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Review

Vascular Endothelial Growth Factor: An Overview Across Multiple Disease Conditions

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Abstract: Vascular Endothelial Growth Factor (VEGF) is the major player in the regulation of physiological angiogenesis as well as it has also been implicated in pathological angiogenesis, associated with cancers and other conditions, among which psoriasis, autoimmune diseases and visual loss in macular degeneration. Interestingly, three regulatory Single Nucleotide Polymorphisms (rSNPs) in the promoter region of VEGF-A gene have been significantly associated with different human diseases and it is possible that, in the near future, the cumulative effect of several high-risk Single Nucleotide Polymorphisms (SNPs) may prove useful in a clinical setting. Currently, new VEGF inhibitors are undergoing clinical testing in various disease conditions, given that VEGF inhibition has also been contemplated as a possible strategy for prevention of angiogenesis and vascular leakage to decrease inflammation. This review focuses mainly on the role of Vascular Endothelial Growth Factor in several pathological contexts, highlighting the emerging association of the most common VEGF polymorphisms with disease risk. An update on the therapeutic implications of VEGF has also been documented.

Keywords: Vascular Endothelial Growth Factor (VEGF), Angiogenesis, Inflammation, VEGF Polymorphisms, Regulatory Single Nucleotide Polymorphisms (rSNPs), Single Nucleotide Polymorphisms (SNPs), VEGF Inhibitors

Introduction

For over a decade, the role of Vascular Endothelial Growth Factor (VEGF) in the regulation of angiogenesis was the object of intense investigation (Ferrara *et al*., 2003b). Recent evidence indicates that new vessels growth and maturation are highly complex and coordinated processes, requiring the sequential activation of a series of receptors by numerous ligands (Ferrara, 2002) and VEGF signaling often represents a critical rate-limiting step in physiological angiogenesis (Ferrara, 2004). However, more recently, vascular endothelial growth factors have been shown to also play a role in atherosclerosis, arteriogenesis, cerebral edema, neuroprotection, neurogenesis, post-ischemic brain and vessel repair and to mediate the effects of transplanted stem cells in experimental stroke (Lee *et al*., 2007; Greenberg and Jin, 2013).

Angiogenesis, the formation of new blood vessels from a pre-existing vascular bed, is a common component in physiological conditions as well as in several pathogenic mechanisms including cancer (Ferrara, 2002), inflammatory joint disease (Brenchley, 2000) and psoriasis (Detmar *et al*., 1994). VEGF is well established as a primary mediator of pathological angiogenesis (Ferrara and Davis-Smyth, 1997; Ferrara, 2004); the first cytokine identified within the VEGF family was originally referred to as vascular permeability factor (Crawshaw *et al*., 2012) and it is now more commonly named VEGF-A. It functions as a selective endothelial mitogen as well as regulator of increasing permeability of the vasculature (Canavese *et al*., 2010; Ferrara *et al*., 2003b). VEGF also influences the immune system by promoting monocyte activation and chemotaxis (Clauss *et al*., 1990). VEGF ligands exert their effects on cells by binding to specific receptor tyrosine kinases (VEGFRs) VEGFR-1 and VEGFR-2 (Ferrara *et al*., 2003b; Hasan and Jayson, 2001). It is through binding with these VEGFRs that VEGF exerts its effects on vascular endothelium and peripheral monocytes (Crawshaw *et al*., 2012).

In the early ninety, important advances have been made regarding the biological role of VEGF. It was discovered that VEGF is expressed in spatial and temporal association with physiological events of angiogenesis *in vivo* (Neufeld *et al*., 1999). Inhibition of VEGF activity by neutralizing antibodies or by the introduction of dominant negative VEGF receptors into endothelial cells of tumour-associated blood vessels resulted in the inhibition of tumour growth and in tumour regression, indicating that VEGF is a major initiator of tumour angiogenesis (Kim *et al*., 1993; Millauer *et al*., 1994). Furthermore, it was found that VEGF expression is potentiated by hypoxia and that the potentiation of VEGF production in hypoxic areas, (e.g.: in solid tumours), contributes significantly to VEGF-driven tumour angiogenesis (Plate *et al*., 1992; Neufeld *et al*., 1999). Moreover, VEGF-induced angiogenesis was also found to play an important role in the a etiology of several additional diseases (Canavese *et al*., 2010; Detmar *et al*., 1994; Medana *et al*., 2010), some of them associated with abnormal angiogenesis (Aiello *et al*., 1994) and wound repair (Brown *et al*., 1992).

