Tie1: an orphan receptor provides context for angiopoietin-2/Tie2 signaling

Sarah B. Mueller[1,2] and Christopher D. Kontos[1,2,3]

[1]Duke University School of Medicine, Medical Scientist Training Program, [2]Department of Pharmacology and Cancer Biology, and [3]Division of Cardiology, Department of Medicine, Duke University Medical Center, Durham, North Carolina, USA.

Angiopoietin-1/Tie2 (ANG1/Tie2) signaling is well documented as regulating angiogenesis and vessel maturation. This pathway is complicated by involvement of the orphan receptor Tie1, which has been implicated as both a positive and negative regulator of ANG1/Tie2 signaling, and ANG2, which can serve as both a Tie2 agonist and antagonist, depending on the context. Two papers in this issue of the *JCI* provide new insight into this complicated pathway. Korhonen et al. reveal that Tie1 acts to modulate the effects of ANG1 and ANG2 on Tie2 in vitro and in vivo. Kim et al. demonstrate that ANG2 acts as a Tie2 agonist in non-pathological conditions, whereas in the setting of inflammation, ANG2 functions as a Tie2 antagonist and promotes vascular dysfunction. Both studies indicate that inflammation promotes cleavage of the ectodomain of Tie1 and that this cleavage event corresponds with the switch of ANG2 from a Tie2 agonist to an antagonist. The results of these studies lay the groundwork for future strategies to therapeutically exploit this pathway in diseases characterized by adverse vascular remodeling and increased permeability.

Angiopoietin/Tie receptor signaling: a complex relationship

Tie receptors (tyrosine kinases with immunoglobulin and epidermal growth factor homology domains) are a family of receptor tyrosine kinases (RTKs) that are expressed primarily in endothelial cells (ECs) (1, 2). Both Tie1 (encoded by *TIE1*) and Tie2 (encoded by *TEK*) are required for appropriate developmental angiogenesis and vessel maturation in mice and are therefore attractive therapeutic targets for a variety of diseases characterized by vascular dysfunction (3–5).

Angiopoietin-1 (ANG1), the first Tie2 ligand identified, behaves as a canonical RTK agonist when bound to Tie2 (6). ANG1/Tie2 signaling promotes EC survival and vascular integrity, is associated with a stable, quiescent EC phenotype, and is likely the primary factor responsible for baseline

Tie2 activation in resting adult tissues (7–10). ANG1 also plays a role in angiogenesis, serving as an EC chemoattractant, and acts as a weak EC mitogen to promote vascular enlargement (11–13). ANG2, which has homology to ANG1, was originally described as a competitive antagonist of ANG1/Tie2 signaling based on the fact that it binds Tie2 with nearly the same affinity as ANG1 (~3 nM) and blocks ANG1-induced Tie2 phosphorylation (14, 15). However, ANG2 was later shown to induce Tie2 activation in a variety of contexts, including at high concentrations, in stressed ECs, and in a fibrin matrix model of angiogenesis (16–19). Thus, ANG2 is now largely accepted as a context-dependent agonist/antagonist for Tie2.

Unlike Tie2, Tie1 is considered to be an orphan receptor, as it is unable to bind directly to any of the angiopoietins, and no other natural ligands have been identified.

This lack of a natural ligand for Tie1 has significantly complicated investigation of its function (20). However, in the presence of Tie2, Tie1 activation by ANG1 or a modified recombinant oligomeric ANG1 protein (COMP-ANG1) has been shown to occur (21, 22). Prior in vitro studies suggested that Tie1 functions primarily as a negative regulator of Tie2 signaling (reviewed in ref. 23), but recent in vivo data show that Tie1 can both negatively and positively regulate Tie2 signaling during angiogenesis, depending on the cellular context. For example, Tie1 expression in stalk cells stabilizes Tie1-Tie2 heteromultimers at the cell membrane, perpetuating ANG1/Tie2 signaling, while Tie1 expression in tip cells negatively regulates Tie2 surface presentation (24).

In addition to vascular development, angiogenesis, and vessel stabilization, the angiopoietins and Tie receptors have been shown to play important roles in inflammation, both in vitro and in vivo. ANG1/Tie2 activity is generally associated with antiinflammatory signaling. For example, ANG1 reduces the adhesion and transendothelial migration of leukocytes, in part via decreased expression of inflammatory adhesion molecules and cytokines following recruitment of the NF-κB inhibitor ABIN-2 (7, 25, 26). Conversely, Tie1 has been associated with EC activation and inflammation. In vitro, Tie1 positively regulates the expression of proinflammatory genes (27, 28), while EC-specific deletion of *Tie1* reduces expression of inflammatory adhesion molecules and results in a dose-dependent improvement of atherosclerosis in *Apoe*^{−/−} mice (29).

