

The Complex Role of Angiopoietin-2 in the Angiopoietin–Tie Signaling Pathway

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The angiopoietin–Tie signaling system is a vascular-specific receptor tyrosine kinase pathway that is essential for normal vascular development. Although the basic functioning of the pathway is understood, many uncertainties remain about the role of certain members of the pathway, particularly angiopoietin-2 (Ang2), in pathological vascular remodeling and angiogenesis. We summarize the components of the angiopoietin–Tie pathway and then focus on studies that highlight the role of Ang2 in disease settings, including cancer and inflammation. The expression of Ang2 is elevated in many cancers and types of inflammation, which prompted the development of specific reagents to block its interaction with the Tie2 receptor. The application of these reagents in preclinical models of inflammation and cancer has begun to elucidate the role of Ang2 in vascular remodeling and disease pathogenesis and has led to emerging clinical tests of Ang2 inhibitors.

The angiopoietin–Tie signaling system was identified as a vascular-specific receptor tyrosine kinase pathway that is essential for vessel development. This signaling system has many important parallels to the better understood VEGF system. For example, the Tie receptors (Tie1 and Tie2, or Tek) are expressed selectively by endothelial cells, similar to what has been found with VEGF receptors. Signaling by Tie receptors appears to complement the VEGF pathway by contributing to later stages of vascular development. Thus, whereas VEGF signals promote initiating events in angiogenesis such as endothelial cell sprouting, angiopoietin–Tie signals appear to promote endothelial cell survival and vascular assembly, stability, and maturation.

The core components of the signaling system appear to be angiopoietin-1 (Ang1) and Tie2, in that Ang1 is a definitive activating agonist of the pathway and Tie2 is the cognate receptor. However, perhaps because of their regulated expression patterns, other members of the pathway have emerged as desirable therapeutic targets for drug development. For example, several approaches have been developed to selectively block Ang2. Despite much research over the past decade, our understanding of the role of Ang2 in the angiopoietin–Tie signaling system, and vascular biology in general, is particularly murky. For example, it is still unclear whether Ang2 is an antagonist or agonist of Tie2 in settings of vascular remodeling. Increased understanding of Ang2 will become

Editors: Michael Klagsbrun and Patricia D'Amore
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Cite this article as *Cold Spring Harb Perspect Med* 2012;2:a006650

G. Thurston and C. Daly

especially important as these inhibitors move forward in the clinic and are tested in combination with other anti-angiogenic agents. This work summarizes the components and basic biology of the angiopoietin–Tie pathway, describes in more detail studies that reveal the increased expression of Ang2 in human disease as well as mechanistic studies that shed light on its role in preclinical disease models, and then attempts to highlight the outstanding questions for our understanding of the role of Ang2 in angiopoietin–Tie2 signaling and vascular biology. For a more general summary of the angiopoietin–Tie pathway, the reader is directed to an excellent recent review (Augustin et al. 2009).

BASIC BIOLOGY OF THE ANGIOPOIETIN–Tie2 PATHWAY

Receptors—Tie1 and Tie2

The receptors Tie1 and Tie2 are expressed selectively by endothelial cells, although other cell types including early hematopoietic cells and subsets of monocytes also express Tie2. Despite a high degree of structural homology, the two receptors have markedly different properties (Sato et al. 1993; Augustin et al. 2009). Structurally, in the extracellular portion, both receptors are composed of two immunoglobulin (Ig)–like domains, followed by three EGF-like domains, another Ig-like domain, and three fibronectin type III domains (Fig. 1). In the cytoplasmic portion, both Tie1 and Tie2 contain split tyrosine kinase domains.

Functionally, Tie2 binds directly to angiopoietins and has strong kinase activity. In contrast, Tie1 does not bind directly to angiopoietins under normal conditions and has weak kinase activity. Following binding to Ang1, Tie2 becomes phosphorylated on several cytoplasmic tyrosine residues, which results in activation of downstream signaling pathways including the PI3-kinase/AKT and ERK pathways. In comparison, although Tie1 does not directly bind to angiopoietins, it forms a complex with angiopoietins and Tie2 and also becomes phosphorylated on cytoplasmic tyrosine residues (Saharinen et al. 2005).

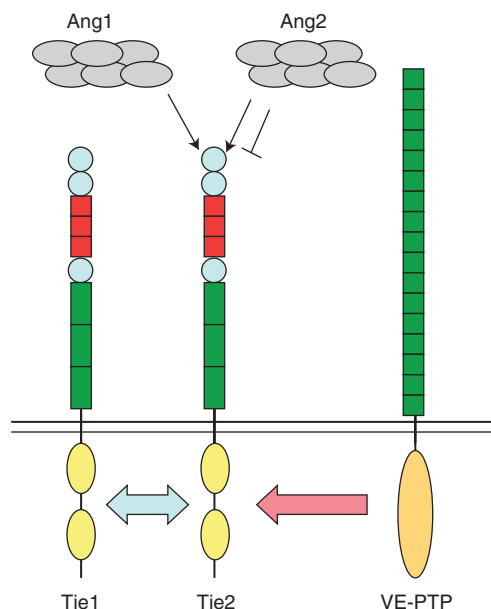


Figure 1. Molecular components of the angiopoietin–Tie pathway. The multimeric ligands Ang1 and Ang2 bind to Tie2 receptor. Tie1 receptor can interact with Tie2, although it apparently does not bind directly to Ang1 or Ang2. The receptor tyrosine phosphatase VE-PTP dephosphorylates Tie2. In the extracellular regions of receptors: (blue circles) Ig-like domains; (green boxes) fibronectin type III domains; (red boxes) EGF-like domains. Components are not drawn to scale.

Knockdown of Tie1 by siRNA indicates that Tie1 is not required for Ang1-dependent activation of the AKT or ERK pathways (Yuan et al. 2007). Thus, the functional role of Tie1 in angiopoietin signaling remains unclear.

Genetic deletion of Tie2 confirms its role as the core signaling component, because mice null for Tie2 exhibit severe vascular and cardiac abnormalities that lead to embryonic lethality at approximately embryonic day 10.5 (~E10.5) (Dumont et al. 1994; Sato et al. 1995). In comparison, genetic deletion of Tie1 leads to vascular perturbation later in development and embryonic lethality that is somewhat variable in onset (E13.5 to birth) (Puri et al. 1995; Sato et al. 1995).

Important insights have come from work linking human venous malformations to mutations in the Tie2 gene. Initially, heritable venous

malformations in two families were found to be associated with a missense mutation in the kinase domain of Tie2 (Vikkula et al. 1996). This mutation results in increased activity of Tie2. Subsequent studies have found that sporadic venous malformations are also associated with somatic point mutations in Tie2, which again are activating (Limaye et al. 2009).

Ligands—Angiopoietin-1 and Angiopoietin-2

Angiopoietins are secreted, multimeric ligands. There are three genuine angiopoietins (Ang1, -2, and -4 in human) that bind to Tie2, plus several angiopoietin-like molecules that share the same structure and have sequence homology to the angiopoietins but do not bind Tie receptors. Ang1 and Ang2 have been the most extensively studied and are described here (Fig. 1), whereas Ang4 has been much less studied and does not yet have a clearly defined role in physiology or pathology.

