1 Introduction

The present chapter aims to disclose the cancer vascularization processes, giving primary emphasis to the role of vascular endothelial growth factor (VEGF) and its receptors.

The formation of blood vessels can be driven by several mechanisms: 1) vasculogenesis, which defines the embryonic formation of blood vessels; 2) angiogenesis that names the de novo formation of blood vessels, in adults, through the proliferation of endothelial cells (ECs); 3) neovasculogenesis that defines the de novo formation of a primary vascular plexus from endothelial progenitor cells (EPCs), and 4) vascular mimicry, which consists of a process through which cancer cells can behave as ECs by establishing vascular structures (Figure 1).

The processes underlying blood vessels formation in cancer will be further depicted in this chapter, integrating the relevance of VEGF signaling whenever appropriated. Vasculogenesis will not be addressed, since it is a physiological mechanism occurring during the development of the embryo.

Nevertheless, it is important to refer that angiogenesis is the term often used to denote overall tumor neovascularization, though scientifically this is not fully correct. Angiogenesis and neovasculogenesis refer both to the formation of new vessels, but through different processes. However, in several circumstances, particularly when anti-vascularization therapy in cancer is addressed the designation of anti-angiogenesis therapy is often or almost exclusively used.

1.1 Angiogenesis

In healthy adults, the vasculature is mostly quiescent (Witmer et al., 2003). In a few situations, however, ECs may experience some proliferation: wound healing (Paavonen et al., 2000; Witmer et al., 2003), skeletal growth and the female reproductive cycle (Ferrara et al., 2003). Some pathophysiological processes may also encompass the de novo formation of new blood vessel capillaries from an existing vasculature, as result of the imbalance in the demand and supply of oxygen and nutrients (Verbridge et al., 2009; Witmer et al., 2003). These diseases include diabetic retinopathy (Witmer et al., 2003), rheumatoid arthritis (Robinson & Stringer, 2001), pathology of the female reproductive tract and cancer (Ferrara et al., 2003). The formation of new capillaries from pre-existing vessels, by their sprouting or splitting, is called angiogenesis (George et al., 2011; Kovacic et al., 2008; Risau, 1997).
Figure 1: The main processes of tumor vascularization and their intervenients. During embryogenesis the *de novo* formation of vessels occurs by the differentiation of EPC into mature ECs. This process of EPC recruitment to new vessels formation happens after birth in a process called *neo*-vasculogenesis. At the opposite, angiogenesis is characterized by the division of ECs towards pro-angiogenic stimuli. Vascular mimicry is another mechanism that involves cancer cells that mimics ECs and co-expresses some of their receptors, allowing the formation of a structure similar to vessels. Pro-angiogenic stimuli like VEGF, FGF, TGFβ, PlGF, Endostatin, Angiopoetins and their specific receptors (VEGFRs, Neuropilin, FGFR, TGFR and Tie2) play a critical role in vasculogenesis/ neovasculogenesis/ angiogenesis. Extracellular matrix is degraded by MMPs (which inhibitors are TIMPs) during the vessels formation.

The switch from the normal quiescent vasculature to angiogenesis is the result of a dynamic and tightly controlled balance between angiogenic activators and inhibitors, released predominantly by surrounding pericytes and lymphocytes, and ECs themselves, which induce a number of signal transduction systems that activate ECs (Carmeliet & Jain, 2000; Folkman, 1997; Hanahan & Folkman, 1996; Robinson & Stringer, 2001). These angiogenic factors are increasingly receiving attention, especially in the field of malignant neoplastic vascularization (Nishida et al., 2006).

Angiogenesis is a complex multi-step process, characterized by a cascade of events: firstly, vasodilatation of existing vessels and an increase of vascular permeability along with the localized degradation of the surrounding extracellular matrix (ECM), enabling further activation of ECs which proliferate and migrate to form tubes; subsequently, these cells undergo
a maturation phase and remodel into capillary structures, and a new ECM is deposited (Hanahan & Folkman, 1996; Nishida et al., 2006; Robinson & Stringer, 2001).

1.1.1 Tumor Angiogenesis

Tumor cells initially lack angiogenic ability (Folkman, 1992; Hanahan & Weinberg, 2011). In order to sustain survival and to expand in size, incipient neoplasias must develop angiogenic ability, which seems to be acquired in a discrete step during tumor development, via an "angiogenic switch" (Folkman & Hanahan, 1991; Hanahan & Folkman, 1996; Verbridge et al., 2009). Tumor vessels play an essential role in supplying nutrients, oxygen and immune cells, and also in the removal of waste products, enabling tumors to grow beyond the limitations of passive diffusion. In addition, and very importantly, newly formed vessels also afford the possibility of primary tumor to invade adjacent tissues, and circulate, through bloodstream, to distant sites, where they may form secondary tumors, known as metastases (Sporn, 1996; Kawaguchi, 200; Nishida et al., 2006). Angiogenesis represents, therefore, a crucial step in cancer progression.

As mentioned above, angiogenic switch is induced by several factors – Figure 1. Such angiogenic factors (positive regulators) include fibroblast growth factors (FGFs), thymidine phosphorylase (TP), transforming growth factor β (TGF-β), tumor necrosis factor α (TNF-α), platelet-derived growth factors (PDGFs), angiopoietins, interleukin-8 (IL-8) and vascular endothelial growth factors (VEGFs) (Ferrara et al., 2003; Nishida et al., 2006). Among these, VEGFs, particularly VEGFA (VEGF), and their receptors assume particular relevance, VEGF being the only growth factor observed almost ubiquitously at sites of angiogenesis and representing a critical rate-limiting step in physiological angiogenesis, and fundamental in pathophysiological angiogenesis as well (Robinson & Stringer, 2001; Ferrara et al. 2003; Gerber & Ferrara, 2003; Cebe-Suarez et al., 2006). In the field of neoplastic neovascularization, VEGFs and their receptors families have received increased attention.

Angiogenesis stimulation however depends also on the downregulation of angiogenic inhibitors, besides the upregulation of angiogenic activators. Such suppressors include angio- statin, endostatin, interferon, platelet factor 4, trombospondin and tissue inhibitor of metalloproteinase-1, -2 and -3 (TIMP-1, 2 and3). Levels of expression of angiogenic factors are usually correlated to the aggressiveness of tumor cells (Nishida et al., 2006).

1.2 Neovasculogenesis – Endothelial Progenitor Cells (EPCs)

Recent data have disclosed the occurrence of a new mechanism for formation of vessels in adult, different from angiogenesis, termed postnatal/adult vasculogenesis or neovasculogenesis. This mechanism differs from angiogenesis by comprising the de novo formation of a primary vascular plexus from EPCs (George et al., 2011; Kovacic et al., 2008).

