Review

Orchestral actions of angiopoietin-1 in vascular regeneration

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Among many proangiogenic growth factors, angiopoietin-1 (Ang1, or ANGPT1) is unique because it can induce distinctive vascular remodeling through highly organized angiogenesis and tightening of endothelial cell (EC) junctions. These effects are mediated by synchronous activation of both vascular Tie2 and nonvascular integrin signaling, making Ang1 a viable candidate for therapeutic neovascularization and vascular protection. Ang1 helps delay diabetic complications by restoring microvascular function and can maintain the quiescence of some adult stem cells. Interestingly, Ang1 is dispensable for maintaining normal vasculature throughout adulthood, challenging the original concept of its functions in cell survival and stabilization in quiescent vasculature. This review summarizes recent advances in understanding the biomedical implications of Ang1 and discusses its multifaceted roles in vascular diseases, the mechanisms underlying its effects, and potential therapeutic applications.

Molecular structure of Ang1 and its variants

Ang1 [1,2] is a secreted protein ligand for 'tunica interna endothelial cell kinase', Tek (also called Tie2; see Glossary), which is primarily expressed in growing vascular ECs and a subset of hematopoietic cells [1,2]. The structure of Ang1 consists of a carboxyl-terminal fibrinogen-like domain that binds to the Tie2 receptor, a central coiled-coil domain that enables oligomerization of these fibrinogen-like domains, and a short amino-terminal domain that superclusters the oligomers into variably sized multimers [1–4] (Figure 1a). To induce the activation of Tie2, Ang1 uses oligomeric and multimeric structures (Figure 1b) that are distinct from most other known growth factors. Recombinant Ang1 is produced as heterogeneous multimers of basic trimeric, tetrameric, and pentameric oligomers [3,4]. However, the multimeric forms of native Ang1 in various tissues under different conditions remain to be clarified. A dimeric variant of Ang1 activates Tie2 less than Ang1, whereas monomeric Ang1 is dramatically less able to bind to and activate Tie2 [3,4] (Figure 1b). Thus, clarification of the molecular interaction between the Tie2 and Ang1 binding domains would provide the insight needed to develop a desirable Tie2 agonist or antagonist. Moreover, how Ang1 binds to and activates integrin subtypes in both vascular and nonvascular cells remains to be elucidated [2].

Importantly, recombinant Ang1 is noted to be sticky, binds nonspecifically to extracellular matrix, and aggregates easily because of its unique molecular structure. These unwanted characteristics of recombinant Ang1

Glossary

Angiopoietin-2 (Ang2): a Tie2 ligand that acts in context-dependent antagonistic or agonistic manner. Ang2 inhibits Ang1-induced Tie2 phosphorylation, leading to disrupted blood vessel formation in the embryo. Ang2 also facilitates sprouting of tumor vessels in the presence of VEGF-A.
Angiogenesis: a process of new blood vessel formation via sprouting of preexisting blood vessels. It is indispensable for organ deveopment during embryonic growth, wound healing, endometrial growth, and tumor progression. The VEGF-A/VEGFR2 system plays a central role in angiogenesis.
Bone marrow-derived circulating progenitor cells (BMCPCs): hematopoietic stem cell- and mesenchymal stem cell-derived progenitor cells that are mobilized from bone marrow into systemic circulation.
***db/db* diabetic mouse**: a genetically modified mouse strain with a point mutation in the gene for leptin receptor. This mouse strain can be used as a model for obesity.
Hematopoietic stem cells (HSCs): multipotent stem cells capable of giving rise to all the blood cell types from both myeloid and lymphoid lineages. HSCs are usually found in the bone marrow, and constitute approximately 0.01% (1:10 000) of the myeloid tissue.
Integrin: transmembrane heterodimer cell-surface receptors that consist of one α subunit and one β subunit. Integrins function as adhesion molecules as well as receptors for transmitting biochemical and mechanical signals from the extracellular matrix.
Microbiota: aggregate of microorganisms in the digestive tract. They are known to carry out various functions for their host organisms, such as carbohydrate fermentation, immunity promotion, and proliferation and differentiation of intestinal epithelial cells.
Neovascularization: formation of new microvascular networks. Neovascularization in the form of choroidal neovascularization or retinal neovascularization is characteristic of certain diseases such as age-related macular degeneration and diabetic retinopathy.
Sepsis: a potentially lethal medical condition characterized by whole-body inflammatory state caused by foreign microbes in the blood, lungs, etc. Sepsis may lead to the failure of multiple organs and death.
Satellite myoblasts: mononuclear muscle stem or progenitor cells that reside between the basement membrane and the plasma membrane of mature multinucleated skeletal muscle cells. These cells are able to proliferate and differentiate into mature muscle cells and fuse to existing muscle fibers in response to injury or mechanical strain.
Tie2: a receptor tyrosine kinase mainly expressed in growing vascular ECs, megakaryoblasts, subsets of hematopoietic stem cells, and a subset of tumor-associated monocytes. Ang1 and Ang2 are agonistic ligands of Tie2. PI3 kinases, Erk, and Dok-R are its representative intracellular downstream signaling pathways.
Vascular endothelial cadherin (VE-cadherin): cell–cell adhesion glycoprotein that plays an important role in EC adherence in a homophilic manner. VE-cadherin is indispensable for proper vasculogenesis and for the maintenance of newly formed vessels. In addition, it plays an essential role in regulating vascular permeability.
VEGF-A: a secretory glycosylated protein that specifically acts on ECs and has various effects, including promoting the proliferation, differentiation, migration, and survival of ECs. It is the most potent ligand for inducing angiogenesis and vasculogenesis, as well as increasing vascular permeability in blood capillaries. Most actions of VEGF-A are mediated through activation of the receptor VEGFR2.

