Molecular control of angiopoietin signalling

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Abstract

The angiopoietins act through the endothelial receptor tyrosine kinase Tie2 to regulate vessel maturation in angiogenesis and control quiescence and stability of established vessels. The activating ligand, Ang1 (angiopoietin-1), is constitutively expressed by perivascular cells, and the ability of endothelial cells to respond to the ligand is controlled at the level of the Ang1 receptor. This receptor interacts with the related protein Tie1 on the cell surface, and Tie1 inhibits Ang1 signalling through Tie2. The responsiveness of endothelium to Ang1 is determined by the relative levels of Tie2 and the inhibitory co-receptor Tie1 in the cells. Tie1 undergoes regulated ectodomain cleavage which is stimulated by a range of factors including VEGF (vascular endothelial growth factor), inflammatory cytokines and changes in shear stress. Ectodomain cleavage of Tie1 relieves inhibition of Tie2 and enhances Ang1 signalling. This mechanism regulates Ang1 signalling without requiring changes in the level of the ligand and allows Ang1 signalling to be co-ordinated with other signals in the cellular environment. Regulation of signalling at the level of receptor responsiveness may be an important adaptation in systems in which an activating ligand is normally present in excess or where the ligand provides a constitutive maintenance signal.

Introduction

The angiopoietins are a family of secreted glycoprotein ligands of approximately 70 kDa acting primarily on the vasculature to control blood vessel development and stability [1]. These ligands bind the endothelial RTK (receptor tyrosine kinase) Tie2 and this is the primary dedicated receptor for the angiopoietins mediating their effects [2]. Four distinct angiopoietins have been described: Ang1 (angiopoietin-1)–Ang4, with Ang3 and Ang4 being mouse and human orthologues respectively [2–4]. Ang1 and Ang2 are the best characterized of these ligands. In vascular development, Ang1 acts to control vessel branching and diameter [5], whereas post-development, the ligand is a potent inhibitor of vascular permeability, suppresses vessel inflammation, inhibits endothelial apoptosis and promotes vessel survival [6,7]. In contrast, Ang2 can act as a context-dependent antagonist of Ang1, suppressing its pro-stabilizing effects and promoting vessel remodelling [3]. Ang1 is produced by cells surrounding the blood vessel and also binds extracellular matrix on the vessel, providing a pool of ligand to act on the Tie2 receptor [2,8,9]. Ang1 provides a constitutive pro-stabilizing signal to underlying endothelial cells [10]. Recent data show that, although levels of Ang1 may not be actively regulated, the ability of the ligand to act on its receptor is tightly controlled, allowing Ang1 signalling to be closely co-ordinated with other signals in the cellular microenvironment.

Key words: angiogenesis, angiopoietin, endothelium, inflammatory cytokine, receptor tyrosine kinase (RTK), Tie2.

Abbreviations used: Ang1 (etc.), angiopoietin-1 (etc.); FReD, fibrinogen-related domain; NF-κB; nuclear factor κB; RTK, receptor tyrosine kinase; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor.

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Angiopoietins

The angiopoietins share a similar overall structure, comprising a C-terminal FReD (fibrinogen-related domain), upstream of which is a flexible linker sequence, then a coiled-coil domain and a superclustering region at the N-terminus [2,3]. Binding to Tie2 occurs via the FReD domain, specifically a 74-residue portion at the C-terminus of the domain designated the P-motif [11]. At the amino acid level, sequence identity between Ang1 and Ang2 is approximately 60% overall and approximately 73% in the P-domains. The coiled-coil domains in the angiopoietins along with the N-terminal superclustering region mediate homo-oligomerization of the proteins [12,13]. Ang1 exists predominantly as tetrameric and higher-order oligomers, and this oligomerization state of Ang1 is important for its activity [13,14]. The ligand must present to its receptor as a tetramer or higher-order oligomer in order to activate the receptor [13,14].

As indicated above, the primary receptor specifically binding and mediating angiopoietin signalling is the RTK Tie2 [2]. This receptor is expressed primarily on endothelial cells [15,16]. However, angiopoietins can also bind integrins, including α2β1, α5β1, αvβ3 and αvβ5, and this interaction does mediate some actions of the ligands [17–20]. Indeed, integrin binding allows Ang1 to induce signalling and functional effects on non-endothelial cells lacking the Tie2 receptor, such as cardiac and skeletal myocytes [17].

Ang1 is an activating ligand for Tie2 and acts to stimulate endothelial migration and cytoskeletal reorganization, inhibiting apoptosis [6,7]. These cellular effects translate into protective effects on blood vessels in vivo, including suppression of vessel inflammation and permeability, promoting vessel...
survival [6,7]. In angiogenesis, Ang1 is necessary for correct vessel patterning and promoting formation of quiescent functional vasculature [21]. Expression of Ang1 occurs in perivascular cells, including pericytes and vascular smooth muscle cells [2,8,9]. Hypoxia, VEGF (vascular endothelial growth factor) and PDGF (platelet-derived growth factor B) have been reported to elevate Ang1 expression in pericytes or smooth muscle cells [22,23], although the ligand is suppressed by growth factors in some other cell types [24].