The emerging evidence seems, thus, to unsettle the dogma that, for a long time, stated EPCs would contribute to vessel growth exclusively in the embryo (vasculogenesis), whereas in the adult that growth would occur only from division of differentiated ECs (angiogenesis) (Ribatti et al., 2005). Both phenomena are represented in Figure 1.

EPCs are a minor population of mononuclear non-endothelial cells capable to proliferate, migrate, and differentiate into endothelial lineage cells, but have not yet acquired characteristics of mature ECs (Fadini et al., 2008; Medina et al., 2010; Ribatti, 2007).
Asahara et al. (Asahara et al., 1997) was the first to isolate putative EPCs from human peripheral blood on the basis of cell surface expression of CD34 and VEGFR-2 markers, observing experimentally EPCs differentiation into ECs. Since then, increasing knowledge on EPCs has emerged. Although some questions persist regarding the precise panel of cell surface markers defining EPCs, the combinations of CD133+/CD34+/VEGFR-2+, CD34+/VEGFR-2+, or CD114+/CD34low are now widely used to define or select cells expressing properties attributed to EPCs (Peichev et al., 2000; Romagnani et al., 2005; Schmidt-Lucke et al., 2010).

Most circulating EPCs reside in the bone marrow in close association with hematopoietic stem cells and the stroma. EPCs circulating in the peripheral blood may correspond to cells derived from the bone marrow, not yet incorporated into the vessel wall (Ribatti et al., 2005).

Tumor vascularization seems also to be supported by the mobilization and functional incorporation of EPCs. A study focusing EPCs revealed the upregulation of tumor endothelial markers on EPCs, supporting the hypothesis of the involvement of EPCs in cancer (Bagley et al., 2008). Moreover, increased circulating levels of EPCs have been detected in cancer patients (Ahn et al., 2010; Mancuso et al., 2001; Pircher et al., 2008; Richter-Ehrenstein et al., 2007; Sakamori et al., 2012). A strong correlation has also been observed between EPCs number and tumor growth and progression in several neoplastic contexts (Gao et al., 2009; Monestiroli et al., 2001; Real et al., 2011; Shaked et al., 2005; Yu et al., 2007). Tumor secretion of VEGF was also found to be correlated with EPC mobilization (Real et al., 2011; Shaked et al., 2005; Young et al., 2002).

The recruitment of EPCs to tumors is a multistep process, involving the arrest and homing of the circulating EPCs within the vasculogenic microvasculature, transendothelial extravasation into interstitial space, extravascular formation of cellular clusters, creation of vascular sprouts and cell networks and, finally, the EPCs incorporation into a functional microvasculature (Ribatti et al., 2005). VEGF appears to be an inducing factor for these events (Aicher et al., 2003; Ferrara et al., 2003; Greenberg et al., 2008; Ribatti et al., 2005).

Interestingly, increasing evidence regarding neovasculogenesis and EPC sources has now introduced the blood mononuclear cell population as a putative intervenient, both in normal and pathological conditions. Peripheral blood mononuclear cells collected from humans were shown to be enriched in EPCs after addition of VEGF, FGF-2, insulin-like growth factor (IGF) and epidermal growth factor (EGF) to the culture medium for 7-10 days. Afterwards, these cells contributed to the formation of new vessels in ischemic limbs in mice (Kalka et al., 2000). Another study showed that EPCs isolated from peripheral blood mononuclear cell fraction, although unable to proliferate, contributed to vascularization by secreting angiogenic growth factors (Rehman et al., 2003). Monocytes cultured under angiogenic conditions also displayed an EPC phenotype with expression of specific surface markers and even formed cord-like structures (Rohde et al., 2006; Schmeisser et al., 2001). The incorporation of bone marrow-derived cells displaying characteristics of macrophages has been observed in brain vascularization (Hao et al., 2008). Moreover, tumor-associated macrophages, derived from circulating monocytes, have been shown to accumulate in hypoxic regions of tumors (Ribatti et al., 2007; Talks et al., 2000). The involvement of monocytes/macrophages in tumor blood vessels formation was observed in numerous neoplastic contexts, either directly, as EPCs, and/or indirectly by secreting angiogenic activators, such as VEGF (Barbera-Guillem et al., 2002; Bingle et al., 2006; Chen et al., 2011; Hao et al., 2008; Ribatti et al., 2007; Wang et al., 2013). Macrophage migration inhibitory factor (MIF), a cytokine implied, namely, in the recruitment of EPCs and
macrophages activation (Asare et al., 2013; Chesney et al., 1999) has been presented as an important factor in tumorigenesis, its high expression being widely associated to enhanced tumor vascularization (Bacher et al., 2003; Girard et al., 2012; Hira et al., 2005).

The rising data on EPCs seem to introduce EPCs as powerful tools for diagnostic and prognostic purposes, and, additionally, putative targets for interventive therapies.

Both angiogenesis and neovasculogenesis are known to be part of tumor vascularization. Each relative contribution might, though, vary within the various neoplastic contexts and still remains to be clarified.

1.3 Vascular Mimicry

One of the new emerging paradigms in tumor vascularization is called “vascular mimicry”, which describes the de novo formation of perfusable, matrix-rich, vasculogenic-like networks by aggressive malignant tumors – Figure 1. This phenomenon was first characterized in human melanoma where the tumor cells were shown to co-express endothelial and tumor markers (like VEGFR-2 that has been shown to play an essential role in vascular mimicry formation) and formed channels, networks, and tubular structures rich in laminin, collagens IV and VI, and heparin sulfate proteoglycans containing plasma and red blood cells. This could be a way of tumor cells to escape from immune system, which allows a faster pathway for metastasis. The vascular mimicry is nowadays described in a variety of cancers, namely sarcomas (Ewing, mesothelial, synovial, osteosarcoma, alveolar rhabdomyosarcoma); carcinomas of the breast, ovary, lung, prostate, bladder and kidney; laryngeal squamous cell carcinoma, gliomas, glioblastoma, and astrocytoma (Kirschmann et al., 2012).

Recently, epithelial-to-mesenchymal transition (EMT) has been reported to contribute to the formation of vascular mimicry and the upregulation of EMT-associated transcription factors has been demonstrated in vascular mimicry-forming tumor cells (Z. Liu et al., 2012; Sun et al., 2010).

