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Review

Tie2: a journey from normal angiogenesis to cancer and beyond

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Summary. The tyrosine kinase receptor Tie2 was initially identified as a specific vascular growth factor that governed several properties of endothelial cells under both physiological and pathological conditions. It was subsequently found that angiopoietins, the natural ligands of Tie2, modulate Tie2-dependent signaling, which in turn regulates the survival and apoptosis of endothelial cells, controls vascular permeability, and regulates the capillary sprouting that occurs during normal angiogenesis such as through development and ovarian remodeling. Tie2 also seems to play a crucial role in several vascular abnormalities, such as familial venous malformations. Beyond its critical role in angiogenesis, Tie2 also appears to maintain the long-term population and quiescent status of hematopoietic stem cells in the bone marrow stem cell niche. In cancer, Tie2 was originally found to be overexpressed in tumoral vessels. More recently, our laboratory and others have found that Tie2 is also expressed outside the vascular compartment in several types of cancer, including leukemia and solid neoplasms such as gastric tumors, breast tumors, and gliomas. The role of Tie2 in these tumoral cells is currently being explored. In this regard, our group reported the importance of Tie2-expressing glioma cells in their adhesion to the tumoral microenvironment. Because cancer may be considered as a complex organ with several cellular lineages coexisting in the same tumor, the expression of Tie2 by different tumoral compartments makes this cellular receptor an attractive target for cancer therapy.

Key words: Tie2, Angiopoietins, Angiogenesis, Cancer, Hematopoiesis

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Introduction

Inherited diseases of the vasculature can provide insights into the process of vessel formation. One particularly informative and the most common error of vascular morphogenesis in humans, the venous malformations (VMs), are vascular masses composed of dilated channels lined by endothelial cells. Vessels that make up VMs have an abnormal ratio of endothelial to smooth muscle cells, with very few supporting smooth cells, leading to functionally low resistance vessels. Patients with VMs usually present with rubbery, compressible, bluish purple nodules or a tumor-like vascular mass, often manifesting at birth. Lesions may also appear or enlarge throughout life, possible influenced by physical trauma, hormonal changes, or hemodynamic changes. Although the majority of VMs are sporadic, the anomaly can also be inherited. One particularly informative finding that was made in families with inherited forms of VM is that the malformations harbor mutations in the coding sequence of the receptor kinase Tie2 (Vikkula et al., 1996; Calvert et al.,1999), which gives Tie2 a decisive role in angiogenesis. However, Tie2 expression and activity is not restricted to vascular structures. In this review, we will cover the journey of Tie2 from its discovery as a specific endothelial growth factor to the various roles of Tie2 outside the vascular compartment, under both physiologic and pathologic conditions.

Discovery of Tie2, its ligands, and its roles

Tie2 was originally described as the second member of an orphan subfamily of tyrosine kinase receptors that were found to be expressed predominantly in the embryonic endothelium (Dumont et al., 1993). The Tie2 sequence is highly conserved across vertebrate species, with the greatest amino acid homology occurring in the kinase domain, a characteristic that denotes the importance of its biological function. Its structure

involves an unusual amino-terminal ligand binding domain composed of immunoglobulin domains, epithelial growth factor repeats, and fibronectin-like 3 repeats; a single transmembrane domain; and an intracellular tyrosine kinase domain (Jones et al., 2001a) (Fig. 1). Tie2's orphan status was changed, however, with the discovery of its natural ligands, the angiopoietins (Ang), which include Ang1, Ang2, and the interspecies orthologs Ang3 (mouse) and Ang4 (human) (Davis et al., 1996; Maisonpierre et al., 1997; Valenzuela et al., 1999).

