Available online at www.sciencedirect.com

SCIENCE DIRECT®

Experimental Cell Research 312 (2006) 630 – 641

Experimental Cell Research

www.elsevier.com/locate/yexcr

Review Article

Tie receptors and their angiopoietin ligands are context-dependent regulators of vascular remodeling

Lauri Eklund, Bjorn R. Olsen*

Harvard School of Dental Medicine, Department of Oral and Developmental Biology, 188 Longwood Avenue, REB, 4th floor, Boston, MA 02115, USA

Received 16 August 2005, revised version received 1 September 2005, accepted 1 September 2005
Available online 12 October 2005

Abstract

Angiopoietins are ligands of the Tie2 receptor that control angiogenic remodeling in a context-dependent manner. Tie signaling is involved in multiple steps of the angiogenic remodeling process during development, including destabilization of existing vessels, endothelial cell migration, tube formation and the subsequent stabilization of newly formed tubes by mesenchymal cells. Beyond this critical role in blood vessel development, recent studies suggest a wider role for Tie2 and angiopoietins in lymphangiogenesis and the development of the hematopoietic system, as well as a possible role in the regulation of certain non-endothelial cells. The outcome of Tie signaling depends on which vascular bed is involved, and crosstalk between different VEGFs has an important modulating effect on the properties of the angiopoietins. Signaling through the Tie1 receptor is not well understood, but Tie1 may have both angiopoietin-dependent and ligand-independent functions. Changes in the expression of Tie receptors and angiopoietins occur in many pathological conditions, and mutations in the Tie2 gene are found in familial cases of vascular disease.

Keywords: Angiopoietin; Tie1; Tie2; Endothelial cell; Mural cell; Blood vessel; Angiogenesis

Contents

* Corresponding author. Fax: +1 617 432 0638.
E-mail address: bjorn_olsen@hms.harvard.edu (B.R. Olsen).

0014-4827/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.yexcr.2005.09.002

Introduction

Tie1 and Tie2 constitute a distinct family of receptor tyrosine kinases found to be expressed mainly in endothelial cells [1,2]. The Tie receptors have many interesting cellular effects and potential therapeutic applications, which make them molecules of significant interest to vascular biologists.

Angiopoietins 1–4 (Ang1–Ang4) constitute a family of growth factors known to function as ligands for the Tie2 receptor [3–5]. The characteristic features of angiopoietins are opposing effects on Tie2 receptor activation and signaling outcome [3–5]. Ang1 has been shown to act as an obligatory agonist promoting structural integrity of blood vessels [3,6], whereas Ang2 has been found to function as a naturally occurring antagonist promoting either vessel growth or regression depending on the levels of other growth factors, such as VEGF-A [4,7]. However, in certain in vitro models, Ang1 and Ang2 have similar responses, and in vivo studies, including studies of Ang2-deficient mice [8], also suggest that Ang2 can have an agonistic role depending on the tissue environments and the experimental conditions [9–13]. The effects of Ang3 and Ang4 have been less characterized, but they also show context-dependent actions as antagonistic and agonistic ligands, respectively [5,14]. The mechanisms underlying the different effects of angiopoietins on Tie2 activation are not yet known.

The formation of a functional vasculature is a complex process requiring spatial and temporal coordination of multiple angiogenic factors, receptors, intracellular signaling pathways and regulatory factors. One emerging theme is that VEGFs and angiopoietins play complementary roles. In many contexts, Ang1 and VEGF-A have been shown to have synergistic effects [15–20], but Ang1 can also counteract the permeability [15,21] and proangiogenic effects of VEGF-A [22–24], and VEGF-A can change the proangiogenic outcome of Ang2 signaling through Tie2 [7,22,25,26]. Thus, the net effect of Tie signaling is dependent not only on angiopoietins, but also on other growth factors and likely also on other, as yet unidentified regulatory factors.

Signaling through Tie2 has been extensively studied, and the results suggest that signaling involving phosphatidylinositol 3′ kinase (PI3-K) activation is a major pathway [18,27–32]. However, the lack of an identified ligand has made it difficult to characterize the function of Tie1. Intriguingly, recent studies suggest a novel ligand-independent function for Tie1. This involves shedding of the receptor [33–38] and heteromeric complex formation with Tie2 [36,37,39,40]. Additionally, although previous studies failed to demonstrate binding of angiopoietins to Tie1 [3–5], it has recently been found that Ang1 and Ang4 can in fact activate Tie1 [40].

Mutations in Tie2 are found in vascular anomalies, including venous malformations [41,42] and intramuscular hemangiomas [43]. Changes in the expression of Tie2 and angiopoietins are found in a wide range of diseases including psoriasis [44], pulmonary hypertension [45,46], infantile hemangiomas [47] and different tumors [48]. However, the exact mechanisms by which Tie signaling may contribute to the diseases are not yet known.