Vascular mimicry has been, additionally, presented as one of the putative reasons underlying anti-angiogenic therapy resistance in cancer, once this therapy is directed to “bona-fide” epithelium (Dunleavey & Dudley, 2012) (see 3. Therapy anti-angiogenesis).

2 Vascular Endothelial Growth Factors (VEGFs)

The VEGF family includes placental growth factor (PGF), VEGF-A, VEGF-B, VEGF-C, VEGF-D and VEGF-E (viral homologue of VEGF-A). VEGF-A, also known as vascular permeability factor (VPF) or just VEGF, was the first to be discovered and has been studied most extensively (Ferrara, 1999; Ferrara et al., 2003).

VEGF is produced by a broad variety of cell types, including endothelial, hematopoietic and stromal cells (Bachelder et al., 2001; Bates et al., 2003; Ferrara et al., 2003; Mercurio et al., 2005; Stutfeld & Ballmer-Hofer, 2009). Most tumor types, solid and hematological (tumor cells and stroma), overexpress VEGF, being this expression directly correlated to regions of angiogenesis and high vascular density (Carmeliet & Jain, 2000; J. Chen et al., 2014; P. Chen et al., 2014; H. F. Dvorak, 2002; Ferrara, 2005; Ferrara & Davis-Smyth, 1997; Gerber & Ferrara, 2003; Obermair et al., 1997; Xu et al., 2013; Yuan et al., 2000).
VEGF is encoded by a gene located in chromosome 6p21.3, organized in eight exons, separated by seven introns. Through alternative splicing, this gene gives rise to several distinct VEGF isoforms, which differ in their expression patterns and their biochemical and biological properties (Gerber & Ferrara, 2003; Robinson & Stringer, 2001; Vincenti et al., 1996). Five human isoforms have been identified, having 121, 145, 165, 189 and 206 amino acids, respectively VEGF121, VEGF145, VEGF165, VEGF189 and VEGF206. Additionally, minor splice variants have been reported. The different isoforms vary in their ability to bind to heparin, which affects their diffusion rates and concentration gradients in relation to their secretor cell. VEGF206 exhibits the strongest binding to heparin in contrast to VEGF121, the most diffusible isoform (Ferrara et al., 1992; Gerber & Ferrara, 2003; Grunstein et al., 2000; Robinson & Stringer, 2001).

VEGF is a basic, heparin-binding, homodimeric glycoprotein of about 45 kDa. These properties correspond to those of VEGF165, the most abundant isoform (Ferrara et al., 1992; Gerber & Ferrara, 2003; Grunstein et al., 2000).

VEGF plays several roles, important under both physiological and pathophysiological conditions. VEGF is a potent mitogen for vascular ECs and also a critical factor for their proliferation and survival (Ferrara, 2001; Leung et al., 1989; Neufeld et al., 1999). During embryonic development, the disruption of a single VEGF allele lead to embryonic lethality due to impaired vessel formation and function (Carmeliet et al., 1996; Ferrara, 1996). In vitro, VEGF was shown to prevent apoptosis, by activation of phosphatidylinositol(PI)3 kinase(A)/Akt pathway (Gerber, McMurtry, et al., 1998) and induction of the expression of anti-apoptotic proteins Bcl-2 and A1 in ECs (Gerber, Dixit, et al., 1998).

VEGF is also able to induce vascular permeability (hence its alternative term VPF), which appears to be mediated by the formation of vesicular-vascular organelles, within the ECs, that ultimately fuse with the cell membrane, creating the vascular lumen (A. M. Dvorak et al., 1996; H. F. Dvorak et al., 1995; George et al., 2011; Risau, 1997; Witmer et al., 2003).

VEGF is also implicated in the degradation of ECM, required for the proliferation and migration of ECs, and finally the establishment of new tubes. That event demands the activity of matrix degrading proteases, such as matrix metalloproteinases (MMPs) and urokinase-type plasminogen activator (uPA). VEGF acts as one of the main inducers of the expression of these proteolytic enzymes (Gerber & Ferrara, 2003; Witmer et al., 2003).

The vessel formation seems to be additionally controlled by VEGF on the recruitment of bone marrow-derived cells and by increasing procoagulant activity and by recruiting pericytes (Clauss et al., 1990; Ferrara et al., 2003; Greenberg et al., 2008). VEGF is still implicated in hematopoiesis, under both normal and neoplastic conditions (Gerber & Ferrara, 2003).

Regarding VEGF role in cancer progression, some functions may be independent of neovascularization. VEGF produced by tumor cells engages VEGF receptors on tumor cells (autocrine signaling), initiating a signaling response that promotes survival, and, thus, providing tumor cells with a degree of self-sufficiency, and, ultimately facilitating their ability to migrate and metastasize (Bachelder et al., 2001; Mercurio et al., 2005) (see “2.3. VEGF autocrine signaling”). Studies related this self-sufficiency to the activation of P13-kinase (P13K) pathway (Bachelder et al., 2001; Mercurio et al., 2005). This self-sufficiency seemed to rely, additionally, on the capacity of VEGF to mitigate apoptosis, that being mostly induced by the expression of the anti-apoptotic protein Bcl-2 (Mercurio et al., 2005; Pidgeon et al., 2001). Moreover, migration and invasion of tumor cells have also been related to VEGF signaling, likely due to regulation of the expression of C-X-C chemokine receptor type 4 (CXCR4) by VEGF (Bachelder et al., 2002).
2.1 Regulation Mechanisms of VEGF Expression

The molecular mechanisms governing VEGF expression in normoxia by extracellular mediators are poorly understood. A vast body of literature has demonstrated that, in hypoxia, hypoxia-inducible factor-1 (HIF-1) is a key regulator responsible for the induction of genes that facilitate adaptation and survival of tumor cells (Semenza, 2003). As a heterodimeric complex, HIF-1 consists of a hypoxia inducible subunit HIF-1α and a constitutively expressed subunit HIF-1β. The overexpression of HIF-1α was found in various types of human and mouse cancers (Semenza, 2003). Song et al. (Song et al., 2009) showed, in ovarian cancer cells, that lysophosphatidic acid (LPA), a natural phospholipid that is a ligand for protein G coupled receptors, induces VEGF expression through c-Myc and Sp-1 transcription factors independently of HIF-1α. This mechanism can involve VEGF receptor-2 (VEGFR-2) since regulation of VEGFR-2 signal transduction also occurs through the trimeric G proteins Gaq/Ga11, which was shown to interact with VEGFR-2 in vitro (Shibuya, 2013). It was previously demonstrated, in glioblastoma, the implication of IL-6 in the regulation of VEGF expression, again through Sp1 transcription factor as a partner of signal transducer and activator of transcription 3 (STAT3) (Loeffler et al., 2005). Sp1 and STAT3, together with HIF-1α, are also involved in the expression of VEGF when the epidermal growth factor receptor (EGFR) is stimulated by the proteoglycan decorin in mouse brain ECs (Santra et al., 2008). In mouse embryonic fibroblasts NIH3T3 cell line, it was shown that PDGF (platelet-derived growth factor) pathway was important to activate VEGF expression mediated by Sp1 and Sp3 that, in these cells, are constitutively bound to VEGF promoter (Finkenzeller et al., 1997).