Angiopoietin proteins are comprised of an amino-terminal domain that serves to promote higher-order clustering of the molecules, followed by a coiled-coil domain that promotes multimerization, and a carboxy-terminal fibrinogen homology domain that contains the binding sites for Tie2 (Davis et al. 2003). Angiopoietins form homomeric higher-order multimers containing from three to six (or more) individual proteins. In general, they are rather sticky proteins, particularly Ang1, with reported interactions with extracellular matrix (Xu and Yu 2001). As a result, much effort has been made to generate artificial recombinant forms of Ang1 (Davis et al. 2003; Cho et al. 2004).

Angiopoietin-1 was initially identified as an activating ligand for Tie2 that is expressed by perivascular cells (Davis et al. 1996). Genetic deletion of Ang1 results in embryonic lethality, with severe heart and vascular defects, very similar in phenotype to Tie2-null mice, and death at ~E12.5 (Suri et al. 1996). The blood vessels in Ang1-null mice form but lack proper attachment of pericytes and fail to mature. Ang1 is strongly expressed in the heart during mid-gestational development and is moderately

expressed by pericytes and perivascular cells in normal vessels. Its expression is not strongly changed by most vascular stimuli. Interestingly, Ang1 is stored at high levels in platelet granules (Li et al. 2001).

Ang2 was initially identified by homology with Ang1 (Maisonpierre et al. 1997). Ang2 was found to bind to Tie2 with a similar affinity as Ang1. However, unlike Ang1, exogenous Ang2 provided only a very weak activation of Tie2 on endothelial cells. When exogenous Ang2 and Ang1 were added together, the levels of Tie2 phosphorylation were decreased compared with addition of Ang1 alone. This and other results (see below) led to the model that Ang2 was an antagonist of Tie2 (Hanahan 1997; Maisonpierre et al. 1997).

Ang2 is expressed predominantly by endothelial cells and some smooth muscle cells. Unlike Ang1, the expression of Ang2 is strongly regulated: Its expression is normally low in quiescent mature vessels but is strongly increased in many inflammatory and angiogenic settings. For example, Ang2 expression in cultured endothelial cells is increased by TNE, VEGF, and hypoxia (Mandriota and Pepper 1998; Kim et al. 2000c). In the ovary of mature female rats, which is a site of physiologic angiogenesis and vascular remodeling, the expression of Ang2 was shown to have dramatic variations during the course of the follicular cycle, which mirror similar dramatic changes in expression of VEGF (Maisonpierre et al. 1997).

In cultured endothelial cells, Ang2 protein is often stored in intracellular granules (Fiedler et al. 2004). These granules can release Ang2 in response to stimuli such as PMA. However, more documentation is needed as to whether normal quiescent or angiogenic endothelial cells in vivo store Ang2 protein in granules. Regardless of whether Ang2 is stored in granules or not, it is clearly secreted by activated endothelium and has been shown to be elevated in the plasma in a variety of inflammatory and angiogenic diseases, including sepsis, malaria, and cancer (see below).

Genetic manipulation of Ang2 in mice has validated its role in vascular development but has also revealed its complexity. The initial

G. Thurston and C. Daly

studies with genetic manipulation described transgenic overexpression of Ang2 in endothelial cells (via a Tie2 promoter) (Maisonpierre et al. 1997). No viable mice were found, but, rather, this manipulation yielded embryonic lethality at ~E9.5–E10. Embryos analyzed at E9–E9.5 showed dramatic disruption of the vasculature and heart. The effects of overexpression of Ang2 were described as similar to those seen in embryos lacking either Tie2 or Ang1, thus providing evidence that Ang2 was acting as an antagonist for Tie2.

Mice genetically deficient for Ang2 showed complex lymphatic and vascular phenotypes (Gale et al. 2002). Knockout (KO) mice were born at approximately normal Mendelian ratios but soon developed chylous ascites and edema, and most mice died within the first 2 wk postnatally. An underlying defect was found in the central lacteals of the intestinal villi and throughout the lymphatic system, which likely accounted for the perinatal lethality. In addition, defects were found in the early postnatal remodeling of the vasculature in the eye: The hyaloid vessels failed to regress properly, and the retinal vessels grew abnormally. Subtle defects were also noted in the patterning of the vasculature in several organs, although the mechanism for these defects was not determined.

Receptor Phosphatase—VE-PTP

The angiopoietin–Tie pathway appears to have another key player, namely, the receptor tyrosine phosphatase VE-PTP (also called PTPR β). VE-PTP is an endothelial cell-specific transmembrane phosphatase that consists of a large extracellular domain with 17 fibronectin type III repeats, a transmembrane domain, and a cytoplasmic phosphatase domain (Fig. 1). Genetic inactivation of VE-PTP in mice results in embryonic lethality, with defects in vascular remodeling and heart development (Baumer et al. 2006; Dominguez et al. 2007). The extracellular domain of VE-PTP appears to be important for its function because antibodies to this region can cause defects in vascular remodeling similar to those seen with genetic inactivation of VE-PTP (Winderlich et al. 2009). The

activity of VE-PTP has been linked to its dephosphorylation of Tie2 (Li et al. 2009; Winderlich et al. 2009) and subsequent downregulation of ERK signaling. Thus, VE-PTP appears to be an essential negative regulator of angiopoietin–Tie signaling. It is worth noting that the reported phenotype of the VE-PTP knockout mice, which is likely mediated by overactivation of Tie2, is somewhat similar to that of the Ang1 and Tie2 knockout mice, making the interpretation of the Ang2-overexpressing mice more difficult. A more detailed and contemporaneous examination of these multiple genetic manipulations would be valuable.

Cellular and Vascular Effects of Angiopoietin–Tie Signaling

Several studies in mice and with cultured endothelial cells suggest that Ang1 promotes vessel stabilization and enlargement without inducing vascular sprouting. Genetic overexpression of Ang1 in the skin of mice resulted in enlarged dermal vessels (Suri et al. 1998; Thurston et al. 1999). Treatment of normal mice with exogenous Ang1 protein can also induce vascular enlargement, associated with an increased number of endothelial cells (Baffert et al. 2004; Thurston et al. 2005). Importantly, vessels exposed to Ang1 are resistant to leak induced by VEGF or inflammatory agents (Thurston et al. 1999, 2000). In addition to its anti-leakage effects, acute exposure of vasculature to Ang1 can reduce vascular activation and dysfunction. For example, Ang1 inhibits leukocyte infiltration in models of diabetic retinopathy (Joussen et al. 2002) and sepsis (Witzenbichler et al. 2005). These results suggest a role for Ang1–Tie2 signaling in maintaining a quiescent, well-functioning vasculature.

The effects of Ang1 on leukocyte infiltration in inflammatory models may reflect, at least partly, inhibition of endothelial cell adhesion molecule expression. Decreased expression of adhesion proteins including ICAM-1, VCAM-1, and E-selectin has been attributed to activation of the PI3-kinase/AKT pathway (Kim et al. 2001). With respect to permeability, several



reports suggest that Ang1 works by inhibiting calcium influx and/or activation of the small GTPase Rho (Li et al. 2004; Jho et al. 2005). Both calcium flux and Rho are believed to promote permeability through effects on the actin cytoskeleton.

The effects of Ang1–Tie2 signaling on the inflammatory response may be more complex than described above. A recent study has shown that chronic activation of Tie2 by exogenous Ang1 in mice can change airway microvessels from a “capillary-like” phenotype to that of a “venule-like” phenotype (Fuxe et al. 2010). Such phenotypic changes in the microvasculature also occur in chronic inflammation. Because the postcapillary venules are typically the site of the inflammatory response, the expanded venule-like phenotype produced by Ang1 stimulation produced a more vigorous response to acute inflammatory challenge (Fuxe et al. 2010). The anti-inflammatory effects of Ang1 may depend on the dosage or duration of exposure to Ang1 and the nature of the acute inflammatory stimuli.