Structural characteristics of angiopoietins

Angiopoietins contain an amino-terminal angiopoietin-specific domain followed by a coiled-coil domain, a linker peptide and a carboxy-terminal fibrinogen homology domain [3–5]. The fibrinogen homology domain is responsible for receptor binding, the coiled-coil domain is required for dimerization of angiopoietin monomers, and the short amino-terminal region forms ring-like structures that cluster dimers into variable sized multimers necessary for Tie2 activation [49–51]. One intriguing question has been how the Ang2 antagonist effect is mediated. The ability of Ang2 to bind to Tie2 without inducing receptor phosphorylation is not due to a lack of clustering of Ang2 oligomers [50]; instead, it appears that the Ang2 antagonistic function is derived from the structure of its fibrinogen homology motif [49,50]. Ang1 and Ang2 have indistinguishable binding sites on the Tie2 receptor, suggesting that differential receptor binding is unlikely to be responsible for Ang2 antagonistic function [52]. Angiopoietins have different properties in terms of extracellular and cell surface binding. While Ang1 is incorporated into the extracellular matrix (ECM), Ang2 is secreted but not associated with the ECM [53], and it can be stored in Weibel-Palade bodies in the cytoplasm of endothelial cells [54]. In contrast to Ang1 and Ang2, Ang3 is tethered on the cell surface via heparin sulfate proteoglycans [55]. The differences in binding to ECM and cell membranes may regulate availability and biological activity of the angiopoietins. The characteristics of angiopoietins are summarized in Table 1.

Tie2/Ang1 signaling

Genetic studies using targeted mutations in mice have been used to study the function of Tie signaling. A common feature of mouse models lacking Tie1 [56], Tie2 [56,57], Ang1 [6] or Ang2 [8] is that vasculogenesis, the formation of blood vessels by in situ differentiation of endothelial cell precursors, proceeds normally, but remodeling and maturation of the vessels are defective. *Tie2*$^{-/-}$ mice die between embryonic day 9.5 and 12.5 due to lack of remodeling of the primary capillary plexus. The development of the heart also shows severe defects with poor association between endothelial cells and the underlying matrix and lack of myocardial trabecular projections [56,57]. Ligand-deficient *Ang1*$^{-/-}$ mice die by embryonic day 12.5 due to a similar vascular phenotype [6]. This similarity in phenotypes suggested that Ang1-stimulated Tie2 activation mediates remodeling and stabilization of

Table 1
Characteristics of angiopoietins

Characteristic	Ang1	Ang2	Ang3	Ang4
Binding to Tie2	Yes [3]	Yes [4]	Yes [5]	Yes [5]
Activation of Tie2	Obligatory [3]	Context-dependent [4,9,10]	context-dependent [5,14]	Obligatory [5,14]
Activation of Tie1	Yes [40]	No [40]	No [40]	Yes [40]
Expression pattern in adults	Many tissues [4]	Sites of vascular remodeling [4]	Many tissues [5]	Especially in the lungs [5]
Primary cellular source	Non-endothelial cells [3,6,29]	Endothelial cells [4,86]	ND	ND
Ectopic expression in tumor cells	Reduces tumor size [94,99]	Increases tumor size [94,95]	Reduces tumor size [102]	ND
Association with the ECM	Incorporated [53]	Secreted but not incorporated [53]	ND	ND
Binding to the cell surface HSPGs	No [55]	No [55]	Yes [55]	ND
Storage in Weibel-Palade bodies	No [54]	Yes [54]	ND	ND

Mouse Ang3 and human Ang4 represent interspecies orthologues. ECM, extracellular matrix; HSPGs, heparan sulfate proteoglycans; ND, not determined (references in brackets).

cell–cell and cell–matrix interactions and plays a role in the recruitment of peri-endothelial mesenchymal cells to the vessels.

Ang1 induces migration [58], tube formation [59], sprouting [16,60] and survival [29,61–63,139], but not proliferation, of endothelial cells in vitro [3]. In addition, Ang1 has anti-permeability [15,21,64,140–142] and anti-inflammatory [64–68] functions.

The signal transduction pathways triggered by Ang1 binding to Tie2 have been extensively studied, and several cell signaling cascades and downstream targets have been identified (Fig. 1). Although in vivo functional significance of all these cascades and targets is unclear, signaling through PI3-K appears to be essential for Ang1-induced survival [27–31,69,70], sprouting, migration [28,60] and capillary tube formation [18,32].

Ligand binding to the extracellular domain results in receptor dimerization, allowing activation of the kinase domain and autophosphorylation of specific tyrosine residues, acting as docking sites for a number of effectors that couple the activated receptors to the cytoplasmic signaling pathways [71]. Activated Tie2 associates with Dok-R [72], which in turn establishes binding sites for Nck and p21-activated kinase (PAK). The Dok-R-PAK pathway is involved in the Ang1-induced migratory effect [72–74]. Tie2 also associates with protein tyrosine phosphatase SHP2 [75,76] and adaptor protein GRB2 [76], both of which have roles in activation of the ras-mitogen-activated protein kinase (MAPK) pathway. Ang1 can activate the MAPK signaling cascade [17,28,30,77,78], which may have a role in Ang1-mediated cell survival [28,30] and migration [78,79]. Binding of the p85 subunit of PI3-K to Tie2 is required for activation of PI3-K [80], which in turn activates the serine–threonine kinase Akt [28,29] to mediate the anti-apoptotic effect of Ang1 [27,28,59]. PI3-K-dependent activation of focal adhesion kinase (FAK) [60] and Rho GTPases RhoA and Rac1 [81] may mediate Ang1-induced endothelial cell motility. PI3-K also mediates the activation of endothelial nitric oxide synthase (eNOS) [82]. Inhibition of eNOS or PI3-K results in defective tube formation and endothelial cell migration and sprouting [32,82]. Ang1 may also regulate the MAPK signaling cascade by modulating phosphorylation of ERK1/2 and p38 MAPKs by PI3-K [30]. Moreover, Tie2 binds the Src homology domain containing adaptor proteins Grb7 and Grp14 [75] and adapter ShcA protein [83]. ShcA may regulate Ang1-induced motility and morphogenesis [83]. Tie2 was shown to interact with ABIN-2, which functions as a regulatory protein for the transcription factor NF-κB [84]. Ang1 is also able to activate signal transducers and activators of transcription factor-3 (STAT3) and factor-5 (STAT5) and upregulate expression of the p21 cell cycle inhibitor [85].