Angiopoietins contain an amino-terminal angiopoietin–specific domain followed by a coiled-coil domain, a linker peptide, and a carboxy-terminal fibrinogen homology domain that is responsible for receptor binding (Davis et al., 1996; Maisonpierre et al., 1997; Valenzuela et al., 1999). Of interest, these ligands show different expression patterns and extracellular and cell surface binding characteristics. In particular, Ang1 is widely expressed, mainly secreted by periendothelial cells in quiescent vasculature (Davis et al., 1996), and incorporated into the extracellular matrix (ECM). Ang2 is expressed during angiogenesis in areas of endothelial cell activation/regression (Stratmann et al., 1998). It can be stored in the Weibel-Palade bodies in the cytoplasm of endothelial cells and, once secreted, is not associated with the ECM (Xu and Yu, 2001; Fiedler et al., 2004). Ang3 binds to the cell surface via heparan sulfate proteoglycans (Xu et al., 2004). Ang4 is highly expressed in the lung (Valenzuela et al., 1999). These differences may partially determine the availability and biologic activity of angiopoietins. Other differences result from their interactions with their receptor. For example, Ang1 binding to Tie2 stimulates autophosphorylation of the Tie2 kinase domain, and by blocking Ang1-mediated Tie2 activation, Ang2 is a naturally occurring inhibitor of Tie2 activation (Maisonpierre et al., 1997). Consistent with this finding, mice lacking functional Ang1 expression and mice overexpressing Ang2 both displayed a phenotype similar to that of Tie2-null mice, which will be discussed later in this review (Suri et al., 1996; Maisonpierre et al., 1997). However, it has also been demonstrated that, under some circumstances, Ang2 can stimulate Tie2, suggesting that the action of Ang2 as a Tie2 agonist or antagonist is context dependent (Kim et al., 2000; Teichert-Kuliszewska et al., 2001). The effects of Ang3 and Ang4 have been less characterized, but they too have been observed to behave as antagonistic and agonistic ligands, respectively, depending on the context (Valenzuela et al., 1999).

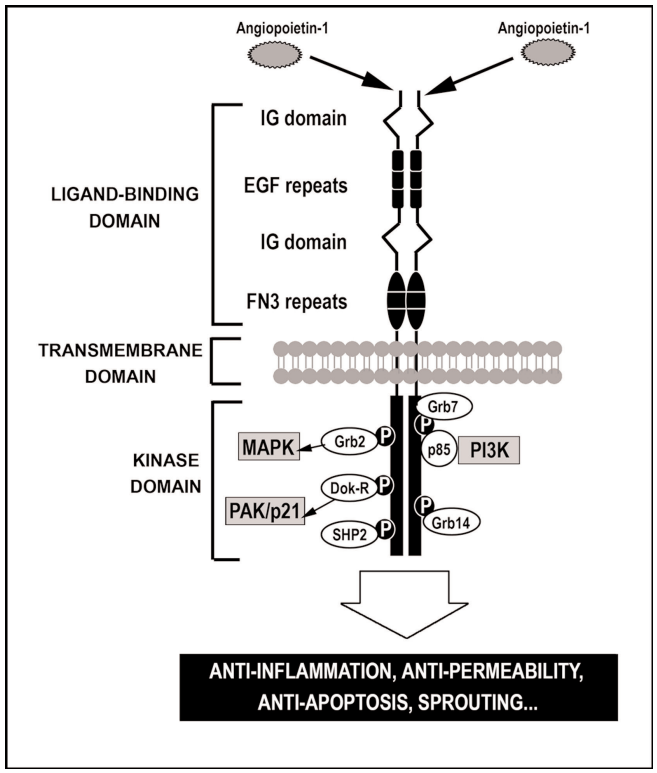

Fig. 1. Tie2 structure and signaling. Extracellular ligand-binding domain includes immunoglobulin (IG) domains, epithelial growth factor (EGF) repeats, and fibronectin-like 3 (FN3) repeats. Angiopoietin 1 binding results in receptor dimerization and autophosphorylation of different tyrosine residues in the intracellular kinase domain, which act as docking sites for many effectors of cytoplasmatic signaling pathways. MAPK, mitogen-activated kinase; PAK, p21-activated kinase; PI3K, phosphatidylinositol 3' kinase. The Tie2-dependent signaling cascade regulates several phenotypic characteristics of the vascular structures, such as vessel permeability and sprouting, endothelial cell survival and apoptosis, as well as properties related with inflammation.

The signal transduction pathways triggered by the binding of Ang1 to Tie2 have been extensively studied (Jones et al., 2001a). This has shown that the binding of Ang1 to the Tie2 extracellular domain results in receptor dimerization, which leads to activation of the Tie2 kinase domain and autophosphorylation of specific tyrosine residues, which then act as docking sites for a number of effectors, including p21-activated kinase (PAK); SHP2 and the adaptor protein GRB2, which is implicated in the MAPK pathway; and the p85 subunit of PI3K (Fig. 1). Signaling through PI3K appears to be essential for Ang1-induced cell survival, sprouting, migration, and capillary tube formation.

