one of the most vascularized tumors; therefore antiangiogenic therapeutic strategies are very appealing. Bevacizumab, a humanized monoclonal antibody against vascular endothelial growth factor A, was the first FDA approved angiogenesis inhibitor, based on impressive response rates in recurrent GBM. Recent trials have shown that the combination of bevacizumab with standard radiotherapy–TMZ for the treatment of newly diagnosed glioblastoma resulted in improved median progression-free survival, without gain in overall survival. Data regarding quality of life and functional status are conflicting. Not surprisingly, there was an increase in adverse

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events associated with bevacizumab therapy, namely thrombosis, bleeding and hypertension. Therefore, the efficacy of antiangiogenic therapy in the management of GBM remains unclear. To improve outcomes, it has been made a huge effort to better understand the biology underlying angiogenesis and tumor survival, as well as the mechanisms of antiangiogenic resistance in GBM.

**Keywords:** Angiogenesis, Antiangiogenic, Bevacizumab, Biomarkers, Central nervous system, Glioblastoma, Glioma, Quality of life, Resistance, Response, Safety, Survival, VEGF.

## 1. INTRODUCTION

Primary central nervous system (CNS) tumors constitute the leading cause of cancer-related death in the pediatric population and the fourth in middle-age men, while accounting for less than 2% of all malignancies. Gliomas are the most common CNS tumors, representing approximately 70% of all brain tumors. Astrocytomas are the most frequent gliomas and are divided into four grades of malignancy. The World Health Organization (WHO) classifies them on the basis of histologic features into four prognostic grades: grade I (pilocytic astrocytoma), grade II (diffuse astrocytoma), grade III (anaplastic astrocytoma), and grade IV (glioblastoma). Grade III and IV tumors are considered high-grade gliomas presenting increased cellularity, nuclear atypia, and mitotic activity and glioblastomas also contain areas of microvascular proliferation, necrosis, or both [1 - 3].

Glioblastoma multiforme (GBM), or grade IV astrocytoma, is the most common and aggressive malignant primary brain tumor in adults. Standard treatment consists of maximal surgical resection followed by radiotherapy (RT) plus concomitant and adjuvant TMZ [4]. However, this regimen offers modest benefits with a median overall survival (OS) of only 14–15 months [5]. When relapse or progression occurs, there is no standard treatment and OS ranges from 3 to 9 months [6]. Despite advances in conventional treatment, the outcome remains almost universally fatal. Therefore, a better understanding of glioblastoma biology and more effective therapeutic options are warranted.

Primary GBM, responsible for 90% of glioblastoma cases, typically occurs in

patients older than 50 years of age and is characterized by EGFR (epidermal growth factor receptor) amplification and mutations, loss of heterozygosity of chromosome 10q, PTEN deletion or mutations, and p16 deletion, in addition to *TERT* promoter mutations. Secondary GBM is manifested in younger patients as diffuse or anaplastic astrocytoma, which transforms over a period of several years into glioblastoma. It is characterized by mutations in the *TP53* tumor suppressor gene, overexpression of the PDGFR (platelet derived growth factor receptor), abnormalities in the p16 and retinoblastoma (Rb) pathways, loss of heterozygosity of chromosome 10q and, *IDH1* or *IDH2* mutations [1, 7 - 11].

During embryogenesis, the development of the vasculature involves the birth of new endothelial cells and their assembly into tubes (vasculogenesis) in addition to the sprouting (angiogenesis) of new vessels from existing ones. Following this morphogenesis, the normal vasculature becomes largely quiescent. In the adult, as part of physiologic processes, such as wound healing and female reproductive cycling, angiogenesis is turned on, but only transiently. In contrast, during tumor progression, an ‘‘angiogenic switch’’ is almost always activated and remains on, causing normally quiescent vasculature to continually sprout new vessels that help sustain expanding neoplastic growths [12]. This “angiogenic switch” is mediated by the release of a wide array of proangiogenic factors, mainly vascular endothelial growth factor (VEGF), and by endothelial, stromal, and tumor cells, which cause vessel growth and tumor expansion [12]. VEGF consists of a family of 5 glycoproteins named VEGFA, VEGFB, VEGFC, VEGFD, and placenta growth factor. They bind with their corresponding tyrosine kinase receptors (VEGFR1, VEGFR2, and VEGFR3), activating a downstream signal that results in the development of angiogenesis, increased vascular permeability, and lymphangiogenesis. Of the 5 glycoproteins, VEGFA plays the most important role in tumor angiogenesis, as elevated levels in patients with cancer, specifically breast, lung, colon, uterus, and ovary cancers, confer a worse prognosis [13, 14].

Given that GBM is one of the most vascularized human tumors, it is not surprising that GBM cells produce a wide variety of proangiogenic factors, including VEGF. Tumor vessels in GBMs are disorganized, highly permeable and present abnormal endothelial walls, different from normal blood vessels morphologically and functionally [15, 16]. Angiogenesis is induced by

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