Angiopoietin-2 functions as a context-dependent antagonist or agonist

Ang2 was found to bind to Tie2 but failed to induce autophosphorylation in endothelial cells [4]. Transgenic overexpression of Ang2 led to a phenotype similar to that observed in Ang1- or Tie2-deficient mice leading to the hypothesis that Ang2 blocks the stimulatory effects of Ang1, thereby acting as a natural antagonist for Ang1 [4]. In contrast to the relatively stable expression of Ang1, expression of Ang2 was found predominantly at sites of vascular remodeling, including female reproductive tract and tumors. In these tissues, Ang2 was upregulated together with VEGF-A at sites of angiogenic sprouting, whereas reduced VEGF-A expression relative to Ang2 was associated with vascular regression [4,86–88]. These findings suggested a model in which binding of Ang2 to Tie2 does not induce any specific function but rather interferes with the Ang1-induced stabilization effect, making vessels more sensitive to the actions of other angiogenic cytokines such as VEGF-A [7]. Consistent with the model is the finding that Ang2 promotes VEGF responsiveness in different

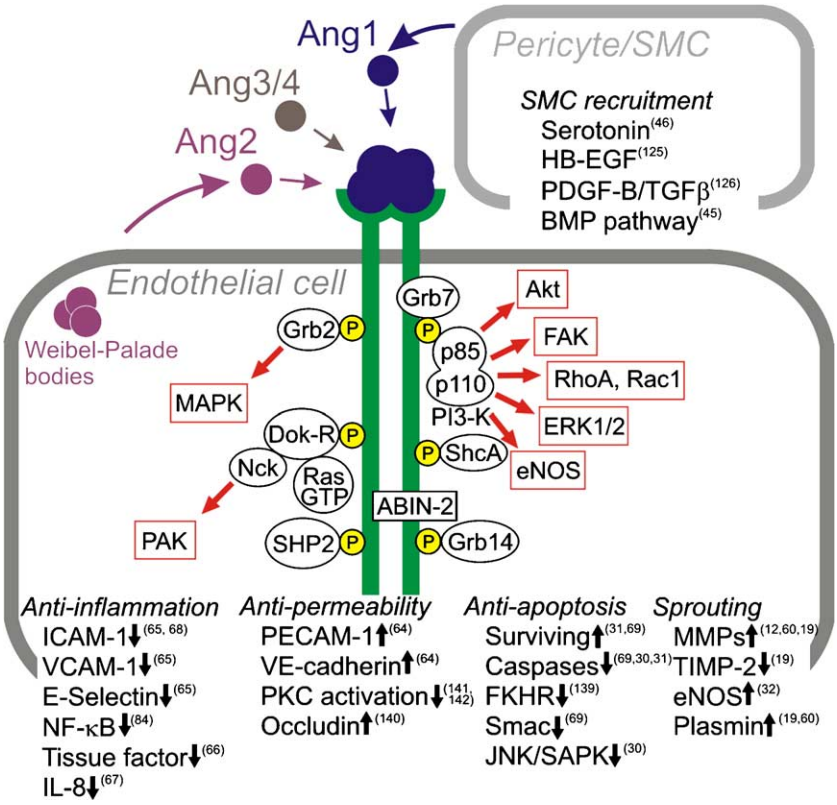

Fig. 1. Schematic representation of Ang1/Tie2 signaling. Ang1 is produced by non-endothelial cells, whereas a primary source of Ang2 is endothelial cells where it can be stored in cytoplasmic Weibel-Palade bodies. Cellular origin of Ang3/4 is not known. Signaling molecules interacting with phosphotyrosine residues (yellow) are encircled, and downstream effectors are framed. Signaling through Tie2 receptor increases survival, vascular permeability and sprouting and may regulate pericyte/SMC recruitment. The exact molecular mechanisms of how Tie2 signaling elicits downstream effects are not known, but several potential downstream target molecules are identified as listed at the bottom; arrow down indicates downregulation; arrow up indicates upregulation. Secreted growth factors possibly involved in Ang1-induced SMC recruitment are listed at top right (references in parenthesis), other references appear in the text. It should be noted that because of the poor solubility of native Ang1 most studies have been done with modified Ang1/Ang2 chimeric protein called Ang1*, believed to have the same biological activity as native Ang1 [4,16]. Abbreviations: Ang, angiopoietin; SMC, smooth muscle cell; HB-EGF, heparin binding epidermal growth factor-like growth factor; PDGF, platelet-derived growth factor; TGF, transforming growth factor; BMP, bone morphogenetic protein pathway; MAPK, mitogen-activated protein kinase; PAK, p21-activated kinase; PI3-K, phosphatidylinositol 3′ kinase; Akt, protein kinase B; FAK, focal adhesion kinase; ERK, extracellular signal-regulated kinase; eNOS, endothelial nitric oxide synthase; ICAM, intercellular adhesion molecule; VCAM, vascular cell adhesion molecule; IL-8, interleukin-8; PECAM, platelet/endothelial cell adhesion molecule; VE-cadherin, vascular/endothelial-cadherin; PKC, protein kinase C; FKHR, forkhead transcription factor; Smac, second mitochondrial-derived activator of caspases; JNK/SAPK, c-Jun N-terminal kinase/stress-activated protein kinases; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinases.

experimental conditions, including the mouse corneal pocket assay [89] and rat papillary membrane assay [90], in transgenic mice overexpressing both Ang2 and VEGF in the heart [22] or in the eye [25,26] and in a 3-dimensional spheroid coculture system [13,91].