Tie2's roles in the vascular compartment: health and pathology

Role in normal angiogenesis

During development and throughout an organism's life, vessels undergo several processes of angiogenic remodeling, stabilization and maturation, destabilization, regression, and sprouting. Tie2 and its ligands, angiopoietins, play a critical role in these vascular changes (Jones et al., 2001a), in many circumstances in coordination with the pro-angiogenic vascular endothelial growth factor.

Inmunohistochemical studies and work done in transgenic mice expressing reporting genes driven by the Tie2 promoter have revealed that Tie2 is expressed during embryonic vessel development and in the quiescent adult vasculature in rodent models (Schlaeger et al., 1997; Puri et al., 1999). Genetically modified animal models have also provided important information regarding the in vivo role of Tie2 and angiopoietins, particularly that this signaling system is indispensable for normal blood vessel development. In this regard, Tie2-deficient mice are not viable and have severe vascular abnormalities (Dumont et al., 1994). Thus, Tie2-null embryos show insufficient sprouting and remodeling of the primary capillary plexus, which results in incomplete development of the heart region, characterized by detachment of the endocardium from the underlying myocardial wall and the absence of myocardial projections or trabeculae. Moreover, Tie2-null embryos show widespread vascular hemorrhage, and the remaining vasculature is characterized by a simplification of vessel branching patterns, a decrease in the survival of endothelial cells, and a lack of recruitment of nearby periendothelial cells (Sato et al., 1995; Puri et al., 1999). In combination, these defects result in embryonic lethality between embryonic day 9.5 and 12.5.

These findings suggest that Tie2 and its downstream signaling events are absolutely necessary for the normal maintenance of endothelial cells and the function of the developing heart. However, this absolute requirement for Tie2 signaling in endocardial development may be restricted to an early development phase, as Jones et al. (2001b) have demonstrated that normal heart development does not require Tie2 signaling. However, Tie2 signaling seems to also have an ongoing role in transducing a cell survival stimulus to endothelial cells, at least in some quiescent adult endothelium, such as in the region surrounding the leptomeninges (Wong et al., 1997; Puri et al., 1999). Ang1-null mice exhibit a similar vascular phenotype, suggesting that Ang1 is a primary physiologic ligand for Tie2. Ang1 seems to play a crucial role in mediating reciprocal interactions between the endothelium and surrounding matrix and mesenchyme, specifically in the recruitment of periendothelial mesenchymal cells to the vessels (Suri et al., 1996).

Role in pathologic angiogenesis

As noted earlier, in one common error of vascular morphogenesis in humans gleaned from studies of naturally occurring mutations in humans, an activating mutation in Tie2 has been found to cause inherited VMs that are characterized by enlarged thinned-walled vessels in the venous segment of the vasculature, which have a reduced coverage with pericytes and smooth muscle cells (Vikkula et al., 1996; Calvert et al., 1999). They are also found in intramuscular hemangiomas (Wang et al., 2004). In addition, changes in the expression of Tie2 and angiopoietins are also found in a wide range of diseases with a vascular component, including psoriasis, cancer and pulmonary hypertension (Eklund and Olsen, 2006). Tie2 has also been implicated in inflammatory processes. However, the exact role of Tie2 signaling in these diseases is not yet known. We describe here some of the pathologic conditions linked to Tie's role in angiogenesis that stem from abnormal Tie2 expression and/or activity.

Increased levels of activated (phosphorylated) Tie2, as well as increased expression of Ang1 at the mRNA and protein level, have been observed in patients with pulmonary hypertension, a disease in which pulmonary arterial pressure is increased and is associated with increased smooth muscle coverage of pulmonary arterioles (Du et al., 2003). However, the precise role of this pathway in the pathogenesis of pulmonary hypertension has yet to be identified.