The expression of a constitutively active mutated form of PI3-kinase in ovarian cancer cells results in increased expression of VEGF. PKC zeta, which is the major kinase downstream of PI3-kinase, regulates VEGF expression in a phosphoinositide-dependent kinase-1 (PDK-1)-dependent manner. The inducible expression of Sp1 mutated in the two phosphorylation sites compromises VEGF expression in response to extracellular signal-regulated kinases (ERK) stimulation. This finding strongly establishes that ERK phosphorylation of Sp1 is a major determinant in VEGF expression in response to RAS activation. Sp3 is also an ubiquitous factor that is highly homologous to Sp1. Under hypoxic conditions, the expression of Sp3 decreases, whereas Sp1 is unchanged. A concomitant induction of transcription is observed in the absence of HIF-1. In this case, Sp1/Sp3 ratio represents a putative HIF-1α independent regulatory mechanism in hypoxia (Pages & Pouyssegur, 2005).

In gastric cancer, the overexpression of FoxM1b directly and significantly correlates with transactivation of VEGF expression and increased angiogenesis (Li et al., 2009).

Recent studies have indicated that cells undergoing insufficient oxygen and nutrients supply experience endoplasmic reticulum (ER) stress. ER needs energy and oxygen for the protein folding process, thus nutrient depletion and hypoxia caused by insufficient blood supply lead to inefficient protein folding and ER stress in cells, especially cells that grow and spread rapidly (Adelman et al., 2000; Drogat et al., 2007; Genbacev et al., 1997; Koumenis et al., 2007; Romero-Ramirez et al., 2004). UPR (unfolded protein response), IRE1a (Serine/threonine-protein kinase/endoribonuclease IRE1a), PERK (Proline-rich receptor-like protein kinase) and ATF6a (Activating transcription factor 6 alpha) mediate transcriptional regulation of VEGF under ER stress, during normal development of trophoblast cells in the placenta as well as in cancer cells (Ghosh et al., 2010). In ovary, carbon and energy metabolism also
plays a role in the regulation of VEGF:VEGFR-2 autocrine loop. Some studies showed that glucose can increase the levels of VEGF and consequently potentiate the autocrine loop, whereas glucose depletion decreases VEGF levels and increases VEGFR-2 degradation with concomitant increase of VEGFR-2 mRNA synthesis (Adham & Coomber, 2009). This study suggests that initiation and/or progression of ovarian surface epithelial cells towards a neoplastic phenotype might be modulated by dietary conditions.

More recently, studies have been published revealing the importance of micro-RNAs (miRs) in the regulation of angiogenesis. Lei et al. (Lei et al., 2009) and Cascio et al. (Cascio et al., 2010) have shown a molecular mechanism of regulation of HIF-1α and VEGF, involving miR-20b through which tumor cells are able to adapt to different oxygen concentrations. This way, HIF-1α is capable of regulating VEGF expression before and after transcription, since the expression of miR-20b itself is regulated by HIF-1α. The downregulation of VEGF mRNA by miR-20b under a hypoxic environment was associated with reduced levels of nuclear HIF-1α subunit and STAT3. STAT3 is necessary for CoCl2-mediated HIF-1α nuclear accumulation and recruitment on VEGF promoter. MiR-126 is also downregulated under hypoxic conditions and may halt the hypoxia-induced neovascularization by suspending cell cycle progression and inhibiting the expression of VEGF and MMP-9 (Ye et al., 2014).

It was shown that miR-126 is complementary to VEGF 3’ UTR (untranslated region) and this interaction may inhibit the overexpression of VEGF in tumor cells both in vitro and in vivo (B. Liu et al., 2009). More recently, it has been shown that this miR induces angiogenesis by activating VEGF in patients with oral cancer (Sasahira et al., 2012).

In bone marrow ECs, miR-363-5p was the first miR identified. It regulates (directly and indirectly) the expression and availability of angiocrine and hematopoietic factors. Reduced miR-363-5p in ECs promotes hematopoietic precursors adhesion and expansion (Costa et al., 2013).

### 2.2 VEGF Receptors

The biological functions of VEGF are mediated upon binding to tyrosine kinase receptors (TKRs) VEGFR-1 (FLT-1), VEGFR-2 (kinase domain region (KDR)/Flk-1) (Cebe-Suarez et al., 2006; Ferrara et al., 2003; Vieira et al., 2010).

Similar to other TKRs, signaling by VEGFRs is initiated upon binding of a covalently linked ligand dimer to the extracellular receptor domain of the TKR. This interaction promotes receptor homo and heterodimerization followed by phosphorylation of specific tyrosine residues located in the intracellular juxtamembrane domain, the kinase insert domain, and the carboxyterminal tail of the receptor. A variety of signaling molecules is then recruited to VEGFR dimers giving rise to the assembly of large molecular complexes that activate different cellular pathways (Stuttfeld & Ballmer-Hofer, 2009). Some autophosphorylation sites have also been identified in both VEGF TKRs (Shibuya, 2006).

VEGF binding sites were initially identified on the cell surface of ECs. VEGF receptors expression is now recognized to be wider, occurring also on bone marrow-derived cells (Ferrara et al., 2003), including hematopoietic stem cells (Peichev et al., 2000) and circulating EPCs (Eichmann et al., 1997), pericytes (Witmer et al., 2002), dendritic cells (Gabrilovich et al., 1996), monocytes (Barleon et al., 1996; Rohde et al., 2006; Sawano et al., 2001; Schmeisser et al., 2001), retinal progenitor cells (Suzuma et al., 1998) and several types of tumor cells (Bates et al., 2003; Dias et al., 2001; Santos & Dias, 2004; von Marschall et al., 2000).
VEGFR-1 and VEGFR-2 are 180 and 200 kDa glycoproteins, respectively (Cebe-Suarez et al., 2006; Ferrara et al., 2003; Vieira et al., 2010). These TKRs comprise seven immunoglobulin-like domains in the extracellular domain, a single transmembrane region and a conserved intracellular tyrosine kinase sequence interrupted by a kinase-insert domain (Ferrara et al., 2003; Shibuya et al., 1990; Terman et al., 1991).