However, in certain in vitro assays, Ang1 and Ang2 have similar functions. When Tie2 is ectopically expressed in non-endothelial cells [4] or if used at higher concentrations in endothelial cells [9], Ang2 is able to induce Tie2 phosphorylation. Moreover, Ang2 can induce tube formation in a fibrin clot and Tie2 phosphorylation at prolonged exposure times [10] and stimulate chemotaxis and tube formation of endothelial cells in collagen gels [11]. Moreover, treatment of endothelial cells with Ang1 or Ang2 stimulates matrix metalloproteinase-9 (MMP-9) expression [12], and Ang2 stimulates migration and sprouting of endothelial cells in gel-embedded endothelial cell spheroids [13]. These studies suggest that Ang2 can act as an agonist depending on cell type and experimental context. The dual function of Ang2 was further supported by the eye phenotype suggestive of a function in vessel remodeling and a lymphatic disorder suggestive of a function as a stimulating factor in *Ang2*$^{-/-}$ mice [8]. In the eye, lack of Ang2 causes defective regression of the fetal vasculature in the vitreous body, abnormal outgrowth of retinal capillaries and lack of ischemia-induced neovascularization [8,92], while the lymphatic phenotype is characterized by disorganization and hypoplasia of the intestinal and dermal lymphatic capillaries [8]. Interestingly, the lymphatic but not the eye phenotype can be rescued by replacement of the Ang2 gene with Ang1 cDNA, suggesting distinct functions for Ang1 and Ang2 in vascular remodeling in the eye, but redundant roles in lymphatic development [8]. In *Ang2*$^{-/-}$ mice, phenotypic changes in vascular capillaries can also be found in the kidney, suggesting that lack of Ang2 causes subtle vascular phenotypes more widely in the body [93].

The results of ectopic overexpression of Ang2 in tumor cells and pharmacological inhibition experiments support the role of Ang2 as a proangiogenic factor. Overexpression of Ang2 in cancer cells in mice promotes growth and vascularization of tumors [94,95]. In contrast, pharmacological inhibition specific to Ang2 suppresses angiogenesis and growth of tumors [96] and inhibits neovascularization in the rat cornea [96,97]. In contrast to Ang2, ectopic overexpression of Ang1 in tumor cells may inhibit tumor growth in mice, which might be related to Ang1-induced stabilization of the tumor vasculature [94,98,99].

The primary source of Ang2 is endothelial cells, and its expression is related to their activation [8,54,86,87,100,101]. The expression pattern and observed functions of Ang2 suggest that it may act in an autocrine manner to control endothelial cell quiescence and responsiveness.

Angiopoietin-3 and angiopoietin-4

Ang3 and Ang4 were found by homology cloning by virtue of primary structure similarity to Ang1 and Ang2 and by their ability to bind to Tie2 [5]. Mouse Ang3 and human Ang4 represent interspecies orthologues, but they have relatively low (65%) amino acid identity [5]. Their functions are far less characterized than those of Ang1 and Ang2. Mouse Ang3 is expressed at low levels in many tissues [5], whereas Ang4 mRNAs are expressed at high levels in the lungs, with lower levels in other tissues [5]. A chimeric protein made by inserting the Ang4 fibrinogen-like domain into the Fc portion of human IgG1 or the N-terminal and coiled-coil segments of Ang2 was found to induce autophosphorylation of Tie2 in mouse and human endothelial cells and when Tie2 was ectopically expressed in mouse fibroblasts. In contrast, the Ang3 fibrinogen-like domain did not activate Tie2 in endothelial cells but induced phosphorylation in mouse fibroblasts, suggesting an agonistic role for Ang4 and an antagonistic one for Ang3 [5], as observed with Ang2 [4]. However, when using recombinant proteins corresponding to full-length mouse Ang3 and human Ang4, Ang3 was found to have activating effects, depending on the target species. Human Ang4 induced autophosphorylation of Tie2 in cultured human endothelial cells, but mouse Ang3 failed to act as agonist to human Tie2 but induced phosphorylation of Tie2 in mouse lungs and mouse-derived endothelial cells in culture [14]. Moreover, both human Ang4 and mouse Ang3 stimulated corneal angiogenesis in mouse [14]. Interestingly, unlike Ang1 or Ang2, Ang3 is tethered on the cell surface via heparan sulfate proteoglycans, which regulate the bioactivities of Ang3 [55,102].

Tie signaling in late development

Tie2 is constitutively expressed and phosphorylated at a low level in adult mice, suggesting that Tie2 activation is required in adult tissues to maintain the mature quiescent phenotype of the vasculature [103]. While studies of gene-deficient mice have demonstrated the importance of Tie signaling in early development, analysis of mosaic mouse models [104,105] and different blocking experiments using soluble Tie2 receptors [12,106,107] and inhibitors against Tie2 [78] and Ang2 [96,97] have been used to obtain insights into the role of Tie signaling in late development and pathological angiogenesis.

The importance of Tie signaling in organogenesis was suggested in analyses of chimeric mice having endothelial cells with wild-type and null Tie1 and Tie2 alleles [105]. Double-null endothelial cells lacking both Tie1 and Tie2 were significantly underrepresented at embryonic day 15.5 and absent in adult mice, which also lacked endothelial cells doubly heterozygous for Tie1 and Tie2 null alleles [105].