Recent studies indicate that signaling downstream from Tie2 activation is influenced by the subcellular localization of the receptor, which is different in confluent versus sparse endothelial cells (Fukuhara et al. 2008; Saharinen et al. 2008). In these studies, Ang1 produced stronger AKT signaling in confluent cells, in which Tie2 was localized at cell/cell junctions. In comparison, Ang1 produced stronger ERK signaling when the cells were sparse, in which case, Tie2 was localized at sites of cell/substratum contacts. Thus, the effects of Ang–Tie2 activation on endothelial cell function may depend on the nature of the cell–cell and cell–matrix contacts.

As described above, several lines of evidence suggested that Ang2 acts as a Tie2 antagonist. However, several other experimental results indicate that Ang2 can act as an agonist for Tie2. First, exogenous Ang2 can clearly promote phosphorylation of Tie2 in cultured endothelial cells (Kim et al. 2000b; Teichert-Kuliszewska et al. 2001), although its effects are not as potent as exogenous Ang1. In another model, endogenous expression of Ang2 was induced in

cultured endothelial cells by low AKT signaling and the transcription factor FOXO1, and in this setting Ang2 was shown to activate Tie2 (Daly et al. 2006). Tie2 activation in this model was blocked by antibodies to Ang2, indicating an autocrine or juxtacrine role for Ang2. Thus, Ang2 expression and secretion may be a compensatory response by endothelial cells to low AKT activity, for example, when Ang1/Tie2 signaling is weak (Fig. 2). Conversely, strong AKT activation in response to Ang1–Tie2 activity leads to inhibition of Ang2 expression (Daly et al. 2004). These findings suggest a model in which endothelial cells that actively produce Ang2 can respond by robust activation of Tie2.

In addition to these effects in cultured endothelial cells, Ang1 and Ang2 can produce similar effects in vivo. For example, both Ang1 and Ang2 rapidly activated Tie2/AKT signaling in mouse heart and inhibited expression of FOXO1 target genes, including Ang2 itself (Daly et al. 2006). Additionally, replacement of the Ang2 gene with a cDNA encoding Ang1 led to a partial rescue of the phenotype in Ang2-deficient mice (Gale et al. 2002), consistent with an activating role for Ang2. Finally, in several models of inflammation, acute treatment with either Ang1 or Ang2 can inhibit components of the inflammatory process (Daly et al. 2006; Nykanen et al. 2006).

EXPRESSION OF ANG2 IS INCREASED IN SEVERAL DISEASE SETTINGS

Since the initial description of dramatic regulation of Ang2 expression in the cycling ovary, many studies using a variety of approaches have shown that Ang2 is, indeed, highly up-regulated in many diseases and settings of vascular remodeling. Because of a paucity of antibodies to Ang2 that allow robust immunohistochemistry, very few studies have documented the levels or location of Ang2 protein in diseased human tissue. However, several in situ hybridization studies have revealed increased levels of Ang2 expression in cancers of different origin, including neuroendocrine tumors (Detjen et al. 2010), hepatocellular

G. Thurston and C. Daly

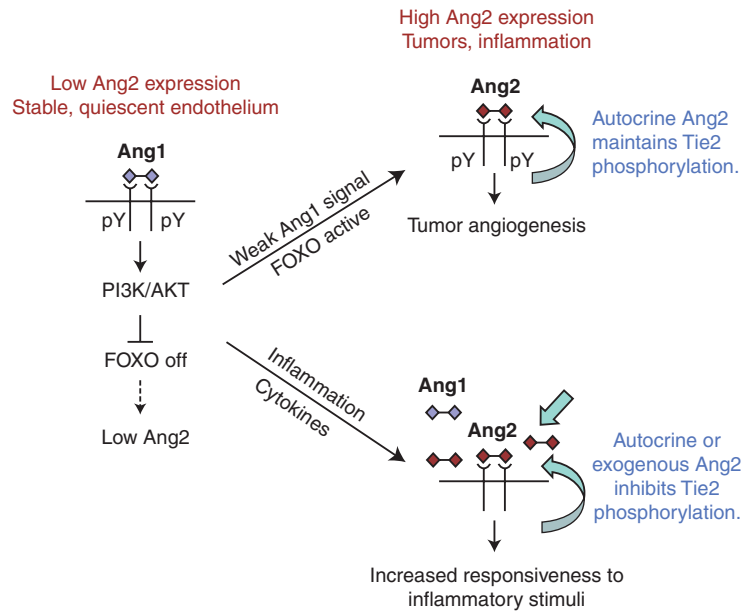


Figure 2. Model of agonist and antagonist roles for Ang2 on Tie2 function. In quiescent, mature vessels, Ang1 promotes strong activation of the Tie2/PI3K/AKT pathway. Ang2 expression is low because of AKT-mediated inhibition of the FOXO1 transcription factor. Low AKT activity, for example, when Ang1/Tie2 signaling is weak, results in activation of FOXO1 and increased Ang2 expression. In this setting, Ang2 activates Tie2 phosphorylation, thereby compensating for the absence of a strong Ang1 signal. As discussed in the text, some evidence indicates that Ang2 promotes tumor angiogenesis via activation of Tie2. Expression of Ang2 can also be induced during inflammation, perhaps by TNF α or other cytokines. In this setting, Ang2 may cause a decrease in Tie2 phosphorylation, by competing with the strong agonist Ang1. Inhibition of Tie2 signaling appears to make the endothelium more responsive to inflammatory stimuli. It should be stressed that only in very few instances have changes in Tie2 phosphorylation been directly measured in vivo. Thus, as discussed in the text, models of Ang2 mechanism of action are based largely on indirect evidence.

cancer (Chen et al. 2001; Scholz et al. 2007), gastric cancer (Moon et al. 2006), angiosarcoma (Brown et al. 2000), carcinosarcoma (Emoto et al. 2004), and astrocytoma (Zagzag et al. 1999). Interestingly, some studies report increased expression of Ang2 in both endothelial cells and tumor cells (e.g., Helfrich et al. 2009; Detjen et al. 2010), whereas other studies have shown Ang2 expression limited to the vasculature and not in tumor cells (Stratmann et al. 1998; Zagzag et al. 1999; Moon et al. 2006; Goede et al. 2010). In addition to in situ hybridization methods, analyses of RNA from whole and microdissected tissue have shown increased Ang2 in tumors versus corresponding normal tissue (e.g., Durkin et al. 2004; Helfrich et al. 2009; Goede et al. 2010).

A reliable commercial enzyme-linked immunosorbent assay (ELISA) for human Ang2 has spawned numerous studies that have documented increased levels of Ang2 protein in plasma or serum in many disease settings, including cancer and inflammation. Using this ELISA, the absolute levels of Ang2 in human plasma reported across a spectrum of studies are reassuringly consistent; plasma from healthy controls contains levels of Ang2 of $\sim 1\text{--}3$ ng/mL, and seldom more than 4 ng/mL, whereas plasma from disease shows a range of Ang2 levels from 5 to 10 ng/mL and as high as 20 ng/mL or more. For example, in a study of melanoma, circulating Ang2 levels were increased in patients with Stage III and IV disease, with median levels from Stage



IV patients almost threefold that of control levels and individual values as high as 8 ng/mL (Helfrich et al. 2009).