Finally, Vascular Endothelial Growth Factor can not only promote angiogenesis, but may also exert certain effects to alter the rate of atherosclerotic plaque development (Celletti *et al*., 2001). Recently, it has been demonstrated an association between VEGF promoter polymorphisms and a vast number of diseases (Buroker, 2014; Prakash *et al*., 2015; Che *et al*., 2015; Kapahi *et al*., 2015; Fauser and Lambrou, 2014; Tie *et al*., 2014; Gora-Tybor *et al*., 2015; Vajtr *et al*., 2014; Sun *et al*., 2014; Qi *et al*., 2014). This has led to investigate whether VEGF could represent a potential early disease biomarker (Canavese and Schauber 2011; Canavese and Spaccapelo, 2014) and to evaluate its role in predicting treatment response to anti-angiogenic therapy, particularly in cancer patients (Otrock *et al*., 2011).

This paper mainly focuses on the role of VEGF across multiple disease conditions, both reviewing the emerging association of most common VEGF polymorphisms with disease susceptibility and giving an up-date on the clinical advances in the development of novel VEGF inhibitors, as possible therapeutic intervention.

Biology of VEGF and its Role in Pathologic Conditions

Vascular Endothelial Growth Factor (VEGF) had been characterized as a heparin binding angiogenic growth factor displaying high specificity for Endothelial Cells (ECs) (Gospodarowicz *et al*., 1989). During embryogenesis, VEGF promotes differentiation and proliferation of ECs and the formation of immature vessels. Then, Angiopoietin-1 induces the remodelling and stabilization of blood vessels, which involves interaction with the extracellular matrix (Bisht *et al*., 2010).

VEGF (referred to also as VEGF-A) belongs to a gene family that includes Placental Growth Factor (PLGF), VEGF-B, VEGF-C and VEGF-D. VEGF-A is a key regulator of blood vessel growth. VEGF-C and VEGF-D regulate lymphatic angiogenesis (Karkkainen *et al*., 2002), emphasizing the unique role of this family in controlling growth and differentiation of multiple anatomic components of the vascular system (Ferrara *et al*., 2003b). Initially, VEGF binding sites were identified on the cell surface of vascular ECs in vitro and in vivo. Subsequently, it became apparent that receptors for VEGF also occur on bone marrow-derived cells (Ferrara and Davis-Smyth, 1997). VEGF binds two related Receptor Tyrosine Kinases (RTKs), VEGFR-1 and VEGFR-2. Both have seven immunoglobulin-like domains in the extracellular domain, a single transmembrane region and a consensus tyrosine kinase sequence that is interrupted by a kinase-insert domain (Terman *et al*., 1991). A member of the same family of RTKs is VEGFR-3 (Pajusola *et al*., 1992), which, however, is not a receptor for VEGF-A, but instead binds VEGF-C and VEGF-D (Karkkainen *et al*., 2002; Ferrara, 2004). In addition to these RTKs, VEGF interacts with a family of co-receptors, the neuropilins (Ferrara *et al*., 2003b).

A well-documented in vitro activity of VEGF is the ability to promote growth of vascular endothelial cells derived from arteries, veins and lympathics (Ferrara *et al*., 2003b).

Blood vessels proliferate by sprouting from existing vessels (angiogenesis) in inflammatory diseases, tumors and many other chronic conditions. Changes in newly formed and remodelled blood vessels are disease-specific, as they reflect vascular adaptations to environmental cues unique to each condition. Remodelling of ECs into a venular phenotype, typical of sustained inflammation, is accompanied by expression molecules that promote endothelial gap formation and leukocyte rolling, attachment and migration. Blood vessels in tumors differ from those in inflammation. Endothelial cells in tumors undergo disorganized sprouting, proliferation and regression and become dependent on VEGF for survival (Mc Donald, 2008). The physiological activity of VEGF as survival factor for ECs has been demonstrated both in vitro and in vivo (Gerber *et al*., 1998a; 1998b).