Although both are high affinity receptors for VEGF, VEGF binds with higher affinity to VEGFR-1 than to VEGFR-2 (Cebe-Suarez et al., 2006; Ferrara et al., 2003; Shibuya & Claesson-Welsh, 2006; Vieira et al., 2010). Another interesting feature of the two TKRs is their difference in tyrosine kinase activity in response to ligand binding: VEGFR-2 is a stronger kinase (Shibuya & Claesson-Welsh, 2006; Waltenberger et al., 1994). These distinct molecular features may explain their different biological activities (Shibuya & Claesson-Welsh, 2006).

VEGFR-2 is considered to be the predominant VEGF receptor in mediating functional VEGF signaling in ECs, including mitogenesis, cytoskeleton organization and cell migration, survival, and vascular permeability (Cebe-Suarez et al., 2006; Shibuya & Claesson-Welsh, 2006). VEGFR-2 is also implicated in playing a distinct role in the activation of PI3K-Akt pathway, a crucial signaling pathway in the process leading to EC survival induced by VEGF (Gerber, McMurtry, et al., 1998; Witmer et al., 2003). VEGFR-2 is still involved in hematopoiesis (Gerber & Ferrara, 2003). These roles were supported by experiments in which, in VEGFR-2 knockout mice, both hematopoiesis and vasculogenesis were blocked (Shalaby et al., 1995).

VEGFR-1 function in mediating effective biological responses in ECs is more elusive. In fact, in ECs, it may function as a negative regulator of VEGFR-2, at least during embryogenesis, serving a “decoy” purpose, by sequestering VEGF and thus rendering it less available to bind to VEGFR-2 (Ferrara et al., 2003; Shibuya & Claesson-Welsh, 2006), as suggested by studies in which VEGF165 mice displayed overgrowth of ECs and disorganized vascular channels (Fong et al., 1995). Moreover, VEGFR-1 signaling may also be involved in hematopoiesis and EPCs recruitment (Clauss et al., 1996; Hattori et al., 2002).

Furthermore, synergism between the two VEGFR receptors, VEGFR-1 and VEGFR-2, enabling the modulation of a variety of VEGFR-dependent signals has been demonstrated (Carmeliet et al., 2001).

VEGF-induced biological signaling is still influenced by the interaction with coreceptors, as neuropilins (NRP-1 and -2) and heparan sulphate proteoglycans (HSPGs), integrins and cadherins.

Neuropilins, broadly expressed transmembrane molecules, traditionally known to be involved in axonal guidance, have been identified as VEGF-binding proteins and part of the VEGF-VEGFR signaling complex (Ellis, 2006; Robinson & Stringer, 2001; Vieira et al., 2010).

These receptors recognize the exon 7-encoded domain of VEGF, binding, therefore, mostly to VEGF165 but not to VEGF121, which misses that domain (Ellis, 2006; Soker et al., 1996; Vieira et al., 2010). The lack of this association has been hypothesized to be related to the lesser potency of this isoform in comparison to VEGF165 (Ellis, 2006; Rollin et al., 2004).

NRP-1 and NRP-2 are respectively 120 to 130 kDa non-tyrosine kinase receptors that share an identical structure, containing both a large N-terminal extracellular domain, a short membrane spanning domain and a small cytoplasmic domain (Ellis, 2006; Soker et al., 1996; Vieira et al., 2010). Their wide distribution includes, besides the developing nervous system, ECs and tumor cells (Cebe-Suarez et al., 2006; Ellis, 2006; Shibuya & Claesson-Welsh, 2006).
NRP-1 and NRP-2 role in mediating VEGF signaling seems to occur mainly through association with VEGFR-2 and VEGFR-1, respectively, stimulating signaling through these TKRs (Cebe-Suarez et al., 2006).

HSPGs are abundant and highly conserved components in the cell surface and ECM, playing an important function in the formation and modulation of gradients of heparin-binding growth factors, as VEGF, and they have been shown to be implicated as modulators of VEGF signaling, by interaction with VEGFR-1 and VEGFR-2 (Bernfield et al., 1992; Vieira et al., 2010). Besides, these molecules are still involved in the restoration of function of damaged VEGF, prolonging its biological activity (Gitay-Goren et al., 1996; Robinson & Stringer, 2001).

Other potential co-receptors for VEGFRs include integrins, as αβ1 and αβ2, and cell adhesion molecules, such as vascular endothelial (VE)-cadherin (Cebe-Suarez et al., 2006; Shibuya, 2006; Stupack & Cheresh, 2004).

2.2.1 Regulation of VEGFR-1 Expression

VEGFR-1 mRNA and protein are specifically found in most of the vascular ECs. As an exception, VEGFR-1 mRNA is also found in human peripheral blood activated monocytes, which suggests a role in vascularization of monocyte/macrophage in vivo (Barleon et al., 1996).

Isotope-labeled VEGF assays detect its binding sites in most of the blood vessels from embryo to adult stages. These experiments showed the higher affinity of VEGFR-1 for VEGF with more than half VEGF-binding sites being associated with VEGFR-1 (Jakeman et al., 1992). However, the kinase activity of VEGFR-1 is one-tenth lower than VEGFR-2. VEGF-1 gene produces two proteins, a full length receptor and a soluble form (sVEGFR-1). These facts suggest that VEGFR-1 may negatively regulate angiogenesis under certain conditions (Shibuya, 2001, 2013). VEGFR-2 knockout mice exhibit a lethal phenotype due to the absence of vasculogenesis, whereas VEGFR-1 knockout mice die at the same age (E8.5-9.0) due to the overgrowth of ECs and their disorganization in blood vessels (Fong et al., 1995). This indicates that during embryogenesis VEGFR-2 is a positive signal transducer and VEGFR-1 is a suppressor of VEGFR-2 signaling. There are two possible reasons: VEGFR-1 tyrosine kinase (TK) generates a negative signal against angiogenesis or the ligand-binding site of VEGFR-1 blocks VEGF activity by trapping VEGF. A VEGFR-1-TK mouse was generated by Shibuya et al. (Shibuya, 2013) with a normal vascular growth, which demonstrates that VEGFR-1 negatively regulates vasculogenesis during early embryogenesis by trapping VEGF and decreasing provasculogenic signals from VEGFR-2.