The expression of Tie2 and the angiopoietins has also been shown to be up-regulated in human psoriasis (Kuroda et al., 2001). In psoriatic lesions, the microvessels of the papillary dermis are elongated, tortuous, and dilated, which contributes significantly to the proinflammatory response. To corroborate the causal relationship of Tie2 signaling to psoriasis, Voskas et al. (2005) generated mice overexpressing Tie2 that showed epidermal hyperproliferation, inflammatory cell accumulation, and altered dermal angiogenesis, a phenotype similar to that of human psoriasis. Further unscoring the involvement of Tie2 in inflammatory disorders, the effect of the chimeric monoclonal antibody infliximab on Tie2 expression was analyzed in a cohort of patients with moderate/severe psoriasis, many with an associated arthritis, who had failed systemic therapy. This work showed that intravenous infliximab to produce a dramatic clinical response in this setting, suggesting that this agent directly modulates angiogenic processes by down-regulating growth factors and their receptors, in particular the Tie2 receptor (Markham et al., 2006).

A few recent reports have indicated a role for Tie2 and angiopoietins role in atherosclerosis. For example, levels of endogenous soluble Tie2 in plasma were higher in patients with coronary artery disease than in healthy controls (Chung et al., 2003). Interestingly, however, Ang1 overexpression in a cardiac allograft arteriosclerosis model protected against the formation of arteriosclerosis (Nykanen et al., 2003). Because the studies in this field are very limited, it is difficult to draw firm conclusions from current observations.

Elevated expression of Tie2 has also been observed in the endothelium of the neovasculature in numerous solid tumors. The first indication of a role for Tie2 in tumoral neovascularization came from an analysis of Tie2 expression in a large number of breast cancer tumor specimens (Peters et al., 1998). Although Tie2 was found to be expressed in the vascular endothelium in both normal breast tissue and breast tumors, the proportion of Tie2-positive microvessels was increased

in tumors compared to normal tissue. Moreover, Tie2 expression appeared to be concentrated in "vascular hot spots" at the leading edge of invasive tumors. Subsequently, increased Tie2 levels were found in the vasculature of a number of other human tumors, including non-small cell lung cancer (Takahama et al., 1999), hepatocellular carcinoma (Tanaka et al., 2002), prostate cancer (Caine et al., 2003), hemangioma (Yu et al., 2001), Kaposi's sarcoma (Brown et al., 2000), and astrocytoma (Zadeh et al., 2004), and these levels were found to correlate with increasing malignancy.

To investigate whether Tie2 plays a significant role in the angiogenesis occurring in cancer, several groups utilized a soluble form of the extracellular domain of murine Tie2 (ExTEK) as a Tie2 inhibitor to treat mammary tumor (Lin et al., 1997), melanoma (Lin et al., 1998), and glioblastoma multiforme (Zadeh et al., 2004). This strategy successfully inhibited tumor growth and decreased the proportion of pathologic vascular structures.

Tie2 has also been implicated in inflammation, which is often accompanied by abnormal angiogenesis and is the underlying cause of many diseases, such as cancer, arthritis, and atherosclerosis. In a sort of vicious cycle, inflammation induces angiogenesis and angiogenesis facilitates inflammation (Kobayashi and Lin, 2005). Under physiologic conditions, the angiopoietins and Tie2 prevent the leakage of plasma protein and leukocytes through vessel walls, thereby having an anti-inflammatory effect (Thurston et al., 1999; Gamble et al., 2000). This protection from vessel leakage is speculated to be due to an increase in cell adhesion molecules such as PECAM-1, E-selectin, and V-cadherin and is essential to the proper functioning of organs. For example, resistance to inflammation in testis microvasculature was suggested to result from the constitutively local expression of Ang1 (Haggstrom et al., 2003). Conversely, the down-regulation of Ang1 has been suggested to be the cause of vascular leakage that occurs in acute lung injury (Karmpaliotis et al., 2002). Further with regard to its anti-inflammatory function, Tie2 has also been implicated in such pathologic disorders as rheumatoid arthritis, in which it is expressed at higher levels in endothelial cells in concert with Ang1 overexpression in the synovium and synovial fibroblasts (DeBusk et al., 2003; Gravallese et al., 2003).