VEGF has also effects on bone marrow-derived cells. It promotes monocyte chemotaxis (Clauss *et al*., 1990) and induces colony formation by mature subsets of granulocyte-macrophages progenitor cells (Broxmeyer *et al*., 1995). VEGF delivery to adult mice inhibits dendritic cell development and increases

production of B cells and generation of myeloid cells (Gabrilovich *et al*., 1996; Hattori *et al*., 2001). It is known also as vascular permeability factor, based on its ability to induce vascular leakage (Senger *et al*., 1983; Dvorak *et al*., 1995). It is well established that such permeability-enhancing activity underlies significant roles of this molecule in inflammation and other pathological circumstances (Ferrara *et al*., 2003b). To this end, in situ hybridization studies have shown that VEGF mRNA is up-regulated in many human tumours (Ferrara and Davis-Smyth, 1997; Dvorak *et al*., 1995), so that VEGF inhibitors may be effective for treatment of haematological malignancies and several clinical trials are currently testing this hypothesis (Ferrara *et al*., 2003b).

Diabetes mellitus, occlusion of the central retinal vein or prematurity with subsequent exposure to oxygen can all be associated with intraocular neovascularization, which may result in vitreous haemorrhages, retinal detachment, neovascular glaucoma and blindness. All of these conditions are associated with retinal ischemia. An increase in VEGF in the aqueous and vitreous humor of the eyes, with proliferative retinopathy secondary to diabetes and other conditions have been previously described (Aiello *et al*., 1994; Malecaze *et al*., 1994). Subsequent animal studies using various VEGF inhibitors have directly shown the role of VEGF as a mediator of ischemia-induced intraocular neovascularization (Aiello *et al*., 1995; Adamis *et al*., 1996).

VEGF has also been implicated in various inflammatory disorders (Dvorak *et al*., 1995). It is strongly expressed by epidermal keratinocytes in wound healing and psoriasis, conditions that are characterized by increased microvascular permeability and angiogenesis (Detmar *et al*., 1995; Canavese *et al*., 2011; Crawshaw *et al*., 2012). Transgenic expression of VEGF in the skin results in increased density of tortuous cutaneous blood capillaries and enhanced leukocyte rolling and adhesion in post-capillary skin venules, suggesting that overexpression of VEGF in the epidermis is sufficient to induce features of chronic skin inflammation (Canavese *et al*., 2011; Xia *et al*., 2003). Notably, no changes in lymphatic vessels were detected in these studies (Detmar *et al*., 1998).

VEGF up-regulation has also been implicated in the development of brain edema. Enhanced levels of VEGF and its receptors have been reported in the rat brain after induction of focal cerebral ischemia (Kovacs *et al*., 1996). Van Bruggen *et al*. (1999) have shown that VEGF antagonism has beneficial effects in a mouse model of cortical ischemia, resulting in a significant reduction in the volume of edematous tissue, shortly after the onset of ischemia and in the infarct size, measured several weeks later. Additionally, VEGF has been found overexpressed in brains of cerebral malaria patients (Medana *et al*., 2010; Furuta *et al*., 2010), although, its protective or pathogenic role is still controversial (Canavese and Spaccapelo, 2014).

Hyperplasia and hypervascularity are features of polycystic ovary syndrome, a leading cause of infertility (Ferrara *et al*., 2003b). Several studies suggest that VEGF and Endocrine Gland-Derived Vascular Endothelial Growth Factor (EG-VEGF) may cooperate in the induction of angiogenesis in this condition (Ferrara *et al*., 2003a). Angiogenesis is also important in the pathogenesis of endometriosis, a condition characterized by ectopic endometrial implants in the peritoneal cavity. Large amounts of VEGF have been measured in the peritoneal fluid of patients with endometriosis (McLaren *et al*., 1996). According to the literatures, circulating levels of soluble VEGFR-1 derived from the placenta are increased in pre-eclampsia, resulting in reduced free VEGF and Placental Growth Factor (PGF). Thus, endothelial dysfunction of pre-eclampsia may be a result of excess VEGF or PGF neutralization by circulating soluble VEGFR-1 (Maynard *et al*., 2003; Ferrara, 2004).

Association of VEGF Polymorphisms with Disease Risk

Single nucleotide changes that affect gene expression by impacting gene regulatory sequences, such as promoters, enhances and silencers are known as regulatory Single Nucleotide Polymorphisms (rSNPs). A rSNP within a Transcriptional Factor Binding Site (TFBS) can change a transcriptional factor's (TF's) ability to bind to its TFBS, in which case the TF would be unable to effectively regulate its target gene (Buroker, 2014).