Ang2 is expressed by endothelial cells and its production is regulated at the transcriptional level by factors such as hypoxia and VEGF [25,26]. Expression of Ang2 in vivo is increased in tissues undergoing angiogenesis and vascular remodelling [3]. In addition, Ang2 levels are dramatically elevated in a range of diseases associated with vascular dysfunction, particularly inflammatory disorders such as sepsis [7]. The ligand is stored within the endothelium in Weibel–Palade bodies and can be rapidly released in response to the pharmacological activator phorbol ester as well as thrombin and histamine [27].

As with Ang1, the ligand Ang2 binds to the Tie2 receptor via a C-terminal P-domain within the Ang2 FReD [11,28]. However, the agonist activity of Ang2 for Tie2 is much lower than the Ang1 agonist activity and Ang2 acts as a partial agonist [29,30]. Indeed, Ang2 acts as an antagonist to Ang1 by displacing the more active ligand from Tie2 [29,30]. The basis for the difference in agonist activity between Ang1 and Ang2 is not clear. One possibility is that the lower oligomeric state of Ang2 is insufficient to provide agonist activity. However, it has been shown previously that a chimaeric angiopoietin in which the Ang2 FReD is inserted into the Ang1 oligomerization scaffold is able to oligomerize like Ang1, but still lacks full agonist activity [12].

**Regulation of Ang1 signalling**

As indicated above, constitutive production of Ang1 by perivascular cells, together with ECM (extracellular matrix)-bound Ang1 acts to stimulate Tie2 in underlying endothelial cells. This provides a basal anti-permeability and pro-stabilizing/pro-quiescent effect in vessels under normal conditions [10]. Although vascular maintenance by this Ang1/Tie2 system is clearly important for normal vessel stability, it is necessary for the endothelium to also be able to respond to destabilizing signals under some conditions, for example when mounting a local inflammatory response or when new vessel formation or remodelling is required. Under such conditions, the pro-stabilizing effects of the constitutive Ang1 signalling pathway will potentially antagonize these responses. It is therefore clear that mechanisms must exist to regulate Ang1 signalling and allow the endothelial cell to respond to other signals in its microenvironment.

If Ang1 levels are not actively regulated, how is Ang1 signalling controlled? A number of studies converge on the concept that Ang1 signalling is controlled at the level of the Ang1 receptor Tie2. Both inhibitory and activating mechanisms have been reported for Ang1 signalling through the Tie2 receptor. The antagonistic effects of Ang2, binding to the Tie2 receptor and displacing the more active Ang1, appear to have key roles in suppressing Ang1 signalling under conditions of vessel remodelling and inflammation. As discussed above, tissues undergoing vessel remodelling have increased Ang2 expression and this results in a decrease in the Ang1/Ang2 ratio, leading to suppression of Ang1 signalling and allowing the endothelium to respond to destabilizing signals [3,10]. Similarly, in vessel inflammation, Ang2 is increased, again permitting suppression of the pro-quiescent effects of Ang1 allowing the endothelium to mount an inflammatory response [41]. The dynamic regulation of Ang2 expression as well as the rapid release of Ang2 from the endothelium all serve to allow active control of the
Ang1/Ang2 ratio and therefore control of Ang1 signalling under conditions of endothelial activation.

An additional mechanism by which Ang1 signalling is controlled involves the co-receptor Tie1. The interaction of Tie1 with Tie2 acts to suppress Ang1 signalling through Tie2 [39]. The two receptors have been reported to exist in hetero-oligomeric complexes on the endothelial surface where the inhibitory effect of Tie1 suppresses Ang1 signalling [39,40,42]. Thus the relative levels of Tie1 and Tie2 in endothelial cells would be expected to affect the effectiveness of Ang1 to signal, with an increased Tie1/Tie2 ratio suppressing signalling. Expression of Tie1 and Tie2 is controlled by a range of factors including growth factors and hypoxia [43]. Thus the responsiveness of the endothelium to Ang1 will be determined by levels of Tie1 and Tie2, and this can be controlled by pathophysiologically relevant factors allowing local and dynamic control of Ang1 signalling. For factors regulating receptor expression by transcriptional and translational mechanisms, this control of cellular Ang1 responsiveness will operate over many minutes to hours.

Tie1 undergoes regulated ectodomain cleavage in which the receptor extracellular domain is proteolytically cleaved and released from the endothelium [44]. Tie1 ectodomain cleavage is stimulated by the pharmacological activator phorbol ester, as well as VEGF, inflammatory activators, such as TNF (tumour necrosis factor), and changes in shear stress [45,46]. This cleavage occurs rapidly for some stimuli, within a few minutes for VEGF for example [45,46]. Importantly, loss of Tie1 ectodomain relieves the inhibitory effect of Tie1 on Tie2, leading to increased responsiveness of Tie2 to Ang1 [39]. In human endothelial cells, activation of Tie1 cleavage leads to a 2–3-fold enhancement in Ang1-activation of Tie2 and 5–6-fold increase in activation of downstream signalling [39]. Tie2 has also been reported to undergo regulated ectodomain cleavage, being stimulated by VEGF, and this would be expected to suppress Ang1 signalling by removing the ligand-binding site [47,48]. However, Tie2 cleavage is much slower than Tie1 cleavage, requiring several hours for VEGF to affect Tie2, compared with minutes for effects on Tie1.