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cause difficulty in controlling Tie2 activation. Therefore, the recombinant versions of native Ang1 cannot be used as a therapeutic protein. To overcome these problems and make production easier, two representative chimeric Ang1 proteins have been developed, Ang1* [5] and COMP-Ang1 [6]. Ang1* is a modified form of Ang1 in which amino acids of the N-terminal domain of Ang1 are replaced by 73 amino acids from the N-terminal domain of Ang2, and cysteine 245 of Ang1 is replaced with a serine [5] (Figure 1c). However, Ang1* has problems similar to recombinant Ang1, albeit to a lesser extent. By contrast, COMP-Ang1, which has been generated by my research group, is a soluble, stable, and potent Ang1 variant. By replacing the N-terminal portion of Ang1 (245 amino acids) with the short, pentameric coiled-coil domain (45 amino acids) of the cartilage oligomeric matrix protein (COMP) (Figure 1c) [6], a truncated, pentameric form of Ang1, named COMP-Ang1, has been created that is more potent than native Ang1 in inducing phosphorylation of the Tie2 receptor and Akt in primary EC cultures [6]. COMP-Ang1 has been shown to protect ECs from irradiation injury [7] and to induce distinct vascular remodeling in adults [8]. Indeed, numerous experiments have proven that COMP-Ang1 is an effective alternative to native Ang1 [2]. Although COMP-Ang1 can be produced in mammalian cells, such as CHO cells, this large protein is difficult to produce, purify, and store in a stable form. Therefore, significant advances are required to develop a simple and potent Ang1 variant for therapeutic use.

Angiopoietin-2 (Ang2, also named ANGPT2) was the second angiopoietin family member identified, through a low stringency hybridization screening with Ang1 [5]; Ang1 and Ang2 have similar structures, with ~60% amino acid identity, and both have similar binding affinities for Tie2 [5]. Ang2 was originally thought to act as an antagonist of Ang1 by binding to Tie2 and blocking Ang1-induced Tie2 autophosphorylation. However, accumulating data indicate that Ang2 has context-specific effects; under certain conditions, Ang2 binds and activates Tie2 and integrin, whereas under different conditions it inhibits Tie2 [2,9].

Ang1 is an ideal molecule for therapeutic angiogenesis

For the past two decades, the unmet medical needs of patients with disabling ischemia have propelled the development of therapeutic strategies for inducing neovascularization. At first these attempts focused on angiogenic growth factors such as basic fibroblast growth factor (bFGF), vascular endothelial growth factor A (VEGF-A), and hepatocyte growth factor (HGF), used either as recombinant proteins or expressed via gene therapy. Unfortunately, the beneficial effects of promoting neovascularization with angiogenic growth factors are outweighed by unwanted side effects such as accelerated inflammation and fibrosis and the uncontrolled overgrowth or instability of newly formed blood vessels. Extensive analyses of preclinical animal models have discouraged the further clinical use of these growth factors. We now understand the causes of such side effects, and since the discovery of Ang1, numerous preclinical studies have been conducted on the use of Ang1 for therapeutic neovascularization, leading to novel insights regarding its