Very recently, three rSNPs in the promoter region of VEGF-A gene have been significantly associated with several human diseases or conditions. The rSNP alleles alter the TFBS in non-coding regulatory regions of the gene, which in turn can result in human disease development (Buroker, 2014). Very recently, Buroker has reported on several cases of diseases that have been listed along with the VEGF-A rSNP genotypes and allele frequencies for ill patients versus their controls (Buroker, 2014). As an example, the rs2010963 VEGFA-G allele has been found to significantly increase in patients with severe ischemic complications in Giant Cell Arteritis (GCA) (Rueda *et al*., 2005). This allele generates the potential SP1 binding site, where SP1 can activate or repress transcription in response to physiological and pathological stimuli (Buroker, 2014). As well as the rs1570360 VEGFA-G allele was significantly increased in patients with Sporadic Alzheimer Disease (SAD) (Yuan *et al*., 2009). This allele generates the potential KLF4 and MIZF binding sites, where KLF4 acts as both an activator and repressor

and MIZF plays a role in DNA methylation and transcription repression. The proteins from these genes are individually capable of activating transcription and replication (Buroker, 2014).

Table 1 is summarizing some of the most recently found associations of VEGF polymorphisms with different human diseases.

The contribution of genetic polymorphisms in the VEGF gene to disease risk is still a controversial topic, since further epidemiological studies should investigate interaction among multiple genotypes, different ethnic groups and environmental exposure to better correlate the VEGF polymorphism with the disease susceptibility.

Interestingly, in a very recent work Fauser and colleagues evaluated a meta-analysis that demonstrated that SNPs within VEGF-A (the presence of a C-nucleotide in rs833061 or TT in rs1413711) may be associated with Age-Related Macular Degeneration (AMD) pathogenesis (Fauser and Lambrou, 2014; Huang *et al*., 2013). Given that VEGF SNPs are involved in disease pathogenesis and that the highly successful anti-VEGF neovascular-AMD (nAMD) therapies target VEGF-A, SNPs within VEGF-A were considered to be the most likely to predict how a patient with nAMD will respond to such therapies. The SNPs analyzed for this purpose fall into two broad categories. The first comprises SNPs within genes originally associated with AMD pathogenesis, termed AMD-related SNPs. The second category includes those SNPs specifically associated with the VEGF signaling pathway targeted by anti-VEGF therapies which are collectively termed pharmacologically related SNPs. A total of 17 VEGF-A SNPs were investigated, but only four (rs699947, rs1413711, rs699946 and rs3025000) correlated with patient response to anti-VEGF therapy (Fauser and Lambrou, 2014). However, the correlation between nAMD treatment response and the VEGF SNP rs1413711 remain still under debate. The genotypes of rs1413711 were found to be indicators of anti-VEGF response in a UK study by McKibbin group (McKibbin *et al*., 2012), but other studies did not find this to be the case (Boltz *et al*., 2012; Yuan *et al*., 2013; Fauser and Lambrou, 2014). In the UK study, the presence of a C nucleotide at rs1413711 was associated with a marked improvement in visual acuity (McKibbin *et al*., 2012) and it had a maximum follow-up time of six months, whereas some of the other studies had longer durations. It is then possible that there is a statistically significant effect at month six of treatment, which decreases to a non-significant level by month twelve or month fifteen (Fauser and Lambrou, 2014). Therefore, duration of treatments may need to be taken into account in the definition of responsiveness used in each of the trials. Additionally, patients characteristics, such as the ethnic background, play an important role in nAMD development and progression (Klein *et al*., 2004), however, no association between patient ethnicity and genetic factors predictive of treatment response were identified in the studies reviewed by (Fauser and Lambrou, 2014).