Similar values for circulating Ang2 have been reported in sepsis. For example, circulating levels of Ang2 in severe sepsis (associated with organ failure) were fivefold increased compared with normal (Davis et al. 2010). Another study reported significantly higher levels of circulating Ang2 in patients admitted for septic shock (van der Heijden et al. 2009). Importantly, in this latter study, the levels of Ang2 at admittance were significantly higher in nonsurviving patients than in subsequent survivors.

Recent efforts have made further associations between the levels of Ang2 in plasma and the outcome of the disease. For example, a recent study found that circulating levels of Ang2 were increased in colorectal cancer, with mean Ang2 levels increasing from 2.3 ng/mL in plasma from healthy people to 3.9 ng/mL in patients with Stage IV colorectal cancer (Goede et al. 2010). Importantly, Ang2 levels were much increased in a subset of the patients, with some as high as 12 ng/mL. This report then compared circulating levels of Ang2 with outcome (progression free survival, overall survival) in a separate group of treated colorectal cancer patients. Using a threshold of 3.5 ng/mL of circulating Ang2, the study found significantly worse outcome for patients with high levels of Ang2 compared with those with low levels (Goede et al. 2010).

A study of melanoma also found a correlation between high levels of circulating Ang2 and worse outcome (Helfrich et al. 2009). In this study, both progression-free survival and overall survival were reduced in melanoma patients with high levels of circulating Ang2 (using a threshold of 1.8 ng/mL). Finally, a study of neuroendocrine tumors found that the average level of circulating Ang2 increased from 2.6 ng/mL in control samples to 4.0 ng/mL in patients with neuroendocrine tumors, with values in the cancer group up to 25 ng/mL (Detjen et al. 2010). Again, in this tumor type, higher levels of Ang2 were associated with worse overall survival.

ANG2 PLAYS A ROLE IN INFLAMMATION-INDUCED VASCULAR REMODELING

As described above, early studies on the pathway found that treatment of endothelial cells with recombinant Ang1 reduced the inflammatory response to mediators such as VEGF and TNF (Gamble et al. 2000; Kim et al. 2001). Similarly, exposure of vessels to Ang1 in vivo resulted in vessels that were less leaky in response to VEGF and acute inflammatory mediators (Thurston et al. 2000). These results, combined with recent data showing increased Ang2 expression in several inflammatory diseases, linked the angiotensin–Tie system to the response of endothelial cells to inflammatory stimuli. In the past several years, tools to block Ang2 function in preclinical disease models have become available, and several functional studies have implicated Ang2 more directly in different aspects of inflammation.

In addition to abnormalities in the blood and lymphatic vasculatures, mice that are genetically deficient for Ang2 have deficits in rapid leukocyte recruitment to sites of inflammation (Fiedler et al. 2006). The response of Ang2 KO mice to intraperitoneal injection of thioglycollate, which induces rapid accumulation of neutrophils, was significantly reduced compared with wild-type mice. Ang2 KO mice also showed reduced recruitment of neutrophils in response to intraperitoneal injection of bacteria. The deficits in leukocyte recruitment in Ang2-deficient mice were associated with reduced firm adherence of neutrophils to vessels after exposure to TNF α (Fiedler et al. 2006).

Another study evaluated the role of Ang2 in a model of acute lung injury in mice (Bhandari et al. 2006). Ang2 KO or wild-type mice were exposed to hyperoxia, which induces lung injury, inflammation, and eventually death. The duration of survival in 100% oxygen was somewhat longer in Ang2 KO mice compared with wild-type mice. In addition, mice deficient for Ang2 had slightly reduced cellularity and protein levels in bronchio-aveolar lavage (BAL) fluid and less alveolar damage as revealed by histology.

A role for Ang2 in inflammation was also demonstrated in a model of airway infection

G. Thurston and C. Daly

(Tabruyn et al. 2010). Airway inoculation of *Mycoplasma pulmonis* in mice causes chronic inflammation accompanied by dramatic infiltration of leukocytes and remodeling of the tracheal microvasculature. Blockade of Ang2 during the infection resulted in near-complete inhibition of the vascular remodeling and significant reduction of infiltrating leukocytes. Ang2 blockade also reduced the inflammatory-associated changes in phenotype of the endothelium, such as increased expression of P- and E-selectin. In this study, the investigators used antibodies to immunolocalize phosphorylated Tie2 and reported a decrease in phospho-Tie2 following *Mycoplasma* infection (Tabruyn et al. 2010). Treatment of infected mice with Ang2-blocking antibodies resulted in a restoration of phospho-Tie2, suggesting that Ang2 was contributing to the loss of Tie2 activation. It will be important to validate the phospho-Tie2 immunohistochemistry with other methods for assessing the levels of Tie2 signaling. Interestingly, blockade of Ang2 also reduced the overall *M. pulmonis* load in the airways of infected mice compared with mice treated with control proteins (Tabruyn et al. 2010). This study suggests that Ang2 is a key mediator of the vascular remodeling and phenotypic changes that occur in chronic inflammation.

In the above settings of inflammation, Ang2 has effects that are seemingly consistent with a role as a Tie2 antagonist. Overall, the results suggest that in these models, Ang2 is a pro-inflammatory factor and helps promote vessel destabilization (Fig. 2). By extension of previous work suggesting that Ang1 can act to stabilize vessels and reduce inflammation, the presumption is that Ang2 must be acting via inhibition of Tie2 signaling (although effects on Tie2 phosphorylation and downstream signaling have not been fully documented). One explanation for the apparently contradictory findings is that the response of Tie2 to Ang2 (either activation or inhibition) is regulated in a context-specific fashion by mechanisms that are not yet understood. However, another explanation is that Tie2 activation, by either Ang1 or Ang2, does not universally lead to vessel stabilization but can produce different

phenotypic outcomes depending on the context of other cellular and molecular factors. Because Ang2 is normally produced in settings of low Ang1 and/or high levels of activating factors for endothelial cells, the context of Tie2 signaling during times of Ang2 expression could typically be very different from that of quiescent endothelial cells.

ANG2 PLAYS A ROLE IN TUMOR ANGIOGENESIS AND GROWTH

As detailed above, Ang2 is preferentially expressed in the endothelial cells of remodeling blood vessels, for example, in tumors. Ang2 expression has been documented in a range of human cancers, including glioblastoma, melanoma, prostate adenocarcinoma, and renal cell carcinoma (see above). The initial model for Ang2 function in tumors, based on high Ang2 expression in vessels before regression and on the purported role of Ang2 as a Tie2 antagonist, was that Ang2-mediated inhibition of Tie2 signaling is required for vessel destabilization, leading to vessel growth in the presence of VEGF or to vessel regression in the absence of VEGF (Hanahan 1997; Holash et al. 1999).

Initial studies aimed at defining a functional role for the Tie2 pathway in tumors used a Tie2 extracellular domain-Fc fusion protein, which prevents binding of both Ang1 and Ang2 to Tie2. Systemic delivery of soluble Tie2 provided modest growth inhibition of mammary tumors and melanoma in mice, suggesting that active signaling through Tie2 is required for robust tumor growth (Lin et al. 1998). Because soluble Tie2 blocked both Ang1 and Ang2, these studies did not shed light on the relative importance of Ang1 versus Ang2, nor did they support the model that antagonism of Tie2 activity promotes tumor angiogenesis and tumor growth.