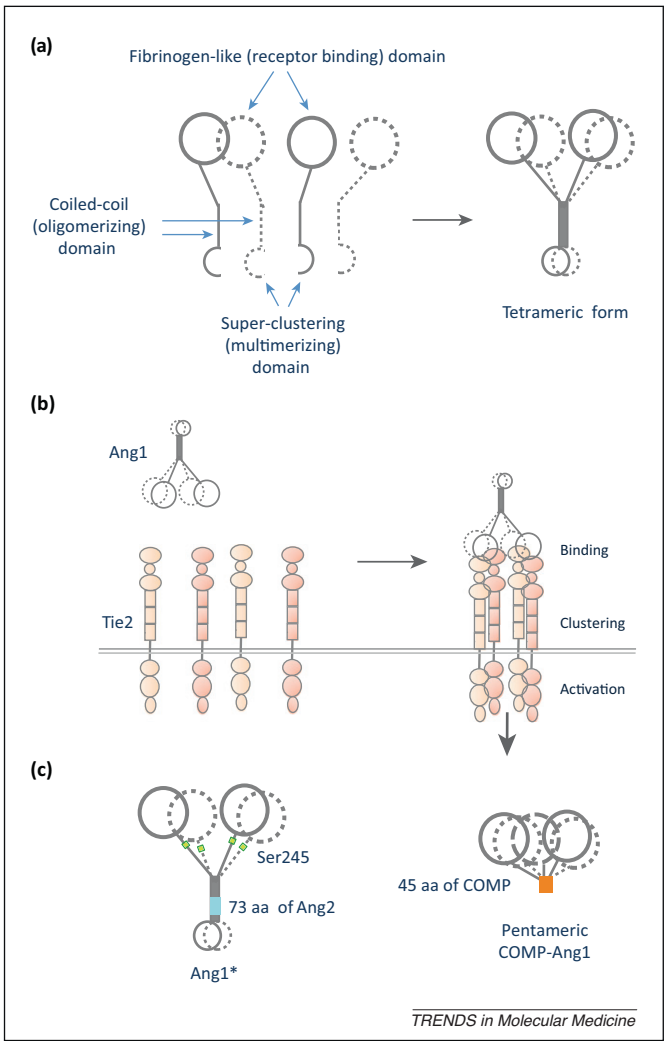

Figure 1. Protein structures of angiopoietin-1 (Ang1) and recombinant angiopoietin-1 (Ang1) variants, and hypothetical molecular interaction between Ang1 and Tie2. **(a)** The structure of Ang1 consists of a carboxyl-terminal fibrinogen-like domain that binds to the Tie2 receptor, a central coiled-coil domain that enables dimerization of these fibrinogen-like domains, and a short amino-terminal domain that superclusters the dimers into tetramers. **(b)** Tetrameric Ang1 binds to and clusters Tie2, leading to its activation. **(c)** Two representative Ang1 variants, Ang1* and cartilage oligomeric protein (COMP)-Ang1. Ang1* is a modified form of Ang1 in which amino acids of N-terminal domain of Ang1 are replaced by 73 amino acids from the N-terminal domain of Ang2 and cysteine 245 of Ang1 is replaced by a serine. COMP-Ang1 is a pentameric, soluble, and potent Ang1 variant in which the N-terminal portion (245 amino acids) of Ang1 is replaced with the short, pentameric coiled-coil domain (45 amino acids) of the COMP protein.

molecular and cellular mechanisms of action. In fact, unlike any other angiogenic growth factor, Ang1 can generate neovascularization with minimal, if any, side effects. Importantly, accumulating evidence clearly explains how Ang1 contributes to healthy and stable neovascularization, as well as vascular maintenance, homeostasis, and protection. Therefore, a second wave of therapeutic angiogenesis trials using Ang1 is likely in the near future.

The first genetic experiment investigating Ang1 established it as an agonistic ligand for Tie2 in the regulation of vessel remodeling, maturation, and stabilization during the mid-period of murine embryonic development [10]. However, a recent genetic study unexpectedly revealed that Ang1 is dispensable in normal vasculature maintenance throughout adulthood [11,12]. Nevertheless, overexpression of Ang1 in normal quiescent vasculature has