Table 1. Association between VEGF-A gene polymorphisms and disease susceptibility

Disease/condition	VEGF-A polymorphisms (SNPs)	Reference
Psoriasis	+405C>G; -460T>C; -2578C>A; -1154G>A	Qi *et al*. (2014)
Acute renal allograft rejection	-2578C>A; -2549Ins>Del; -1154G>A; +936C>T	Prakash *et al*. (2015)
Autoimmune diseases	-1154G>A; +405G>C; -2578C>A; +936C>T; -460T>C	Che *et al*. (2015)
Breast cancer	-2578C>A; -2549I>D; -460T>C;-7C>T	Kapahi *et al*. (2015)
Osteosarcoma	-2578C>A; -1156G>A; +1612G>A; +936C>T	Tie *et al*. (2014)
Chronic lymphocytic leukemia	+405G>C; +936C>T; -271G>A; +1719A>T	Gora-Tybor *et al*. (2015)
Cervical cancer	-2578C>A (rs699947); -1154G>A (rs1570360)	Zidi (2014)
Renal cell carcinoma	-2578C>A; -634G>C	Zhong *et al*. (2014)
Diabetic nephropathy in patients with diabetes mellitus	rs2010963; rs3025039	Sun *et al*. (2014)
Neovascular age-related macular degeneration	rs699947; -2578C>A; +405G>C +936C>T	Habibi *et al*. (2014) Fauser and Lambrou (2014)
Myocardial infarction in patients with type 2 diabetes mellitus	-604T>C (rs2071559)	Kariz and Petrovic (2014)
Endometriosis	-1154G>A	Perini *et al*. (2014)
Acute chest syndrome in pediatric patients with sickle cell disease	-583C>T	Redha *et al*. (2014)
Alzheimer's disease	-2578C>A; -1154G>A	He *et al*. (2013)
Idiopathic Parkinson's disease	-2578C>A; -634C>G; 936C>T	Mihci *et al*. (2011)

rSNPs: Regulatory Single Nucleotide Polymorphisms

A number of human conditions have significantly been associated with VEGF-A rSNPs. Table 1 summarizes the most recent studies investigating the contribution of VEGF polymorphisms as risk factors for the development of several human diseases

Table 2. Therapeutic implications of VEGF

Disease	Therapeutic approach currently implemented	Clinical phase	Reference
Advanced non small cell lung cancer/ Colon rectal cancer/Renal cell cancer	Bevacizumab	Approved	Sullivan and Brekken (2010)
Neovascular Age-related Macular Degeneration (AMD)	Bevacizumab	N/A (Investigational)	Avery *et al*. (2006)
Psoriasis	Bevacizumab	N/A (Investigational)	Akman *et al*. (2009)
Psoriasis	Ramucirumab	N/A (Investigational)	Halin *et al*. (2008)
Gastric cancer/Lung carcinomas	Ramucirumab	Approved	Aprile *et al*. (2013; Spratlin *et al*., 2010)
Metastatic renal carcinoma	Ramucirumab	Phase II Clinical trial	Garcia *et al*. (2014)
Hepatocellular carcinoma	Sorafenib	Approved	Llovet *et al*. (2008)
Advanced renal cell cancer	Sorafenib	Phase III Clinical trial (TARGET)	Escudier *et al*. (2007)
Advanced non small cell lung cancer/Renal cell cancer/Soft tissue sarcoma/gynecological tumors	Pazopanib	Phase III Clinical trial	Sternberg *et al*. (2009)
Metastaic colorectal cancer	Cediranib	Phase III Clinical trial (HORIZON)	Robertson *et al*. (2009)
Metastatic colorectal cancer	IMC-1C11	Phase I Clinical trial	Fontanella *et al*. (2014)
Advanced non small cell lung cancer	Vendetanib Vs Gefitinib	Phase II trial (Investigational)	Natale *et al*. (2009)
Locally advanced or metastatic non-small cell lung cancer	Vendetanib + Docetaxel + Pemetrexed	Phase III trial (ZODIAC and ZEAL)	Morabito *et al*. (2009)
Cytokine-refractory metastatic renal cell cancer	Axitinib	Phase II trial (Investigational)	Rixe *et al*. (2007)

Table 2 summarizes the therapeutic implications of VEGF, giving an overview of the clinical advances of VEGF inhibitors development across multiple disease conditions

Theoretically, SNP variants could affect the severity of the disease to different degrees: Some variants may, e.g., increase inflammation more than others. Expression of different variants could therefore explain differences between study findings; this would be difficult to confirm in a clinical setting, however, as the variants may have relatively little effect. Overall, to date it appears that these AMD-related SNPs are of limited clinical value as predictors of individual patient response to anti-VEGF treatment (Fauser and Lambrou, 2014).