Competitive inhibition using soluble ligand binding domains of Tie2 can reduce pathological neovascularization [12,106,107] but has no detectable morphological effects in normal adult vessels [106,108]. Ang1 does not induce proliferation of endothelial cells in vitro [3] but induces sprouting activity [16,60,82]. In contrast to the findings in vitro, treatment of mice with engineered recombinant forms of Ang1 causes vessel enlargement due to endothelial cell proliferation, without angiogenic sprouting [108–110]. The vessel enlargement was mainly found on the venous side of the circulation [108–110]. The ability of Ang1 to regulate vessel diameter was found to be restricted to a defined developmental stage in most vascular beds, before postnatal day 30 [108]. However, enlargement was also found in certain vessels in adult mice, such as in tracheal venules [108,110], and the treatment with an engineered Ang1 variant, COMP-Ang1, more potent as a Tie2 activator than native Ang1, induced vascular enlargement in adult mice in many tissues [109]. Constitutive overexpression of Ang1 controlled by the K14 promoter in the skin of mice results in increased number of leak-resistant enlarged vessels [15,111]. In contrast, systemic treatment of adult mice with Ang1 expressing adenoviruses did not lead to changes in vessel morphology but reduced vessel permeability [21].

So far, no conditional knockout mouse has been published, but many transgenic mouse models which combine tissue-specific and inducible gene promoters to induce or repress Tie2 receptor or ligand expression have been developed [22–26]. The findings reported for over-expressing mice are not very consistent and do not fit well into a single mechanistic model. In the heart [22] or in the eye [23,24], excess Ang1 has no effect under normal conditions. In contrast, Ang1 promotes angiogenesis in the skin having synergistic effects with VEGF [15,111], but, in the heart [22] and in the eye, Ang1 inhibits the VEGF-induced angiogenic phenotype [23,24].

Overexpression of Ang2 in the heart [22] or in the eye [25,26] does not induce vessel regression, as might have been expected from suggestion that Ang2 is a destabilizing factor [4,7]. In the heart, Ang2 promotes vascularization

with VEGF [22], but, in the eye, Ang2 has different effects depending on the development stage and the vascular beds [25,26]. Newly formed ocular vessels are most sensitive to Ang2, and a high Ang2 to VEGF ratio promotes regression, whereas high levels of Ang2 and VEGF stimulate neovascularization [26].

In addition to ligand overexpression studies, Tie2 has been conditionally overexpressed under its own endogenous promoter in a tetracycline-controlled manner. The Tie2 transgene was found to be expressed in endothelial cells, but also in keratinocytes and epithelial cells. This resulted in increased number of large and tortuous capillaries in the dermis accompanied by inflammation and epidermal hyperplasia, resembling psoriasis-like lesions. The lesions resolved completely following tetracycline-mediated suppression of Tie2 transgene expression, suggesting that maintenance and progression of the lesions depended on Tie2 signaling [44].

Taken together, the data obtained by manipulating Tie signaling in postnatal and adult mice indicate that the vasculature remains sensitive to Tie2 activation after embryonic day 12.5 when *Tie2*$^{-/-}$ or *Ang1*$^{-/-}$ embryos die, suggesting a role in later development and in mature vessels. The apparently conflicting results suggest that the effects are dependent on the nature of the target vascular bed, as well as the dose, timing, source and the modifications of Ang1 used in the experiments. However, they may also reflect real biological differences suggesting that the outcome of Tie2 activation is dependent on the defined developmental stage of specific vascular beds.

Functions of the Tie1 receptor

In spite of the structural homology between Tie2 and Tie1, none of the known angiopoietins was found to bind to Tie1 when native or engineered chimeric ligands were used [3–5]. Because of the lack of an identified ligand, the function of Tie1 has remained enigmatic, although in vivo studies indicate an essential role for Tie1 in vascular development [56,112,113]. Mice lacking Tie1 die between E13.5 and the immediate postnatal period due to severe hemorrhages and edema suggesting defective vessel integrity [56,112]. Moreover, Tie1 deficiency in mice leads to increased vessel density consisting of “hyperactive” endothelial cells, suggesting that Tie1 may regulate endothelial quiescence [113].

In endothelial cells, Tie1 was found to be unphosphorylated, and it failed to induce tyrosine phosphorylation of cellular proteins, suggesting that Tie1 function is not related to ligand-induced kinase activity [35,39]. The extracellular domain of Tie1 can be proteolytically cleaved following treatment with VEGF, protein kinase C, phorbol myristate acetate or by exposure to shear stress [33,34,36–38]. This generates a membrane-bound Tie1 endodomain comprising the intracellular kinase and transmembrane domains. Although the Tie1 endodomain only poorly induced tyrosine phosphorylation, it was found to be associated with signaling proteins such as SHP2, suggesting that Tie1 might signal in a ligand-independent manner [35]. Moreover, in experiments with a chimeric receptor consisting of the Tie1 cytoplasmic domain and colony-stimulating factor-1 receptor extracellular domain subjected to ligand activation with colony-stimulating factor-1, Tie1 was able to induce autophosphorylation and activation of the PI3-K and Akt pathway. These results suggest that Tie1 is capable of signaling and that Tie1 and Tie2 may share a common signaling pathway [114]. Consistent with the finding that Tie1 may have some kinase activity following ligand binding is the recent report that Tie1 can be activated by an Ang1 chimeric protein (COMP-Ang1) as well as native Ang1 and Ang4, but not Ang2 or Ang3 [40]. It has been proposed that a possible function of Tie1 is to co-operate with and modulate signaling through Tie2 through the formation of heterodimeric complexes [36,37,39,40]. Moreover, complex formation with Tie2 enhances Tie1 phosphorylation [40].