The gene regulation of VEGFR-1 is dependent on an Ets-binding motif and on an upstream CRE/ATF-binding motif (Wakiya et al., 1996). In addition, it was reported a hypoxia-response element which may be responsible for the upregulation of VEGFR-1 under hypoxic conditions (Gerber et al., 1997). Zhang et al. (Zhang et al., 2010) observed that VEGF induces potent activation of the JNK-c-Jun pathway and that JNK activity is associated with ubiquitination of VEGFR-2. At the opposite, inhibition of the ubiquitin or proteasome activity is sufficient to enhance the expression of VEGFR-1 in primary ECs. These findings suggest that ECs are continuously producing VEGFR-1 and that ubiquitin–proteasome activity is necessary to maintain its homeostatic levels. Interestingly, the regulation of VEGFR-1 protein levels is dependent on Akt and ERK1/2 phosphorylation and, since these kinases typically inhibit the degradation of proteins by the ubiquitin–proteasome system, they postulate that VEGF induces phosphorylation of Akt and ERK1/2, which in turn prevents degradation of VEGFR-1 by
the ubiquitin–proteasome system. Collectively, these data suggest that VEGF orchestrates an intricate process mediated by the Akt/ERK and JNK/c-Jun that protects VEGFR-1 while VEGFR-2 is degraded, leading to rapid reversal of the protein levels of these two receptors (Zhang et al., 2010).

2.2.2 Regulation of VEGFR-2 Expression

VEGFR-2 gene promoter does not have a TATA box region, but has several DNA binding sites for general and tissue-specific transcription factors. This TATA-less gene contains four upstream Sp1 sites and a single transcription start site that binds multi-functional transcription factor TFII-I for gene expression (Guo et al., 2010). In large vessel ECs TFII-I is a transcription factor that regulates VEGFR-2 expression (T. A. Jackson et al., 2005) and, despite TFII-I deletions being associated with cardiovascular defects (Lucena et al., 2010), its function in angiogenesis remains unknown. Sp1-dependent DNA binding within −77 and −60 region of VEGFR-2 promoter seems to be essential for the regulation of both mRNA and protein in human ECs by Rac-1 (a small Rho-GTPase) (Guo et al., 2010).

GATA2 is another transcription factor that regulates the activity of VEGFR-2 promoter (Minami et al., 2004; Minami et al., 2001) and more recently Mammoto et al. (Mammoto et al., 2009) showed that capillary formation in vitro and in vivo is modulated by p190RhoGAP, a Rho GTPase inhibitor that controls the levels TFII-I and GATA2 antagonistic transcription factors.

Epigenetic modulation of transcription factors has been reported upon VEGF signaling; namely the binding of E2F1 to VEGFR-2, VEGFR-1 and angiopoetin-2 promoters is increased by VEGF stimulation (Pillai et al., 2010) concomitantly with the acetylation of histones and E2F1. Promoter methylation is also described in VEGFR-2 promoter in cancer cell lines from stomach, colon and liver (J. Y. Kim et al., 2009).

In Human Umbilical Vein ECs (HUVECs), NRP-1 si-RNA knockdown causes a marked decrease in VEGFR-2 protein levels and this change is independent of VEGFR-2 mRNA expression. Holmes et al. (Holmes & Zachary, 2008) concluded from these findings that NRP-1 is important for the stability of VEGFR-2 in HUVECs, the loss of NRP-1 enhances degradation of VEGFR-2 both under basal conditions and following activation of VEGFR-2 by VEGF.

Chronic hypoxia is a cause for specific downregulation of VEGFR-2 in human coronary artery endothelial (HCAE) cells due to the decrease of VEGF stimulation. The VEGF signaling seems to be affected upstream of eNOS (endothelial nitric oxide synthase) phosphorylation (Olszewska-Pazdrak et al., 2009). Notch pathway is also responsible for downregulating VEGFR-2 expression in ECs mainly due to the activation of the expression of the Hesr-1 that is a negative regulator of transcription that targets VEGFR-2 (Taylor et al., 2002).

Delta4-Notch signaling has been shown to significantly decrease the expression of VEGFR-2 thus inhibiting the proliferation of angiogenic cells. These two signals have opposite effects, meaning that if the concentration of one signal increases the other will decrease proportionally (Guo et al., 2010).

2.2.3 Regulation of Neuropilins Expression

Neuropilins (NRPs) are mediators of neuronal guidance and angiogenesis. They are expressed in most adult tissues, including in growing blood vessels, for example on the ECs of capillaries, arteries and veins in the postnatal mouse retina and tumor cells (Soker et al., 1998). The extra-
cellular NRP-1 domain has distinct VEGF<sub>165</sub> and semaphoring binding domains to which these two ligands bind non-competitively. VEGF<sub>165</sub> is the VEGF-A isoform with the strongest affinity for NRP-1.

NRP-1 and VEGFR-2 were 50–60% colocalized even in quiescent mouse primary ECs and remained 30 min after stimulation by VEGF-A<sub>165</sub> (Salikhova et al., 2008). The dependence of the NRP-1-VEGFR-2 association on the binding of the PDZ (Post synaptic density protein (PSD95), Drosophila disc large tumor suppressor (Dlg1), and Zonula occludens-1 protein (Zo-1)) adaptor protein synectin suggests that the carboxy-terminus of NRP-1 is required for this association. Since VEGFR-2 is not known to bind synectin, it is not clear why synectin would be required for the association. Lanahan et al. (Lanahan et al., 2010) suggests that APPL (β amyloid protein precursor-like) binds VEGFR-2, so NRP-1 and APPL-bound synectin dimers could cross link the two receptors, thus explaining the dependence of the NRP-1-VEGFR-2 association on synectin.

Several studies suggest that NRP-1 signals are independent of VEGFRs. The contribution of NRP-1 to VEGF signaling would be mediated by the cytoplasmic domain. Since the carboxy-terminus of the same domain is used by synectin to cross-link NRP-1 to myosin VI, the molecular motor that drives NRP-1 trafficking, it is likely that NRP 1-dependent signaling is tightly coupled to NRP-1 trafficking (Horowitz & Seerapu, 2012).

In vivo studies showed that neither semaphoring-3A (SEMA3A) nor semaphorin signaling through NRP-1 and NRP-2 are essential for embryonic vasculogenesis in the mouse (Gu et al., 2003; Vieira et al., 2007). SEMA3A has been reported to also affect pathophysiological angiogenesis in mice. For example, SEMA3A prevents vascular regeneration in a mouse model of oxygen-induced retinopathy and inhibits tumor angiogenesis by eliciting EC apoptosis and normalizing the pericyte coverage of tumor vessels (Fukushima et al., 2011). SEMA3A-induced tumor vessel normalization might be indirectly caused by SEMA3A-mediated recruitment of a subset of NRP-1-expressing monocytes that secrete several factors involved in vessel maturation (Carrer et al., 2012).