Tie2 outside the vascular compartment

Hematopoietic stem cells and the bone marrow niche

Although Tie2 was originally described as a specific receptor in endothelial cells, recently there have been many reports of its expression in non-vascular normal and pathologic tissues. Early studies conducted by Iwama et al. (1993) and Sato et al. (1993) revealed the expression of Tie2 in the more primitive cells in the hematopoietic lineage, the hematopoietic stem cells (HSCs), which constitute a small number of cells in the bone marrow that are essential for the continuous production of hematopoietic cells. Tie2 expression in HSCs was subsequently reported to be specifically required during postnatal bone marrow hematopoiesis (Puri and Bernstein, 2003). In a recent study, Arai et al. (2004) found that Tie2-expressing HSCs were quiescent and antiapoptotic. In addition, these Tie2-expressing HSCs showed high adhesion properties to their microenvironment. In keeping with this, osteoblasts at the surface of trabecular bone, which constitutes the bone marrow niche, were found to secrete Ang1 and stimulate Tie2 activation in HSCs, which in turn resulted in the adhesion of the HSCs to bone (Fig. 2). The quiescence of stem cells is of critical importance in protecting the stem cell compartment from physiologic stress, which is required for an organism's survival. Thus, the Tie2 in the HSCs in the bone marrow niche

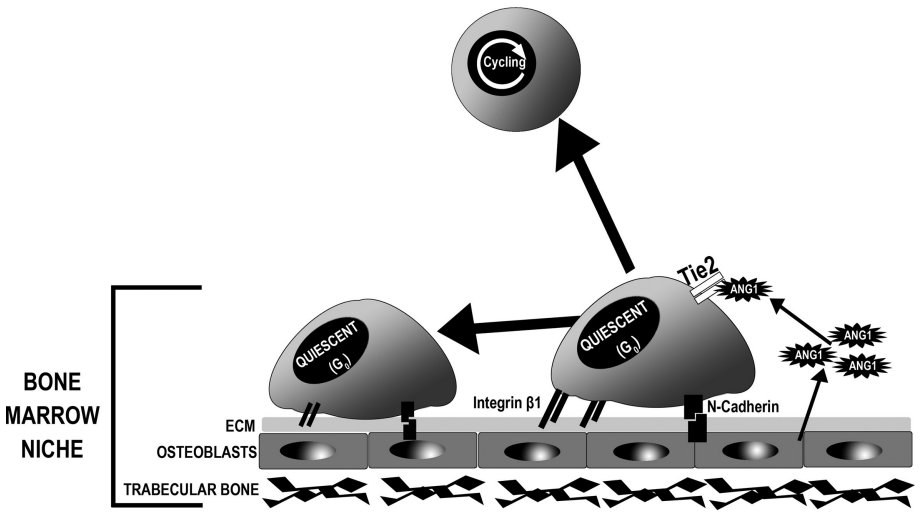

Fig. 2. Regulation of the adult bone marrow stem cell niche by Tie2/Ang1 signaling. Ang1, secreted by osteoblasts (OBs) in the trabecular region of the bone, activates Tie2 in hematopoietic stem cells (HSCs), thereby inducing tight adhesion of the HSCs to the OBs via integrin ß1 and N-cadherin. In addition, the binding of Ang1 to Tie2 promotes the ability of HSCs to be maintained in a quiescent, anti-apoptotic status, which protects them from myelosuppresive stresses, preserving the long-term repopulating activity.

might protect the HSC compartment from myelosuppressive stress.

A newly recognized role for Tie2 in cancer?

As we have already noted, Tie2 expression is being found to be broader than was initially observed. Of interest to cancer researchers, Tie2 has not only been found to be expressed in the vascular structures of tumors, several studies have shown that Tie2 is also expressed in the neoplastic component of several neoplasms. The role of Tie2 in the extravascular component of cancer is still to be revealed.

In this regard, Tie2 and its ligands were found to be overexpressed in acute and chronic myeloid leukemia (AML and CML) cell lines and patient samples (Muller et al., 2002; Schlieman et al., 2006) and in some erythroblastic/megakaryoblastic cell lines (Kukk et al., 1997). In the first study investigating the prognostic relevance of the Tie2/angiopoietin system in hematologic neoplasias, Loges et al. (2005) demonstrated that high Ang2 mRNA expression in peripheral blasts was an independent favorable prognostic factor for overall survival in AML. These findings suggest that Tie2 plays a role in stem cell proliferation or differentiation and possibly in the pathogenesis of AML and CML as well.