A definitive role for Ang2 in tumor growth was established following the generation of specific inhibitors (monoclonal antibodies as well as peptide bodies) that bind to Ang2 and prevent its interaction with Tie2. Systemic delivery of Ang2 blockers (which neutralize both mouse and human Ang2) results in significant, but partial, inhibition of the growth of a range of



human tumor xenografts, including colorectal, breast, and epidermoid carcinoma (Oliner et al. 2004; Brown et al. 2010; Huang et al. 2010). Although some of the blockers used in these studies inhibit both Ang1 and Ang2, the use of completely specific Ang1 and Ang2 inhibitors has clearly established that Ang2 is the dominant Tie2 ligand in tumors. For example, an Ang1-specific peptide body has no effect on tumor growth and only marginally and inconsistently potentiates the effect of an Ang2-specific peptide body (Falcon et al. 2009; Coxon et al. 2010). Similarly, the Ang2-specific peptide body inhibits tumor growth to approximately the same extent as a dual anti-Ang1/Ang2 peptide body (Oliner et al. 2004; Coxon et al. 2010). Providing further support for the role of Ang2 in tumor growth, tumors implanted into Ang2 KO mice grew more slowly than those implanted into wild-type mice (Nasarre et al. 2009).

Consistent with an anti-angiogenic mechanism of action, Ang2 blockers decrease tumor vascularity (Falcon et al. 2009; Brown et al. 2010; Hashizume et al. 2010), apparently via inhibition of vessel sprouting and/or reduction of vessel size (Hashizume et al. 2010). Previous studies have shown that Tie2 activation causes enlargement of blood vessels in normal tissues via increased endothelial cell proliferation (Thurston et al. 2005). In tumors, Ang2 blockade significantly inhibits endothelial cell proliferation and significantly reduces the size of vessels (Falcon et al. 2009), consistent with a decrease in Tie2 activity. Studies in cultured endothelial cells have shown that Tie2 activation promotes endothelial cell survival (Kim et al. 2000a). Whether increased endothelial cell apoptosis also contributes to the decreased tumor vascularity that is observed following Ang2 blockade remains to be determined.

Although the role of Ang2 in tumors was initially believed to be antagonism of Tie2, some data support a model in which Ang2 promotes tumor growth via Tie2 activation. Support for this alternate model comes from studies of tumors (teratomas) formed from embryonic stem cells that lack VE-PTP, a Tie2 phosphatase that is specifically expressed in the vasculature (Li et al. 2009). When implanted

subcutaneously, embryonic stem cells are capable of differentiating into endothelial cells, and they contribute significantly to the vasculature of the teratoma. Teratomas derived from VE-PTP knockout ES cells have elevated levels of phosphorylated Tie2 and much larger blood vessels than wild-type or VE-PTP heterozygous tumors, consistent with chronic activation of Tie2. Interestingly, treatment of the VE-PTP knockout tumors with an Ang2-selective antibody decreased Tie2 phosphorylation and vessel size, suggesting that Ang2 is functioning as a Tie2 activator in this model (Li et al. 2009). This effect of Ang2 blockade on vessel size is consistent with the data from human tumor xenografts mentioned above that demonstrated decreased vessel size following treatment with an Ang2-specific blocker (Falcon et al. 2009).

Further evidence of Ang2 agonist activity comes from the studies cited above that examined the effect of combined treatment with Ang1-specific and Ang2-specific blockers. The fact that the Ang1-specific blocker potentiated (albeit quite modestly), rather than reversed, the effects of the Ang2-specific blocker on tumor growth and tumor vascularity strongly suggests that these effects of Ang2 blockade result from a decrease in Tie2 activity (Falcon et al. 2009). Interestingly, the situation becomes more complex when the effects of Ang2 blockade on “vessel normalization” are examined in the same tumors. Although the Ang2-specific blocker caused a significant increase in pericyte coverage and in PECAM localization at endothelial cell junctions, these effects were reversed by the Ang1-specific blocker (Falcon et al. 2009), suggesting that in this case the effects of Ang2 blockade result from an increase in Tie2 activity. One could imagine that different subsets of tumor vessels are exposed to different local concentrations of Ang2/Ang1 and that this determines the net effect of Ang2 blockade on Tie2 phosphorylation. For example, if most of the Tie2 agonist activity in sprouting vessels is mediated by Ang2, then Ang2 blockade would be expected to decrease Tie2 phosphorylation in these cells, even though Ang2 is a relatively weak agonist. If, on the other hand, there are somewhat higher levels of Ang1 in the vessels that

G. Thurston and C. Daly

are normalized following Ang2 blockade, then Ang2 inhibition would increase Tie2 phosphorylation by allowing greater access of Ang1 to Tie2. Although further work is clearly required to substantiate the agonist role of Ang2 in tumors, the available data strongly suggest that the effects of Ang2 blockade on tumor vascularity and growth result from a decrease in Tie2 signaling.

The picture that has emerged from these preclinical studies is that Ang2 blockade inhibits the growth of a broad range of tumors but that the effects on growth are relatively modest. This is in contrast to anti-VEGF agents, which provide complete growth inhibition in some of the same preclinical tumor models. Given that blockade of VEGF has proven to be beneficial but not curative in several human cancers (Duda et al. 2007), several groups have examined whether Ang2 blockade can potentiate the effects of VEGF inhibitors. Combined treatment with Ang2 and VEGF blockers has been shown to provide better inhibition of tumor growth than the single agents in a number of tumor models, including some that exhibit a very strong response to VEGF blockade (Brown et al. 2010; Hashizume et al. 2010; Huang et al. 2010). Consistent with the more potent effects on tumor growth, the combination treatment reduces tumor vascularity to a much greater extent than the single agents do (Hashizume et al. 2010). Thus, it appears that inhibition of Ang2 imposes an additional stress on tumor endothelial cells beyond that provided by VEGF blockade alone. The precise nature of the molecular events that ensue following Ang2 versus VEGF blockade and how these events combine to impair endothelial cell function remain an exciting subject for further investigation.

SUMMARY AND OUTSTANDING ISSUES

In recent years, the components of the angiopoietin–Tie pathway have been characterized. However, as is evident from this review, the networked effects of these components on vascular biology and pathology are not fully understood. In this review, we have focused on Ang2 because it has been a particularly enigmatic component

and has emerged as a preferred target for therapeutic blockade. Despite our poor understanding of the precise functional role of Ang2, two things are clear: Ang2 expression is very often increased in vascular remodeling and disease; and blocking Ang2 appears to benefit the course of the disease, whether it is airway inflammation, lung injury, or solid tumors.

Several key aspects of Ang2 biology need further investigation. One issue is to determine whether Ang2 produced by endothelial cells and acting in an autocrine manner is functionally similar to recombinant exogenous Ang2 (which has been applied in many experimental settings). If not, then what are the biochemical mechanisms? A second issue is to determine what factors are responsible for the up-regulation of Ang2 in disease settings. Although several factors have been reported to induce Ang2 expression in cultured cells, it is not clear if these are the relevant factors in vivo. Another key issue is to determine whether endogenous Ang2 can have different actions on Tie2 in different cellular contexts. An extension of this issue is to determine whether Tie2 activation (by either Ang1 or Ang2) can have different cellular effects in different contexts. To address this particular issue, we need to understand more about the key factors within tumor and inflammatory environments that determine the “context.” For example, we need to understand more about how angiopoietin–Tie signaling interacts with VEGF signaling. Finally, although several reports have described interactions of other proteins with angiopoietins and Tie2, it will be important to determine whether these interactions contribute functionally to the activity of angiopoietins in disease settings. Led by the ongoing clinical development of Ang2 inhibitors, the next few years promise to be another exciting period for research into the angiopoietin–Tie pathway.