The categorization of the identified SNPs into the two above groups, AMD-related and pharmacologically related SNPs, revealed that the more widely studied AMD-related SNPs have yet to provide a substantiated clinical predictive value (Hagstrom *et al*., 2013). Anti-VEGF-related SNPs have not yet accumulated the same amount of evidence as AMD-related SNPs either to commend or reject them as potentially useful biomarkers. One of the more promising SNPs is VEGF-A rs699947, but because of the variability of the study results to date, it is still difficult to prove it as a useful biomarker. Thanks to the ongoing investigations into the genetics of AMD (Fritsche *et al*., 2013) and the full realization of the efficacy of anti-VEGF treatments, other candidate SNPs may yet emerge. Clinical factors, such as patient baseline characteristics, also show potential as predictive markers of the treatment response (Finger *et al*., 2014; van Asten *et al*., 2014). It may be possible that the cumulative analysis of several high-risk AMD genes could result in the identification of patients with specific response patterns to anti-VEGF treatment (Investigators *et al*., 2012), as van Asten and colleagues showed in a recently published study (van Asten *et al*., 2014).

Therapeutic Implications and Updates on the Clinical Advances of VEGF Inhibitors Development

The VEGF family clearly plays an essential role in the regulation of embryonic and postnatal physiological angiogenesis processes, such as normal growth processes (Gerber *et al*., 1999b; 1999a) and cyclical ovarian function (Ferrara *et al*., 1998). Furthermore, many studies have documented acute neuro-protective effects of VEGF in experimental stroke (Zhang *et al*., 2000; Hayashi *et al*., 1998; Sun *et al*., 2003; Wang *et al*., 2006; Feng *et al*., 2008; Zheng *et al*., 2010; Sun *et al*., 2004), as well as, other reports have described long term VEGF-mediated beneficial effects of stem cell treatment (Lee *et al*., 2007; Chu *et al*., 2005; Zhu *et al*., 2005; Harms *et al*., 2010; Horie *et al*., 2011).

The hypothesis that therapeutic angiogenesis may be beneficial for disorders characterized by inadequate tissue perfusion generated a high level of enthusiasm in the field of cardiovascular medicine and led to many clinical trials (Ferrara, 2004). Early studies have shown that VEGF administration leads to a recovery of normal endothelial reactivity in dysfunctional endothelium (Bauters *et al*., 1995). Furthermore, VEGF gene transfer was also reported to prevent the ischemic peripheral neuropathy associated with lower extremity vascular insufficiency in a rabbit model (Schratzberger *et al*., 2000).

The idea that VEGF may result in therapeutically significant angiogenesis in humans was initially tested

by (Isner *et al*., 1996) using a gene therapy approach. However, a greater understanding of the differential role of VEGF receptors had opened additional avenues. In particular, several studies have emphasized that VEGFR-1 possesses important activities in hematopoiesis and in the recruitment of mononuclear cells. The fact that VEGFR-1 activation is associated with fewer side effects relative to VEGF made it a particularly attractive target. Furthermore, the report that a VEGFR-1 agonist protected the liver from toxic damage, by instructing the quiescent endothelium to produce a series of tissue-specific growth factors, extended the potential clinical applications of VEGFR-1 agonists (LeCouter *et al*., 2003). Other activities of VEGF may have interesting clinical implications. For example, on the basis of the key role played by VEGF in angiogenesis and endochondral bone formation, the application of this factor might be useful to enhance revascularization and healing of fractures and other skeletal conditions (Carano and Filvaroff, 2003). Several studies have shown that both recombinant (Street *et al*., 2002) and adenovirus-delivered-VEGF leads to enhanced blood vessel formation and ossification in models of bone damage (Tarkka *et al*., 2003).

On the other hand, already in 1971 Folkman and colleagues started initial efforts aimed to isolate a tumor angiogenesis factor from human and animal cancers (Folkman *et al*., 1971; Folkman, 1971). Therefore it became clear that VEGF blockage inhibits pathological angiogenesis in a wide variety of tumour models, a phenomenon that has led to the clinical development of a vast number of VEGF inhibitors (Ferrara *et al*., 2003b). Inhibition of VEGF signaling in tumors stop sprouting angiogenesis and triggers regression of some tumor vessels while normalizing others. Some capillaries in normal thyroid, pancreatic islets and intestine may also regress after VEGF blockade, but most remodelled vessels at sites of inflammation do not. Pericytes and empty sleeves of vascular basement membrane persist after endothelial cells regress and provide a scaffold for blood vessel regrowth, which can occur within days after the inhibition ends. The clinical efficacy of VEGF signaling inhibitors in cancer provides proof of concept and stimulates the search for even more effective agents (Mc Donald, 2008).