Shear forces induced by blood flow have essential roles in the regulation of vascular function, remodeling and structure. Interestingly, Tie1 expression is downregulated in cells exposed to changes in shear stress in vitro [36], and upregulation of Tie1 expression in vivo is particularly seen in regions exposed to disturbed flow, including branch points of large vessels and aortic valves and atherosclerotic lesions [115]. This suggests that Tie1 may have a role in mediating cellular responses to changes in shear force.

Angiopoietins stimulate lymphangiogenesis

Tie2 expression in lymphatic endothelial cells [116–118] and the lymphatic phenotype in *Ang2*$^{-/-}$ mice and its rescue by Ang1 [8] suggest a role for Tie signaling in lymphatic vessels. The ability of Ang1 to promote lymphangiogenesis has been demonstrated by viral overexpression of Ang1 in mouse skin [117] and cornea [118]. Ang1-induced lymphangiogenic activity was found to be accompanied by upregulation of VEGFR-3 [117], which functions as a receptor for the lymphangiogenesis regulators VEGF-C and VEGF-D [117,118]. Thus, similarly to Ang1/Ang2 and VEGF cooperation in blood vessel development, angiopoietins appear to cooperate with other members of the VEGF family via VEGFR-3 in the formation of the lymphatic vasculature [117,118].

Tie2 and Ang1 are important in the hematopoietic system

Endothelial and hematopoietic stem cells are derived from a common hemangioblast progenitor. Tie1 and Tie2 are expressed in certain hematopoietic cells, suggesting a

potential role in establishment or maintenance of hematopoietic system [119–122]. In fact, *Tie2*$^{-/-}$ mice show severely impaired hematopoiesis [121]. However, in chimeric mice with both normal and Tie receptor-deficient hematopoietic stem cells, it was found that Tie2 is not required for embryonic hematopoiesis [104]. Instead, cells lacking both Tie1 and Tie2 showed impaired capacity to contribute to adult hematopoiesis in the bone marrow [104]. In the doubly deficient cells, this effect is likely due to lack of Tie2 since Tie1-deficient cells expressing normal levels of Tie2 can contribute to hematopoiesis [123]. Tie2/Ang1 signaling has a role in maintaining hematopoietic stem cells in a quiescent state in the bone marrow niche. This is associated with an increase in the adhesion of Tie2-positive stem cells to Ang1-expressing osteoblasts, resulting in quiescence and enhanced survival [122].

Role of Tie signaling in the regulation of mesenchymal cells

A characteristic feature of *Tie2*$^{-/-}$ and *Ang1*$^{-/-}$ mice, patients with venous malformations due to mutations in Tie2 and lymphatic vessels in *Ang2*$^{-/-}$ mice is defective vessel remodeling and abnormal smooth muscle cell (SMC) layering around affected vessels. The lack of Tie2 expression in SMCs has led to a hypothesis that mesenchymal-derived Ang1 activates Tie2 in endothelial cells in the developing vessels, and the endothelial cells in turn produce and secrete soluble chemoattractants to recruit the mural cells [41,124], although it has also been suggested that the defective SMC layering may be secondary to decreased survival of the endothelial cells [105].

Potential mechanisms by which endothelial cells regulate SMC layering in a paracrine manner via Tie2 activity have recently been characterized and may involve heparin binding EGF-like growth factor (HB-EGF) [125], serotonin [46], growth factors PDGF-B and TGF-β [126] and the bone morphogenetic protein pathway [45]. Although initial experiments suggested endothelial-cell-specific expression pattern for Tie2, there is evidence that under certain conditions Tie2 may be expressed in SMCs or mesenchymal precursor cells [127–130], suggesting the possibility that Tie2 signaling may have direct consequences in SMCs.

Tie signaling in nerves and angiopoietin interactions with integrins

Interestingly, Tie2 expression is found in certain neuronal cells where it has neuroprotective effects and stimulates growth responses in vitro [131–133]. Additionally, overexpression of Ang1 in the forebrain of mice leads to increased vascularization and also to changes in dendrite organization of the neurons [134]. However, Tie2 was not found to be expressed in neurons or dendritic processes in vivo, and it is speculated that the effect of Ang1 on neurons might be mediated by an interaction of Ang1 with integrins [134]. Consistent with this possibility, there is evidence that angiopoietin signaling might be related to integrins. In Tie receptor-negative fibroblasts, direct cell adhesion to Ang1 and Ang2 is mediated by α5β1 and $\alpha_v\beta5$ integrins [135]. Ang1 was found to promote Tie2-negative cardiac and skeletal myocyte adhesion and survival through integrins by the same signaling pathways that are activated in endothelial cells by Ang1 [136]. The ability of Ang1 to bind to integrins was also indicated in experiments using recombinant protein representing a truncated and monomeric Ang1 variant. This was found to bind to Tie2 without inducing autophosphorylation of the receptor, but it also bound with similar affinity to integrin α5β1 and had similar downstream effects to the full-length Ang1, possibly through integrin ligation [137].

Concluding remarks

Although it is evident that Tie signaling is essential for vascular remodeling and maturation, the detailed molecular mechanisms of how Tie signaling contribute to these processes are less clear. The wide range of activities suggested for Tie receptors and angiopoietins with apparently conflicting results make an attempt to generate a unifying model for their biological functions difficult. This may simply reflect the complexity and complex regulation of Tie signaling as well as a lack of detailed understanding of the biological processes themselves. Recent findings suggest that many potential therapeutic applications and modulation of Tie signaling may be a significant clinical value in inhibiting undesirable angiogenesis, e.g. in tumors, preventing inflammation or in promoting the growth of leakage-resistant vessels to treat ischemic diseases.