ACKNOWLEDGMENTS

We thank the many outstanding members of the Regeneron scientific community who have helped shape our understanding of the angiopoietin–Tie system.

REFERENCES

- Augustin HG, Koh GY, Thurston G, Alitalo K. 2009. Control of vascular morphogenesis and homeostasis through the angiopoietin–Tie system. *Nat Rev Mol Cell Biol* **10**: 165–177.
- Baffert F, Thurston G, Rochon-Duck M, Le T, Brekken R, McDonald DM. 2004. Age-related changes in vascular endothelial growth factor dependency and angiopoietin-1-induced plasticity of adult blood vessels. *Circ Res* **94**: 984–992.
- Baumer S, Keller L, Holtmann A, Funke R, August B, Gamp A, Wolburg H, Wolburg-Buchholz K, Deutsch U, Vestweber D. 2006. Vascular endothelial cell-specific phosphotyrosine phosphatase (VE-PTP) activity is required for blood vessel development. *Blood* **107**: 4754–4762.
- Bhandari V, Choo-Wing R, Lee CG, Zhu Z, Nedrelo JH, Chupp GL, Zhang X, Matthay MA, Ware LB, Homer RJ, et al. 2006. Hyperoxia causes angiopoietin 2-mediated acute lung injury and necrotic cell death. *Nat Med* **12**: 1286–1293.
- Brown LF, Dezube BJ, Tognazzi K, Dvorak HF, Yancopoulos GD. 2000. Expression of Tie1, Tie2, and angiopoietins 1, 2, and 4 in Kaposi's sarcoma and cutaneous angiosarcoma. *Am J Pathol* **156**: 2179–2183.
- Brown JL, Cao ZA, Pinzon-Ortiz M, Kendrew J, Reimer C, Wen S, Zhou JQ, Tabrizi M, Emery S, McDermott B, et al. 2010. A human monoclonal anti-ANG2 antibody leads to broad antitumor activity in combination with VEGF inhibitors and chemotherapy agents in preclinical models. *Mol Cancer Ther* **9**: 145–156.
- Chen L, Yang Z, Wang G, Wang C. 2001. Expression of angiopoietin-2 gene and its receptor Tie2 in hepatocellular carcinoma. *J Tongji Med Univ* **21**: 228–235.
- Cho CH, Kammerer RA, Lee HJ, Steinmetz MO, Ryu YS, Lee SH, Yasunaga K, Kim KT, Kim I, Choi HH, et al. 2004. COMP-Ang1: A designed angiopoietin-1 variant with nonleaky angiogenic activity. *Proc Natl Acad Sci* **101**: 5547–5552.
- Coxon A, Bready J, Min H, Kaufman S, Leal J, Yu D, Lee TA, Sun JR, Estrada J, Bolon B, et al. 2010. Context-dependent role of angiopoietin-1 inhibition in the suppression of angiogenesis and tumor growth: Implications for AMG 386, an angiopoietin-1/2-neutralizing peptide. *Mol Cancer Ther* **9**: 2641–2651.
- Daly C, Wong V, Burova E, Wei Y, Zabski S, Griffiths J, Lai KM, Lin HC, Ioffe E, Yancopoulos GD, et al. 2004. Angiopoietin-1 modulates endothelial cell function and gene expression via the transcription factor FKHR (FOXO1). *Genes Dev* **18**: 1060–1071.
- Daly C, Pasnikowski E, Burova E, Wong V, Aldrich TH, Griffiths J, Ioffe E, Daly TJ, Fandl JP, Papadopoulos N, et al. 2006. Angiopoietin-2 functions as an autocrine protective factor in stressed endothelial cells. *Proc Natl Acad Sci* **103**: 15491–15496.
- Davis S, Aldrich TH, Jones PF, Acheson A, Compton DL, Jain V, Ryan TE, Bruno J, Radziejewski C, Maisonpierre PC, et al. 1996. Isolation of angiopoietin-1, a ligand for the TIE2 receptor, by secretion-trap expression cloning. *Cell* **87**: 1161–1169.
- Davis S, Papadopoulos N, Aldrich TH, Maisonpierre PC, Huang T, Kovac L, Xu A, Leidich R, Radziejewska E, Rafique A, et al. 2003. Angiopoietins have distinct modular domains essential for receptor binding, dimerization and superclustering. *Nat Struct Biol* **10**: 38–44.
- Davis JS, Yeo TW, Piera KA, Woodberry T, Celermajer DS, Stephens DP, Anstey NM. 2010. Angiopoietin-2 is increased in sepsis and inversely associated with nitric oxide-dependent microvascular reactivity. *Crit Care* **14**: R89. doi: 10.1186/cc9020.
- Detjen KM, Rieke S, Deters A, Schulz P, Rexin A, Vollmer S, Hauff P, Wiedenmann B, Pavel M, Scholz A. 2010. Angiopoietin-2 promotes disease progression of neuroendocrine tumors. *Clin Cancer Res* **16**: 420–429.
- Dominguez MG, Hughes VC, Pan L, Simmons M, Daly C, Anderson K, Noguera-Troise I, Murphy AJ, Valenzuela DM, Davis S, et al. 2007. Vascular endothelial tyrosine phosphatase (VE-PTP)-null mice undergo vasculogenesis but die embryonically because of defects in angiogenesis. *Proc Natl Acad Sci* **104**: 3243–3248.
- Duda DG, Batchelor TT, Willett CG, Jain RK. 2007. VEGF-targeted cancer therapy strategies: Current progress, hurdles and future prospects. *Trends Mol Med* **13**: 223–230.
- Dumont DJ, Gradwohl G, Fong GH, Puri MC, Gertsenstein M, Auerbach A, Breitman ML. 1994. Dominant-negative and targeted null mutations in the endothelial receptor tyrosine kinase, tek, reveal a critical role in vasculogenesis of the embryo. *Genes Dev* **8**: 1897–1909.
- Durkin AJ, Bloomston M, Yeatman TJ, Gilbert-Barnes E, Cojita D, Rosemurgy AS, Zervos EE. 2004. Differential expression of the Tie-2 receptor and its ligands in human pancreatic tumors. *J Am Coll Surg* **199**: 724–731.
- Emoto M, Charnock-Jones DS, Licence DR, Ishiguro M, Kawai M, Yanaiharu A, Saito T, Hachisuga T, Iwasaki H, Kawarabayashi T, et al. 2004. Localization of the VEGF and angiopoietin genes in uterine carcinosarcoma. *Gynecol Oncol* **95**: 474–482.
- Falcon BL, Hashizume H, Koumoutsakos P, Chou J, Bready JV, Coxon A, Oliner JD, McDonald DM. 2009. Contrasting actions of selective inhibitors of angiopoietin-1 and angiopoietin-2 on the normalization of tumor blood vessels. *Am J Pathol* **175**: 2159–2170.
- Fiedler U, Scharpfenecker M, Koidl S, Hegen A, Grunow V, Schmidt JM, Kriz W, Thurston G, Augustin HG. 2004. The Tie-2 ligand angiopoietin-2 is stored in and rapidly released upon stimulation from endothelial cell Weibel-Palade bodies. *Blood* **103**: 4150–4156.
- Fiedler U, Reiss Y, Scharpfenecker M, Grunow V, Koidl S, Thurston G, Gale NW, Witzernath M, Rosseau S, Suttorp N, et al. 2006. Angiopoietin-2 sensitizes endothelial cells to TNF- α and has a crucial role in the induction of inflammation. *Nat Med* **12**: 235–239.
- Fukuhara S, Sako K, Minami T, Noda K, Kim HZ, Kodama T, Shibuya M, Takakura N, Koh GY, Mochizuki N. 2008. Differential function of Tie2 at cell–cell contacts and cell–substratum contacts regulated by angiopoietin-1. *Nat Cell Biol* **10**: 513–526.
- Fuxe J, Lashnits E, O'Brien S, Baluk P, Tabruyn SP, Kuhnert F, Kuo C, Thurston G, McDonald DM. 2010. Angiopoietin/Tie2 signaling transforms capillaries into venules primed for leukocyte trafficking in airway inflammation. *Am J Pathol* **176**: 2009–2018.
- Gale NW, Thurston G, Hackett SF, Renard R, Wang Q, McClain J, Martin C, Witte C, Witte MH, Jackson D,