The role of ANG2 in inflammation may also be context dependent. Based on the antagonistic effects of ANG2 on ANG1/Tie2 signaling, ANG2 may be considered a proinflammatory factor. Moreover, ANG2 release from Weibel-Palade bodies sensitizes ECs to the inflammatory effects of TNF-α (30), and ANG2 has also been shown to promote β$_2$ integrin–dependent myeloid cell infiltra-

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tion in vivo, even in the absence of inflammatory stimuli (31). Conversely, ANG2 has been shown to promote nitric oxide release and is considered to be atheroprotective in *Apoe*⁻/⁻ mice, supporting an antiinflammatory function in this model (31).

A better understanding of how the angiopoietins and Tie receptors function in inflammation may have clinical implications. For example, targeting this system may be valuable in numerous disorders of dysregulated inflammation and vascular integrity. Indeed, data from several clinical studies indicate that elevated levels of ANG2 are associated with poor prognosis and increased mortality in sepsis, regardless of microbial etiology (32, 33), while elevated levels of ANG1 are associated with decreased mortality (34). Additionally, compared with their WT counterparts, mice heterozygous for *Ang2* exhibit a substantial improvement in survival following both cecal ligation-perforation and LPS-endotoxemia models of sepsis (35).

While much has been learned about angiopoietin/Tie receptor signaling over the last 20 years, two critical questions have remained unanswered. First, what are the mechanisms that are responsible for the context-dependent effects of ANG2 in both inflammation and angiogenesis? Second, how does Tie1 regulate angiopoietin/Tie2 signaling? In this issue, two complementary studies provide critical insight into both of these questions. Specifically, Kim et al. address the issue of the context-dependent effects of ANG2 (36), while Korhonen et al. investigate the role of Tie1 in angiopoietin/Tie2 signaling (37). Together, these studies provide clinically relevant information by focusing on angiopoietin/Tie receptor signaling in the regulation of vascular remodeling in infection and/or inflammation.

The effect of inflammation

Using inhibitory antibodies targeting ANG2 and Tie2 along with powerful mouse models, Kim et al. demonstrated that ANG2 antagonism of Tie2 promotes loss of vascular integrity during infection with *Mycoplasma pulmonis* (36). Moreover, compromised vascular integrity was further exacerbated by antibody-mediated inhibition of Tie2 activation. Consistent with previous studies of septic patients and preclinical sepsis models, Kim and colleagues report a reduc-

tion of vascular remodeling, including vascular leak during infection, by inhibitory ANG2-targeting antibodies or by administration of exogenous ANG1; however, this group also determined that ANG2 acts as a Tie2 agonist under pathogen-free conditions, whether it is expressed systemically via adenovirus or in the endothelium of transgenic mice. Moreover, inflammation (infection, TNF-α, or LPS) was shown to induce a critical contextual switch in the effects of ANG2, promoting the shift from agonist to antagonist. Kim et al. demonstrated that this change in function is mediated through a feedback loop that involves AKT inhibition, FOXO1 activation, increased ANG2 expression, and subsequent adverse vascular remodeling. But the question remains: how does inflammation allow ANG2 to become a Tie2 antagonist? Kim et al. partially addressed this question by capitalizing on the results of Korhonen et al. (37) and have shown that inflammation induces shedding of the Tie1 ectodomain, which renders ANG2 a Tie2 antagonist, although the precise mechanism or mechanisms of this process remain undetermined.

In a complementary paper, Korhonen et al. address the role of Tie1 in the regulation of Tie2 by both ANG1 and ANG2 (37). Using in vitro techniques, including fluorescence (Förster) resonance energy transfer (FRET) and fluorescence lifetime imaging microscopy (FLIM), these authors demonstrated that Tie1 and Tie2 interact in the absence of ligand, confirming previously published results (38). Both in cultured ECs and in conditional *Tie1*-knockout mice, endothelial expression of Tie1 was required for the agonist effects of both ANG1 and autocrine ANG2 on Tie2 activation and vascular remodeling. Furthermore, in support of the Kim et al. study (36), Korhonen and colleagues also have shown that endotoxemia-induced cleavage of the Tie1 ectodomain reduces ANG2-mediated Tie2 phosphorylation, decreases downstream AKT activation, and increases nuclear localization of FOXO1. Based on these findings, Korhonen and colleagues propose a model in which Tie1 interacts with Tie2 at baseline to promote angiopoietin signaling, but is cleaved in the setting of inflammation, resulting in loss of ANG2 agonist activity and thereby promoting adverse vascular remodeling and leakiness. Importantly, although Tie1 cleavage

diminishes the agonist effects of ANG1, these effects are not completely lost.