Future experiments need to address the many remaining basic questions. What are the mechanisms behind the different effects of angiopoietins on Tie2 activation, which seem to be cell-type-specific and dependent on experimental context and the nature of the vascular bed? Is it possible that integrins, or other cell-type specific coreceptors and regulators such as Tie1 or VE-PTP [138], regulate the actions of angiopoietins? Does differential binding to the ECM or to the cell surface modulate the availability and action of angiopoietins in vivo?

It was only very recently demonstrated that Ang1 and Ang4 may function as activating ligands for the orphan Tie1 receptor and that its activation is enhanced by complex formation with Tie2. However, does Tie1 function as an independent receptor and what might be the effect of increased Tie1 expression at the specific sites of the vasculature regulated by shear force? Finally, generation of mice with conditional null alleles or the development of more effective inhibitors may reveal a precise role for Tie signaling in later development and in mature vessels.

Acknowledgments

This work was supported by NIH grants AR36820 and AR048564 (B.R. Olsen) and EMBO Fellowship (L. Eklund). We would like to thank Eileen Boye for helpful comments on the manuscript and Yulia Pittel for excellent secretarial assistance.

References

[1] D.J. Dumont, T.P. Yamaguchi, R.A. Conlon, J. Rossant, M.L. Breitman, Tek, a novel tyrosine kinase gene located on mouse chromosome 4, is expressed in endothelial cells and their presumptive precursors, Oncogene 7 (1992) 1471–1480.

[2] J. Partanen, E. Armstrong, T.P. Makela, J. Korhonen, M. Sandberg, R. Renkonen, S. Knuutila, K. Huebner, K. Alitalo, A novel endothelial cell surface receptor tyrosine kinase with extracellular epidermal growth factor homology domains, Mol. Cell. Biol. 12 (1992) 1698–1707.

[3] S. Davis, T.H. Aldrich, P.F. Jones, A. Acheson, D.L. Compton, V. Jain, T.E. Ryan, J. Bruno, C. Radziejewski, P.C. Maisonpierre, G.D. Yancopoulos, Isolation of angiopoietin-1, a ligand for the TIE2 receptor, by secretion-trap expression cloning, Cell 87 (1996) 1161–1169.

[4] P.C. Maisonpierre, C. Suri, P.F. Jones, S. Bartunkova, S. Wiegand, C. Radziejewski, D. Compton, J. McClain, T.H. Aldrich, N. Papadopoulos, T.J. Daly, S. Davis, T.N. Sato, G.D. Yancopoulos, Angiopoietin 2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis, Science 277 (1997) 55–60.

[5] D.M. Valenzuela, J.A. Griffiths, J. Rojas, T.H. Aldrich, P.F. Jones, H. Zhou, J. McClain, N.G. Copeland, D.J. Gilbert, N.A. Jenkins, T. Huang, N. Papadopoulos, P.C. Maisonpierre, S. Davis, G.D. Yancopoulos, Angiopoietins 3 and 4: diverging gene counterparts in mice and humans, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 1904–1909.

[6] C. Suri, P.F. Jones, S. Patan, S. Bartunkova, P.C. Maisonpierre, S. Davis, T.N. Sato, G.D. Yancopoulos, Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis, Cell 87 (1996) 1171–1180.

[7] J. Holash, S.J. Wiegand, G.D. Yancopoulos, New model of tumor angiogenesis: dynamic balance between vessel regression and growth mediated by angiopoietins and VEGF, Oncogene 18 (1999) 5356–5362.

[8] N.W. Gale, G. Thurston, S.F. Hackett, R. Renard, Q. Wang, J. McClain, C. Martin, C. Witte, M.H. Witte, D. Jackson, C. Suri, P.A. Campochiaro, S.J. Wiegand, G.D. Yancopoulos, Angiopoietin-2 is required for postnatal angiogenesis and lymphatic patterning, and only the latter role is rescued by Angiopoietin-1, Dev. Cell 3 (2002) 411–423.

[9] I. Kim, J.H. Kim, S.O. Moon, H.J. Kwak, N.G. Kim, G.Y. Koh, Angiopoietin-2 at high concentration can enhance endothelial cell survival through the phosphatidylinositol 3′-kinase/Akt signal transduction pathway, Oncogene 19 (2000) 4549–4552.

[10] K. Teichert-Kuliszewska, P.C. Maisonpierre, N. Jones, A.I. Campbell, Z. Master, M.P. Bendeck, K. Alitalo, D.J. Dumont, G.D. Yancopoulos, D.J. Stewart, Biological action of angiopoietin-2 in a fibrin matrix model of angiogenesis is associated with activation of Tie2, Cardiovasc. Res. 49 (2001) 659–670.

[11] Y. Mochizuki, T. Nakamura, H. Kanetake, S. Kanda, Angiopoietin 2 stimulates migration and tube-like structure formation of murine brain capillary endothelial cells through c-Fes and c-Fyn, J. Cell Sci. 115 (2002) 175–183.

[12] A. Das, W. Fanslow, D. Cerretti, E. Warren, N. Talarico, P. McGuire, Angiopoietin/Tek interactions regulate mmp-9 expression and retinal neovascularization, Lab. Invest. 83 (2003) 1637–1645.

[13] T. Korff, S. Kimmina, G. Martiny-Baron, H.G. Augustin, Blood vessel maturation in a 3-dimensional spheroidal coculture model: direct contact with smooth muscle cells regulates endothelial cell quiescence and abrogates VEGF responsiveness, FASEB J. 15 (2001) 447–457.