Wang et al. (2005) described the overexpression of Tie2 and its ligands Ang1 and Ang2 in gastric cancer cell lines as well as in tumor samples, as compared with expression levels in normal adjacent tissue. These researchers studied the expression of these molecules using PCR, immunohistochemistry, and western blotting and concluded that Tie2 and their ligands might be involved in gastric cancer initiation or progression. Moreover, since Ang1 and Ang2 are produced by gastric cancer cells and endothelial cells, and both cell types also express Tie2, involvement of the autocrine/paracrine loop of the Angs/Tie2 system in gastric cancer is also suggested.

Accumulation of Tie2 and Ang1, but not Ang2, in both malignant and benign thyroid tumor cells and in hyperplastic regions of adenomatous goiter has been described (Mitsutake et al., 2002). The lack of a pronounced accumulation of Tie2 in normal thyroid follicular cells suggests that Ang1 and Tie2 are expressed as autocrine/paracrine factors that enhance the proliferation of thyroid cells. Alternatively, the up-regulation of Tie2 and Ang1 expression in thyroid cells may be closely linked to cell transformation.

Tie2 is also expressed in the endothelial cells, tumor-infiltrating monocytes, and tumor cells in inflammatory breast cancer (IBC), which is characterized clinically by rapid tumor enlargement with skin erythema. In contrast, Tie2 is expressed at lower levels in non-IBC specimens. Supporting an oncogenic role for Ang/Tie2 signaling in IBC, the treatment of IBC xenografts with soluble Tie2 (Ad-TEK) resulted in a reduction in tumor growth and suppression of lung metastasis (Shirakawa et al., 2002).

De Palma et al. (2005) observed the expression of Tie2 in glioma xenografts, not only in endothelial cells, but also in proangiogenic monocytes and a rare population of tumor stroma-derived mesenchymal progenitors. Our group recently found Tie2 to be expressed in neoplasic glial cells when we analyzed human glioma specimens in a tissue array and fresh human surgical specimens (Lee et al., 2006). In this study, Tie2 expression was significantly higher in gliomas than in normal brain tissue, and the levels of expression were associated with progression from lower-grade to higher-grade tumors. Since previous researchers have observed the up-regulation of Ang1 expression in glioblastomas (Stratmann et al., 1998; Ding et al., 2001), the expression of Tie2 in neoplastic glial cells points to the existence of an autocrine ligand/receptor signaling loop in these tumors. In fact, in keeping with this, we observed that Ang1 induced Tie2 phosphorylation and increased the adhesion of glioblastoma cells to collagen type I and type IV, main components of the ECM, by upregulating integrin ß1 expression (Lee et al., 2006). Because cell-ECM interactions can influence tumor development, thereby affecting cell proliferation and survival, as well as the ability of the tumor cells to migrate beyond the original location of the tumor, these observations provide evidence of Tie2 signaling in glioma cells that could influence the neoplastic phenotype of these tumors.

Concluding remarks and future directions

Although discovered more than a decade ago and originally thought to be exclusively involved in the development of vasculature, Tie2 continues to take researchers by surprise by showing up in a range of physiological and pathological conditions. Such discoveries have important clinical implications. For example, the discovery of Tie2 expression in a subpopulation of HSCs with long-term repopulation characteristics could be exploited for use in bone marrow transplant or ex vivo procedures. One recent discovery regarding Tie2 that is proving of great interest to experimental oncologists was the finding that Tie2 is also expressed in various cancers, not only by vascular structures, but also by non-vascular cells within the various cancers. These cancers currently include gastric, breast, and brain tumors, as well as leukemia. Why vascular Tie2 is expressed in these neoplastic cells and what its function is in this setting are important questions that have as yet to be answered. The answers will come from studies that examine tissue expression using a more mechanistic approach that involves the use of cellular biology or genetically modified animal models. If these studies support an oncogenic role for Tie2, this could lead to the development of potentially powerful therapies for cancer that target cancer as a multi-compartmental organ. This is an important concept in cancer therapy because it embraces the fact that

cancer cells are as important as their neighboring endothelial and mesenchymal cells. At this stage, however, the main focus of researchers should be on ways to minimize or prevent the toxicity resulting from the targeting of Tie2-expressing endothelial cells. These therapies could then be adapted to the treatment of other diseases such as pulmonary hypertension, familial VMs, and psoriasis. In any event, the multidimensional role of Tie2 seems to provide a multitude of opportunities for the therapy of several groups of diseases.

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