Ang1 signalling therefore is tightly regulated. Even though levels of the ligand are not thought to change markedly, the ability of the ligand to activate its receptor is dynamically controlled by Tie1 and Ang2. Under normal conditions, Ang2 concentrations are low, allowing Ang1 to bind and activate Tie2, although this activation is not maximal due to the interaction between Tie1 and Tie2, with Tie1 suppressing Tie2. Activation of Tie1 cleavage in response to signals in the cellular microenvironment will increase Tie2 responsiveness to the ambient Ang1 and reinforce Ang1 signalling in the cell. In contrast, if Ang2 is elevated, this antagonistic ligand will suppress Ang1 signalling and relieve the pro-quiescent effects of Ang1, allowing the endothelium to undergo remodelling or inflammatory activation.

Regulation of Ang1 signalling at the level of its receptor provides the cell with a mechanism to increase or decrease Ang1 signalling even in the absence of changes in ligand concentration. Furthermore, the inhibitory interaction between Tie2 and Tie1 in endothelial cells ensures that, under basal conditions, even in the presence of Ang1, signalling by Tie2 is submaximal, being set by the relative levels of the two receptors in the cell. The default level of Ang1 responsiveness of cells will be controlled in the medium- and long-term by factors controlling expression of the two receptors. More acutely, signals such as VEGF have the ability to rapidly increase Ang1 responsiveness of cells by stimulating Tie1 cleavage. This control over Ang1 signalling at the level of Tie2 responsiveness means that the endothelium can maintain a tonic pro-quiescent phenotype without requiring maximal signalling through Tie2. This is because transient increases in, for example, VEGF will act to stimulate Tie1 cleavage and increase Tie2 responsiveness, thereby reinforcing the Ang1 signal and preventing the endothelium from becoming destabilized. Where VEGF elevation is sustained, there is a down-regulation of Ang1 responsiveness, as Tie1 expression is actually elevated chronically by VEGF, and Tie2 cleavage is induced over these long time periods [43,48]. Similarly, transient increases in inflammatory activators will activate Tie1 cleavage and reinforce anti-inflammatory Ang1 signalling in the cells, thereby preventing the endothelium from tipping into a pro-inflammatory phenotype. In order for the cells to mount an inflammatory response they therefore require two signals: increased inflammatory activator together with an increase in Ang2 which will act to antagonize the anti-inflammatory activity of Ang1.

Conclusions

Ang1 signalling is tightly controlled at the level of responsiveness of its receptor, Tie2. This is illustrated schematically in Figure 1. The inhibitory effects of Tie1 interacting with Tie2 sets the level of signalling that Ang1 can induce. Rapid cleavage of Tie1 in response to cytokines and ligands, such as VEGF, inflammatory cytokines and changes in shear stress enhance Ang1 signalling without changes in the Ang1 level. This mechanism allows the endothelium to maintain a quiescent phenotype while being only partially responsive to the reservoir of constitutively produced Ang1 adjacent to the cells. Despite the presence of this Ang1 it therefore does not result in maximal Tie2 signalling, which may lead to desensitization of receptor and signalling pathways as well as potentially wasteful receptor turnover. The ability of Tie1 to undergo cleavage in response to factors such as VEGF and inflammatory cytokines provides a mechanism to strengthen the Ang1 signal and thereby adapt the level of Ang1 signalling to the remodelling or inflammatory stimuli without the need to maintain maximal Ang1 signalling at all times. It is possible that in other systems in which ligands are normally present in excess or provide a constitutive maintenance signal, the regulation of signalling by control of receptor responsiveness may be an important adaptation to allow modulation of cellular signalling responses.
Molecular regulation of angiopoietin signalling

Schematic diagram of the regulation of Ang1 signalling at the level of Tie2 by both the co-receptor Tie1 and Ang2. (A) Under basal conditions, constitutively expressed Ang1 produced by perivascular cells acts to stimulate Tie2 in endothelial cells to maintain the endothelium in a quiescent state. Tie2 present in complexes with Tie1 is inhibited by Tie1, ensuring that Ang1 signalling is not maximal. The level of Ang1 signalling in each cell is set by the relative expression of Tie1 and Tie2 in the cell. (B) In the presence of activators such as, in this example, TNFα, Tie1 undergoes ectodomain cleavage, relieving its inhibitory effect on Tie2 and allowing more Tie2 receptors to engage with the Ang1. Increased Ang1 signalling reinforces the pro-quiescent phenotype, preventing the endothelium from responding to the activating ligand. (C) When Ang2 is also induced in the presence of activating ligand, Ang1 signalling is antagonized, suppressing pro-quiescent signalling and allowing the endothelium to respond.

Figure 1

References


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