### 2.3 VEGF Autocrine Signaling

The complex tumor microenvironment implies that cancer cells receive signals from multiple sources, which, conversely, will influence the function of other cells. This describes a paracrine signaling. However, it has become more evident the acquisition by cancer cells of a certain degree of self-sufficiency – autocrine signaling – that will favor tumor progression (Hanahan & Weinberg, 2011). In such autocrine signaling, the secreted growth factors may act on surrounding cells from same type of producing cells as well as on the secretor cells themselves. These autocrine loops may then provide a growth advantage to tumor cells, under limiting conditions (Gerber & Ferrara, 2003).

Furthermore, there is still evident that, in certain conditions, signaling pathways, such as VEGF pathway, may be transduced even without the factor secretion – internal autocrine/intracrine signaling (Gerber & Ferrara, 2003; Gerber et al., 2002).

Such profitable autocrine signaling loops have been described in both hematological and solid neoplastic contexts. Understanding the effector functions of VEGF in malignant neoplasias may be of great utility for the development of new therapeutic strategies.

In hematological malignancies, the role of VEGF/VEGFR autocrine loops is well established and coexpression of VEGF with its receptors is frequently found.
In acute myeloid primary leukemias and cell lines, previous studies have demonstrated that VEGF:VEGFR-2 autocrine loop operates both internally and externally. It was demonstrated that VEGFR-2 is constitutively phosphorylated and located in the nucleus of VEGF-producing leukemia cells. Treatment with anti-VEGF antibody blocked VEGFR-2 nuclear translocation and inhibited the NFKB pathway (Santos & Dias, 2004). In vivo blocking of VEGFR-2 induced long-term remission of xenotransplanted human leukaemia (Dias et al., 2001). In B-cell non-Hodgkin’s lymphomas the overexpression of VEGF and phosphorylated VEGFR-2 has been reported and correlates with expression of hypoxia inducible factor 1α (HIF1α) (Giatromanolaki et al., 2008), the main known regulator of VEGF expression as it will be explored below. In infantile hemangioma, cell survival seems to be related to low levels of VEGFR-1 that promotes a constitutive VEGF-dependent activation of VEGFR-2 and downstream pathways (Jinnin et al., 2008). In patients with multiple myeloma it was shown that bone marrow ECs (MMEC) highly expressed VEGF and VEGFR-2 at both mRNA and protein level. MMEC showed constitutive autophosphorylation in both VEGFR-2 and ERK2. Auto-phosphorylation, proliferation and capillarogenesis were prevented by a neutralizing antibody against VEGF and VEGFR-2. These findings suggested the existence of an autocrine loop of VEGF in MMEC (Ria et al., 2004). In other study focusing hematological malignancies, VEGFR-1 expression was detected in the cytoplasm and nuclei of proliferating multiple myeloma cells. The inhibition of this receptor abrogated those cells proliferation and motility. The results suggested the contribution of an intracrine VEGF:VEGFR-1 signaling to multiple myeloma cells growth (Vincent et al., 2005).

In solid tumours the role of VEGF:VEGFR autocrine loop is less clear. In breast cancer, Weigand and co-workers (Weigand et al., 2005) detected VEGF levels above the range of biological activity in cell lines and primary culture media and they also verified that, in some cases, VEGFR-2 expressed on cell surface was phosphorylated, indicating its activated state. Conversely, other authors stated that in invasive breast cancer VEGFR-2 was not expressed and the VEGF receptor responsible for the autocrine loop is NRP-1 (Bachelder et al., 2001; Bachelder et al., 2002; Mercurio et al., 2005). Intracrine signaling was also detected in human breast carcinoma cells through VEGF binding to VEGFR-1 (Lee et al., 2007).

In colon cancer, a concomitant overexpression of VEGF and VEGFR-1, but not VEGFR-2, was described by Bates et al. (Bates et al., 2003). The same authors showed, by disturbing VEGFR-1 function with VEGFR-1 blocking antibody and dominant-negative assays, that VEGFR-1 establishes an autocrine loop with VEGF in order to promote cell survival. In contrast, in another study, in vitro and in vivo colon cancer models displayed overexpression of both VEGFR-2 and VEGF in cells with an EMT phenotype and, by blocking VEGFR-2 in vivo, it was observed that subcutaneous xenograft colon tumors were dramatically smaller (Serpa et al., 2010). Intracrine VEGF:VEGFR-2 signaling in survival and chemoresistance of human colorectal cancer cells was also observed (Samuel et al., 2011). Evidence for an autocrine VEGF mitogenic loop was also pointed out in pancreatic cancer, although in some cases the VEGF receptor involved is VEGFR-2 whereas in other situations it is VEGFR-1 (von Marschall et al., 2000).

In prostate cancer, Jackson et al. (M. W. Jackson et al., 2002) published that stimulation of LNCaP cells with VEGF165 induces DNA synthesis and recruits quiescent cells into the S-phase of the cell cycle via signaling through VEGFR-2. These findings suggest that VEGF may regulate both angiogenesis and tumor cell growth via autocrine and/or paracrine mechanisms in
prostate cancer. Before that, other authors described a functional autocrine VEGF loop for cell survival but in this case signaling was performed through VEGFR-1 (Soker et al., 2001). In ovarian carcinoma, an autocrine VEGF:VEGFR-2 loop was described as developing a protective role on cells from anoikis and in ascites formation (Boocock et al., 1995; Sher et al., 2009).

Tumor VEGF:VEGFR-2 autocrine loop signaling was shown to trigger angiogenesis in lung cancer (Chatterjee et al., 2013).

3 Therapy Anti-angiogenesis

In 1971, Judah Folkman (Folkman et al., 1971) was the first to postulate that the anti-angiogenic therapy against tumor growth could be clinically relevant. Since then, many studies lead to the development of highly specific therapies with anti-angiogenic factors and their tyrosine kinase receptors as targets.