G. Thurston and C. Daly

- et al. 2002. Angiopoietin-2 is required for postnatal angiogenesis and lymphatic patterning, and only the latter role is rescued by Angiopoietin-1. *Dev Cell* **3**: 411–423.
- Gamble JR, Drew J, Trezise L, Underwood A, Parsons M, Kasminkas L, Rudge J, Yancopoulos G, Vadas MA. 2000. Angiopoietin-1 is an antipermeability and anti-inflammatory agent in vitro and targets cell junctions. *Circ Res* **87**: 603–607.
- Goede V, Coutelle O, Neuneier J, Reinacher-Schick A, Schnell R, Koslowsky TC, Weihrauch MR, Cremer B, Kashkar H, Odenthal M, et al. 2010. Identification of serum angiopoietin-2 as a biomarker for clinical outcome of colorectal cancer patients treated with bevacizumab-containing therapy. *Br J Cancer* **103**: 1407–1414.
- Hanahan D. 1997. Signaling vascular morphogenesis and maintenance. *Science* **277**: 48–50.
- Hashizume H, Falcon BL, Kuroda T, Baluk P, Coxon A, Yu D, Bready JV, Oliner JD, McDonald DM. 2010. Complementary actions of inhibitors of angiopoietin-2 and VEGF on tumor angiogenesis and growth. *Cancer Res* **70**: 2213–2223.
- Helfrich I, Edler L, Sucker A, Thomas M, Christian S, Schadendorf D, Augustin HG. 2009. Angiopoietin-2 levels are associated with disease progression in metastatic malignant melanoma. *Clin Cancer Res* **15**: 1384–1392.
- Holash J, Wiegand SJ, Yancopoulos GD. 1999. New model of tumor angiogenesis: Dynamic balance between vessel regression and growth mediated by angiopoietins and VEGF. *Oncogene* **18**: 5356–5362.
- Huang H, Lai JY, Do J, Liu D, Li L, Del Rosario J, Doppalapudi VR, Pirie-Shepherd S, Levin N, Bradshaw C, et al. 2010. Specifically targeting angiopoietin-2 inhibits angiogenesis, Tie2-expressing monocyte infiltration, and tumor growth. *Clin Cancer Res* **17**: 1001–1011.
- Jho D, Mehta D, Ahmmed G, Gao XP, Tirupathi C, Broman M, Malik AB. 2005. Angiopoietin-1 opposes VEGF-induced increase in endothelial permeability by inhibiting TRPC1-dependent Ca²⁺ influx. *Circ Res* **96**: 1282–1290.
- Joussen AM, Poulaki V, Tsujikawa A, Qin W, Qaum T, Xu Q, Moromizato Y, Bursell SE, Wiegand SJ, Rudge J, et al. 2002. Suppression of diabetic retinopathy with angiopoietin-1. *Am J Pathol* **160**: 1683–1693.
- Kim I, Kim HG, So JN, Kim JH, Kwak HJ, Koh GY. 2000a. Angiopoietin-1 regulates endothelial cell survival through the phosphatidylinositol 3'-kinase/Akt signal transduction pathway. *Circ Res* **86**: 24–29.
- Kim I, Kim JH, Moon SO, Kwak HJ, Kim NG, Koh GY. 2000b. Angiopoietin-2 at high concentration can enhance endothelial cell survival through the phosphatidylinositol 3'-kinase/Akt signal transduction pathway. *Oncogene* **19**: 4549–4552.
- Kim I, Kim JH, Ryu YS, Liu M, Koh GY. 2000c. Tumor necrosis factor- α upregulates angiopoietin-2 in human umbilical vein endothelial cells. *Biochem Biophys Res Commun* **269**: 361–365.
- Kim I, Moon SO, Park SK, Chae SW, Koh GY. 2001. Angiopoietin-1 reduces VEGF-stimulated leukocyte adhesion to endothelial cells by reducing ICAM-1, VCAM-1, and E-selectin expression. *Circ Res* **89**: 477–479.
- Li JJ, Huang YQ, Basch R, Karpatkin S. 2001. Thrombin induces the release of angiopoietin-1 from platelets. *Thromb Haemost* **85**: 204–206.
- Li X, Hahn CN, Parsons M, Drew J, Vadas MA, Gamble JR. 2004. Role of protein kinase C δ in thrombin-induced endothelial permeability changes: Inhibition by angiopoietin-1. *Blood* **104**: 1716–1724.
- Li Z, Huang H, Boland P, Dominguez MG, Burfeind P, Lai KM, Lin HC, Gale NW, Daly C, Auerbach W, et al. 2009. Embryonic stem cell tumor model reveals role of vascular endothelial receptor tyrosine phosphatase in regulating Tie2 pathway in tumor angiogenesis. *Proc Natl Acad Sci* **106**: 22399–22404.
- Limaye N, Wouters V, Uebelhoer M, Tuominen M, Wirkkala R, Mulliken JB, Eklund L, Boon LM, Vikkula M. 2009. Somatic mutations in angiopoietin receptor gene TEK cause solitary and multiple sporadic venous malformations. *Nat Genet* **41**: 118–124.
- Lin P, Buxton JA, Acheson A, Radziejewski C, Maisonpierre PC, Yancopoulos GD, Channon KM, Hale LP, Dewhirst MW, George SE, et al. 1998. Antiangiogenic gene therapy targeting the endothelium-specific receptor tyrosine kinase Tie2. *Proc Natl Acad Sci* **95**: 8829–8834.
- Maisonpierre PC, Suri C, Jones PF, Bartunkova S, Wiegand SJ, Radziejewski C, Compton D, McClain J, Aldrich TH, Papadopoulos N, et al. 1997. Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. *Science* **277**: 55–60.
- Mandriota SJ, Pepper MS. 1998. Regulation of angiopoietin-2 mRNA levels in bovine microvascular endothelial cells by cytokines and hypoxia. *Circ Res* **83**: 852–859.
- Moon WS, Park HS, Yu KH, Jang KY, Kang MJ, Park H, Tarnawski AS. 2006. Expression of angiopoietin 1, 2 and their common receptor Tie2 in human gastric carcinoma: implication for angiogenesis. *J Korean Med Sci* **21**: 272–278.
- Nasarre P, Thomas M, Kruse K, Helfrich I, Wolter V, Deppermann C, Schadendorf D, Thurston G, Fiedler U, Augustin HG. 2009. Host-derived angiopoietin-2 affects early stages of tumor development and vessel maturation but is dispensable for later stages of tumor growth. *Cancer Res* **69**: 1324–1333.
- Nykanen AI, Pajusola K, Krebs R, Keranen MA, Raisky O, Koskinen PK, Alitalo K, Lemstrom KB. 2006. Common protective and diverse smooth muscle cell effects of AAV-mediated angiopoietin-1 and -2 expression in rat cardiac allograft vasculopathy. *Circ Res* **98**: 1373–1380.
- Oliner J, Min H, Leal J, Yu D, Rao S, You E, Tang X, Kim H, Meyer S, Han SJ, et al. 2004. Suppression of angiogenesis and tumor growth by selective inhibition of angiopoietin-2. *Cancer Cell* **6**: 507–516.
- Puri MC, Rossant J, Alitalo K, Bernstein A, Partanen J. 1995. The receptor tyrosine kinase TIE is required for integrity and survival of vascular endothelial cells. *EMBO J* **14**: 5884–5891.
- Saharinen P, Kerkela K, Ekman M, Marron M, Brindle N, Lee GM, Augustin H, Koh GY, Alitalo K. 2005. Multiple angiopoietin recombinant proteins activate the Tie1 receptor tyrosine kinase and promote its interaction with Tie2. *J Cell Biol* **169**: 239–243.