These two groups of investigators used distinct approaches to show that inflammation-mediated loss of Tie1 expression is responsible for the context-dependent effects of ANG2. Furthermore, these studies provide an important step toward better understanding this complex ligand/receptor signaling pathway. It should be noted, however, that a limitation of both reports is that, although the loss of Tie1 was associated with ANG2's antagonist effects, a causal link between Tie1 and ANG2 was not demonstrated. Such a relationship could possibly be demonstrated by generating a noncleavable Tie1 mutant to determine whether sustained expression of Tie1 at the plasma membrane in the setting of inflammation would reverse the antagonistic effects of ANG2. Although the site of TNF-α–induced Tie1 cleavage has been identified (39), the specific protease that mediates this cleavage has not, and targeting receptor cleavage would likely prove challenging. The exact mechanism by which the presence of Tie1 alters ANG/Tie2 signaling is also unclear. For example, does the Tie1-Tie2 interaction alter ANG binding? Are ANG-mediated signals differentially transduced in the presence of Tie1? Or is ANG/Tie2 signaling altered by the Tie1 endodomain? There is some precedent for all of these possibilities. Structural data have shown that a specific domain within ANG1 confers agonist activity, although this was suggested to result in dissociation of Tie1 and Tie2 rather than association of the receptors (40). The Tie1 endodomain has been shown to associate with SHP2, although the functional significance of this interaction remains unclear (41). As noted by Korhonen et al., acute and chronic inflammation may have very different effects on Tie1 cleavage and ANG/Tie2 signaling (37), and understanding these differences may help explain how loss of Tie1 could have beneficial effects in the context of atherosclerosis and detrimental effects in acute infectious or inflammatory processes.

Remaining questions and implications for therapy

In light of these new findings, it is important to recognize that both Kim et al. and Korhonen et al. demonstrated agonist

effects of ANG2 following overexpression of ANG2 in the absence of inflammation or infection (36, 37). Because ANG2 is typically expressed at high levels only in remodeling vessels or in pathological conditions, it seems unlikely to behave as an agonist under physiological conditions. The notion that ANG2 does not routinely serve as an agonist is further supported by the fact that ANG2 is upregulated by FOXO1 following inhibition of ANG1/Tie2/AKT signaling. Prior reports of ANG2 activating Tie2 have been primarily in cultured ECs rather than in vivo; thus, the context in which this ANG2 action on Tie2 has been shown previously may not be physiological. However, the results presented by Kim et al. and Korhonen et al. highlight an important therapeutic opportunity — the potential to convert ANG2 into an agonist in conditions such as sepsis — in which ANG2 concentrations are very high. Further studies to improve our understanding of the mechanisms by which inflammation cleaves Tie1 and alters the function of ANG2 as well as identification of downstream signaling pathways that regulate this switch could provide therapeutic targets for effectively converting high circulating levels of ANG2 into an ANG1 equivalent, thereby promoting vascular normalization, inhibiting vascular leak, and reducing morbidity and mortality in conditions such as sepsis. Korhonen et al. show that, although COMP-ANG1 is able to partially overcome the inhibitory effects of inflammation on ANG1-mediated Tie2 activation, these effects are nonetheless blunted (37), likely in part because of the high levels of antagonistic ANG2. Approaches to promote ANG2 agonist activity may therefore be more effective than delivery of ANG1 or COMP-ANG1 or may act synergistically with ANG1-based therapies.

Recent evidence provides support for the potential efficacy of pharmacological approaches for redirecting ANG2 toward vascular normalization. Because ANG2 fails to induce Tie2 phosphorylation, it has long been speculated that the antagonist effects of ANG2 might be linked to recruitment of a protein tyrosine phosphatase (PTP). One such candidate molecule is vascular endothelial–PTP (VE-PTP), which has been shown to associate with, dephosphorylate, and inhibit Tie2 (42). Although some evidence suggests that VE-PTP is not responsible for ANG2's antagonistic effects (43), pharmacological inhibition of VE-PTP potently activates Tie2, an effect that persists even in the presence of high ANG2 concentrations, such as in diabetic macular edema and cerebral ischemia (44, 45). VE-PTP inhibition has been advanced to clinical trials for eye disease (46, 47) and has shown promise in LPS-induced vascular leak (48). It is unknown whether VE-PTP's effects on Tie2 are influenced by the presence of Tie1 or its cleavage. Elucidation of the detailed molecular mechanisms responsible for ANG2's antagonist effects may result in additional pharmacological approaches to promote vascular normalization in disease states.

Further investigation of the complex and dynamic interactions among the Tie receptors, the angiopoietins, and their molecular regulators, including VE-PTP and other as-yet-unknown modulators, will be critical for maximizing the therapeutic potential of this signaling pathway. In this regard, the results of Kim et al. and Korhonen et al. provide critical new insights into the mechanisms regulating ANG2's context dependence and its regulation by Tie1.

Acknowledgments

Address correspondence to: Christopher Kontos, Department of Medicine, Division of Cardiology, Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, North Carolina 27710, USA. Phone: 919.684.2119; E-mail: cdkontos@duke.edu.

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