Anti-angiogenic drugs can block angiogenesis, inhibit recruitment of proangiogenic bone marrow–derived cells, induce vessel regression, and promote sensitization to radio- and chemotherapy (Welti et al., 2013). One of the most well established anti-angiogenic therapies is the use of VEGF inhibitors. Anti-VEGF, as the main anti-angiogenic molecule that limits tumor growth, has an essential role in pathophysiological and physiological angiogenesis (Ferrara, 2009). A lot of preclinical studies have demonstrated modest tumor-suppression effects in different types of cancers. VEGF and its receptors inhibitors-based therapies prolong progression-free survival and overall survival in a fraction of cancer patients – Table 1.

Bevacizumab is an anti-VEGF recombinant monoclonal antibody that blocks the link between all isoforms of VEGF and its receptors and it is the most used anti-angiogenic therapy. The end result is the blocking of new blood vessels formation and the subsequent nutrients supply will stop tumor growth. Nowadays, this drug is used in colorectal, kidney, non-small cell lung cancer and certain brain tumors (Ferrara, 2005; K. J. Kim et al., 1993). Almost at the same time, pegaptanib which is an aptamer that blocks 165 aminoacid-VEGFA isoform, was approved for the treatment of the wet form of age-related macular degeneration, with great success (Gragoudas et al., 2004). Other good examples of success using anti-VEGF therapy include the treatment of psoriatic mice, which lead to a reduction in the severity of the disease, and the treatment of hereditary hemorrhagic telangiectasia (a disease characterized by widespread hemangiomas formation) with thalidomide, which has shown to normalize vessels and reduce episodes of epistaxis (Schonthaler et al., 2009).

Multi-targeted tyrosine kinase inhibitors as imatinib, sorafenib, sunitinib or pazopanib, which block signaling pathways such as VEGF pathway, were approved for clinical use in various types of cancers including metastatic non-small cell lung cancer, metastatic breast cancer, recurrent glioblastoma, metastatic renal cell carcinoma and melanoma. A number of studies have reported their therapeutic efficacy.

Unfortunately, clinical trials of anti-VEGF monotherapy in patients with solid tumors have been disappointing. But the combination of anti-VEGF therapy with conventional chemotherapy has improved survival in cancer patients compared with chemotherapy alone. These opposite results could be explained by a normalization of tumor neovasculature by anti-VEGF therapy. Preclinical studies have shown that anti-VEGF therapy changes tumor vasculature towards a more mature/normal phenotype (Goel et al., 2011). So, several questions need to be
<table>
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<tr>
<th>Mode of action</th>
<th>Type of cancers</th>
<th>PFS</th>
<th>Limitations</th>
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<tr>
<td>Bevacizumab</td>
<td>Monoclonal anti-VEGF antibody</td>
<td>Colorectal, breast, ovarian, non-small cell lung, renal, glioblastoma, pancreatic, prostate</td>
<td>Most of studies with improvements</td>
<td>No OS improvement</td>
</tr>
<tr>
<td>Aflibercept</td>
<td>Chimeric VEGF/PIGF neutralizing receptor</td>
<td>Colorectal, pancreatic, non-small cell lung, melanoma</td>
<td>Improvements in colorectal, non-small cell lung and melanoma</td>
<td>Small or no OS improvement</td>
</tr>
<tr>
<td>Sorafenib</td>
<td>VEGFR Tyrosine Kinase Inhibitor (TKI)</td>
<td>Renal, Hepatocellular, melanoma, small-cell lung</td>
<td>Improvements in renal and hepatocellular</td>
<td>No improvements in melanoma and small-cell lung</td>
</tr>
<tr>
<td>Sunitinib</td>
<td>VEGFR TKI</td>
<td>Renal, stromal gastrointestinal, breast, hepatocellular, colorectal, pancreatic neuroendocrine, non-small cell lung, prostate, melanoma</td>
<td>Improvements in most studies</td>
<td>No OS improvements in the majority of studies</td>
</tr>
<tr>
<td>Pazopanib</td>
<td>VEGFR TKI</td>
<td>Renal, non-small cell lung, soft tissue sarcoma</td>
<td>Improvements</td>
<td>No OS improvements</td>
</tr>
<tr>
<td>Vandetanib</td>
<td>VEGFR TKI</td>
<td>Non-small cell lung, medullary thyroid</td>
<td>Improvements in medullary thyroid cancer</td>
<td>No OS improvements</td>
</tr>
<tr>
<td>Axitinib</td>
<td>VEGFR TKI</td>
<td>Renal, pancreatic</td>
<td>Improvements in renal cancer</td>
<td>No OS</td>
</tr>
<tr>
<td>Ramucirumab</td>
<td>VEGFR TKI</td>
<td>Gastric, non-small cell lung, melanoma</td>
<td>Improvements in most studies</td>
<td>Little or no OS improvements</td>
</tr>
</tbody>
</table>

Table 1: Anti-angiogenic drugs used in therapy against cancer: mode of action, type of cancer where it is used, progression free survival (PFS) statistics and associated limitations. OS (overall survival). Table adapted from Welti, 2013 (Welti et al., 2013).
answered, namely why the preclinical models showed a good efficacy of most anti-angiogenic drugs but the clinical data has a modest response, showing an increase in the overall survival just in a few months? A possible explanation could be that several proangiogenic molecules become upregulated under selective pressure by these drug inhibitors, which can explain the poor results in overall survival.

Overall, the evidence demonstrates that antiangiogenic therapy has remarkable therapeutic effects in various types of human cancers. However, the molecular bases of cancer type-dependent resistance mechanisms against VEGF blockade, especially VEGF-independent proangiogenic mechanisms, need to be clarified. Targeting these mechanisms would enhance the effects and minimize the required doses of VEGF blockers. There is no doubt that further efforts in this area will yield opportunities to greatly improve anti-angiogenic treatment (Kubota, 2012). Some promising recent studies demonstrate that the combination of antiangiogenic therapies and anti-inflammatory or immunotherapy could improve the overall response. Some of those candidates are IL-17 and a subset of T lymphocytes (cytokine-induced killer cells) that showed an improvement of efficacy in combination with anti-angiogenic drugs (Shi et al., 2013; Wong, 2013).

4 Final Remarks

Overall, the present chapter was dedicated to tumor vascularization aiming to show its complexity and explaining the central role of VEGF and its receptors in this phenomenon.

The formation of blood vessels in a tumor comprises a stepwise sequence of processes accounting for the supply of signaling factors, nutrients and oxygen to sustain tumor growth. Rationally, strategies affecting tumor vessels would deadly disturb cancer cells, culminating in the regression and/or abrogation of disease. However, the therapeutic anti-angiogenesis approaches used so far did not overcome as expected, showing once again that the dynamic network involved in vessels formation into and within a malignant neoplasia is far from being completely understood.

References