- Saharinen P, Eklund L, Miettinen J, Wirkkala R, Anisimov A, Winderlich M, Nottebaum A, Vestweber D, Deutsch U, Koh GY, et al. 2008. Angiopoietins assemble distinct Tie2 signalling complexes in endothelial cell–cell and cell–matrix contacts. *Nat Cell Biol* **10**: 527–537.
- Sato TN, Qin Y, Kozak CA, Audus KL. 1993. Tie-1 and tie-2 define another class of putative receptor tyrosine kinase genes expressed in early embryonic vascular system. *Proc Natl Acad Sci* **90**: 9355–9358.
- Sato TN, Tozawa Y, Deutsch U, Wolburg-Buchholz K, Fujiwara Y, Gendron-Maguire M, Gridley T, Wolburg H, Risau W, Qin Y. 1995. Distinct roles of the receptor tyrosine kinases Tie-1 and Tie-2 in blood vessel formation. *Nature* **376**: 70–74.
- Scholz A, Rehm VA, Rieke S, Derkow K, Schulz P, Neumann K, Koch I, Pascu M, Wiedenmann B, Berg T, et al. 2007. Angiopoietin-2 serum levels are elevated in patients with liver cirrhosis and hepatocellular carcinoma. *Am J Gastroenterol* **102**: 2471–2481.
- Stratmann A, Risau W, Plate KH. 1998. Cell type-specific expression of angiopoietin-1 and angiopoietin-2 suggests a role in glioblastoma angiogenesis. *Am J Pathol* **153**: 1459–1466.
- Suri C, Jones PF, Patan S, Bartunkova S, Maisonpierre PC, Davis S, Sato TN, Yancopoulos GD. 1996. Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis. *Cell* **87**: 1171–1180.
- Suri C, McClain J, Thurston G, McDonald DM, Zhou H, Oldmixon EH, Sato TN, Yancopoulos GD. 1998. Increased vascularization in mice overexpressing angiopoietin-1. *Science* **282**: 468–471.
- Tabruyn SP, Colton K, Morisada T, Fuxe J, Wiegand SJ, Thurston G, Coyle AJ, Connor J, McDonald DM. 2010. Angiopoietin-2-driven vascular remodeling in airway inflammation. *Am J Pathol* **177**: 3233–3243.
- Teichert-Kuliszewska K, Maisonpierre PC, Jones N, Campbell AI, Master Z, Bendeck MP, Alitalo K, Dumont DJ, Yancopoulos GD, Stewart DJ. 2001. Biological action of angiopoietin-2 in a fibrin matrix model of angiogenesis is associated with activation of Tie2. *Cardiovasc Res* **49**: 659–670.
- Thurston G, Suri C, Smith K, McClain J, Sato TN, Yancopoulos GD, McDonald DM. 1999. Leakage-resistant blood vessels in mice transgenically overexpressing angiopoietin-1. *Science* **286**: 2511–2514.
- Thurston G, Rudge JS, Ioffe E, Zhou H, Ross L, Croll SD, Glazer N, Holash J, McDonald DM, Yancopoulos GD. 2000. Angiopoietin-1 protects the adult vasculature against plasma leakage. *Nat Med* **6**: 460–463.
- Thurston G, Wang Q, Baffert F, Rudge J, Papadopoulos N, Jean-Guillaume D, Wiegand S, Yancopoulos GD, McDonald DM. 2005. Angiopoietin 1 causes vessel enlargement, without angiogenic sprouting, during a critical developmental period. *Development* **132**: 3317–3326.
- van der Heijden M, van Nieuw Amerongen GP, Chedamni S, van Hinsbergh VW, Johan Groeneveld AB. 2009. The angiopoietin–Tie2 system as a therapeutic target in sepsis and acute lung injury. *Expert Opin Ther Targets* **13**: 39–53.
- Vikkula M, Boon LM, Carraway KL III, Calvert JT, Diamonti AJ, Goumnerov B, Pasyk KA, Marchuk DA, Warman ML, Cantley LC, et al. 1996. Vascular dysmorphogenesis caused by an activating mutation in the receptor tyrosine kinase TIE2. *Cell* **87**: 1181–1190.
- Winderlich M, Keller L, Cagna G, Broermann A, Kamenyeva O, Kiefer F, Deutsch U, Nottebaum AF, Vestweber D. 2009. VE-PTP controls blood vessel development by balancing Tie-2 activity. *J Cell Biol* **185**: 657–671.
- Witzenbichler B, Westermann D, Knueppel S, Schultheiss HP, Tschope C. 2005. Protective role of angiopoietin-1 in endotoxic shock. *Circulation* **111**: 97–105.
- Xu Y, Yu Q. 2001. Angiopoietin-1, unlike angiopoietin-2, is incorporated into the extracellular matrix via its linker peptide region. *J Biol Chem* **276**: 34990–34998.
- Yuan HT, Venkatesha S, Chan B, Deutsch U, Mammoto T, Sukhatme VP, Woolf AS, Karumanchi SA. 2007. Activation of the orphan endothelial receptor Tie1 modifies Tie2-mediated intracellular signaling and cell survival. *FASEB J* **21**: 3171–3183.
- Zagzag D, Hooper A, Friedlander DR, Chan W, Holash J, Wiegand SJ, Yancopoulos GD, Grumet M. 1999. In situ expression of angiopoietins in astrocytomas identifies angiopoietin-2 as an early marker of tumor angiogenesis. *Exp Neurol* **159**: 391–400.



The Complex Role of Angiopoietin-2 in the Angiopoietin–Tie Signaling Pathway

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Cold Spring Harb Perspect Med 2012; doi: 10.1101/cshperspect.a006650 originally published online April 10, 2012

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