The potential clinical usefulness of VEGF inhibition is not limited to cancer. Phase 3 trials in AMD patients are under way. As already noted, gynaecologic conditions such as endometriosis or polycystic ovary syndrome might also benefit from this treatments (Ferrara *et al*., 2003b), as well as VEGF antagonists have been recently proposed for the treatment of psoriasis (Crawshaw *et al*., 2012). Indeed, it is conceivable that non-neoplastic conditions will show a greater clinical response, given the reduced likelihood of non-transformed cells to activate alternative angiogenic pathways and thus develop resistance (Ferrara *et al*., 2003b).

As summarized in Table 2, over the last decade, a number of monoclonal antibodies and small molecules that specifically target the VEGF pathway have been studied as single agents or in combination with chemotherapies (Sullivan and Brekken, 2010; Fontanella *et al*., 2014). Bevacizumab, for example, is a monoclonal antibody (mAb) that binds VEGF and it has gained worldwide approval for first or second line treatment in several different tumour types (Sullivan and Brekken, 2010) AMD (Avery *et al*., 2006) and in psoriasis (Akman *et al*., 2009). Recent studies indicates VEGFR-2 as a novel target (Lu *et al*., 2002): Biological and preclinical evidence suggests that blockage of VEGFR-2 could be a promising strategy both to inhibit tumor-induced angiogenesis (Hamerlik *et al*., 2012; Witte *et al*., 1998) and to control psoriasis (Halin *et al*., 2008). In order to proof this hypothesis a novel human IgG1 mAb that specifically blocks the VEGFR-2, named Ramucirumab, was developed and it has produced notable results in different diseases including gastric cancers and lung carcinomas (Aprile *et al*., 2013; Spratlin *et al*., 2010). In these poor prognosis diseases, even a small absolute survival benefit of two months is clinically valuable. Disappointing clinical results reported for the ROSE study confirm that breast cancer may limitedly benefit from angiogenic inhibitors (Mackey *et al*., 2014). While ongoing studies will clarify the role of ramucirumab in metastatic colorectal cancer, translational research will provide more details about how to properly select optimal candidates and corroborate the ethnical difference in benefit. Despite huge efforts have been made to identify a predictive biomarker, no validated predictor is currently available for selecting optimal candidates to antiangiogenic therapy or monitoring treatment response. Future research will possibly increase our knowledge on how to select patients who are more likely to be responsive to antiangiogenic treatment. As well, the role of VEGF in reverting immunosuppression should also be better elucidated. Moreover, novel oral VEGFR-2 inhibitors will possibly add some value to this strategy (Fontanella *et al*., 2014).

Conclusive Remarks

Angiogenesis is an important biological process not only under physiological conditions, but also in a variety of diseases, including cancer, diabetic retinopathy, rheumatoid arthritis, AMD, stroke, psoriasis and others (Risau, 1997). The research conducted after

the discovery of VEGF revealed that it is a central regulator of angiogenesis and vasculogenesis. This has consequently led to the development of drugs aimed at the treatment of pathological conditions associated with angiogenic disorders. A high probability of benefit is desirable to justify the choice of anti-angiogenic therapy from an ever-expanding list of expensive new anti-cancer agents. Still, biomarkers of response to anti-angiogenic agents are inconclusive for predicting benefit from these drugs. The contribution of genetic polymorphisms in the VEGF gene to disease risk is still controversial. However, there are several types of genetic variations found within the genome that can act as biomarkers, but single nucleotide polymorphisms are the most common, with over 19 million within the human genome (Wang *et al*., 2012). SNPs are usually found in non-coding-regions and can be used as markers to locate genes associated with disease or drug response. Therefore, it is possible that, in the near future, the combined effect of high-risk SNPs may be beneficial in a clinical setting and that other genetic biomarkers may emerge.

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