[14] H.J. Lee, C.H. Cho, S.J. Hwang, H.H. Choi, K.T. Kim, S.Y. Ahn, J.H. Kim, J.L. Oh, G.M. Lee, G.Y. Koh, Biological characterization of angiopoietin-3 and angiopoietin-4, FASEB J. 18 (2004) 1200–1208.

[15] G. Thurston, C. Suri, K. Smith, J. McClain, T.N. Sato, G.D. Yancopoulos, D.M. McDonald, Leakage-resistant blood vessels in mice transgenically overexpressing angiopoietin-1, Science 286 (1999) 2511–2514.

[16] T.I. Koblizek, C. Weiss, G.D. Yancopoulos, U. Deutsch, W. Risau, Angiopoietin-1 induces sprouting angiogenesis in vitro, Curr. Biol. 8 (1998) 529–532.

[17] W.H. Zhu, A. MacIntyre, R.F. Nicosia, Regulation of angiogenesis by vascular endothelial growth factor and angiopoietin-1 in the rat aorta model: distinct temporal patterns of intracellular signaling correlate with induction of angiogenic sprouting, Am. J. Pathol. 161 (2002) 823–830.

[18] M. Saito, M. Hamasaki, M. Shibuya, Induction of tube formation by angiopoietin-1 in endothelial cell/fibroblast co-culture is dependent on endogenous VEGF, Cancer Sci. 94 (2003) 782–790.

[19] J.K. Chae, I. Kim, S.T. Lim, M.J. Chung, W.H. Kim, H.G. Kim, J.K. Ko, G.Y. Koh, Coadministration of angiopoietin-1 and vascular endothelial growth factor enhances collateral vascularization, Arterioscler., Thromb., Vasc. Biol. 20 (2000) 2573–2578.

[20] K.G. Shyu, H. Chang, J.M. Isner, Synergistic effect of angiopoietin-1 and vascular endothelial growth factor on neoangiogenesis in hypercholesterolemic rabbit model with acute hindlimb ischemia, Life Sci. 73 (2003) 563–579.

[21] G. Thurston, J.S. Rudge, E. Ioffe, H. Zhou, L. Ross, S.D. Croll, N. Glazer, J. Holash, D.M. McDonald, G.D. Yancopoulos, Angiopoietin-1 protects the adult vasculature against plasma leakage, Nat. Med. 6 (2000) 460–463.

[22] R.P. Visconti, C.D. Richardson, T.N. Sato, Orchestration of angiogenesis and arteriovenous contribution by angiopoietins and vascular endothelial growth factor (VEGF), Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 8219–8224.

[23] H. Nambu, R. Nambu, Y. Oshima, S.F. Hackett, G. Okoye, S. Wiegand, G. Yancopoulos, D.J. Zack, P.A. Campochiaro, Angiopoietin 1 inhibits ocular neovascularization and breakdown of the blood–retinal barrier, Gene Ther. 11 (2004) 865–873.

[24] H. Nambu, N. Umeda, S. Kachi, Y. Oshima, H. Akiyama, R. Nambu, P.A. Campochiaro, Angiopoietin 1 prevents retinal detachment in an aggressive model of proliferative retinopathy, but has no effect on established neovascularization, J. Cell. Physiol. 204 (2005) 227–235.

[25] Y. Oshima, T. Deering, S. Oshima, H. Nambu, P.S. Reddy, M. Kaleko, S. Connelly, S.F. Hackett, P.A. Campochiaro, Angiopoietin-2 enhances retinal vessel sensitivity to vascular endothelial growth factor, J. Cell. Physiol. 199 (2004) 412–417.

[26] Y. Oshima, S. Oshima, H. Nambu, S. Kachi, K. Takahashi, N. Umeda, J. Shen, A. Dong, R.S. Apte, E. Duh, S.F. Hackett, G. Okoye, K. Ishibashi, J. Handa, M. Melia, S. Wiegand, G. Yancopoulos, D.J. Zack, P.A. Campochiaro, Different effects of angiopoietin-2 in different vascular beds: new vessels are most sensitive, FASEB J. (2005) (electronic publication ahead of print).

[27] L.M. DeBusk, D.E. Hallahan, P.C. Lin, Akt is a major angiogenic mediator downstream of the Ang1/Tie2 signaling pathway, Exp. Cell Res. 298 (2004) 167–177.

[28] K. Fujikawa, I. de Aos Scherpenseel, S.K. Jain, E. Presman, R.A. Christensen, L. Varticovski, Role of PI 3-kinase in angiopoietin-1-mediated migration and attachment-dependent survival of endothelial cells, Exp. Cell Res. 253 (1999) 663–672.

[29] I. Kim, H.G. Kim, J.N. So, J.H. Kim, H.J. Kwak, G.Y. Koh,

Angiopoietin-1 regulates endothelial cell survival through the phosphatidylinositol 3′-kinase/Akt signal transduction pathway, Circ. Res. 86 (2000) 24–29.
[30] R. Harfouche, J.P. Gratton, G.D. Yancopoulos, M. Noseda, A. Karsan, S.N. Hussain, Angiopoietin-1 activates both anti- and proapoptotic mitogen-activated protein kinases, FASEB J. 17 (2003) 1523–1525.
[31] A. Papapetropoulos, D. Fulton, K. Mahboubi, R.G. Kalb, D.S. O'Connor, F. Li, D.C. Altieri, W.C. Sessa, Angiopoietin-1 inhibits endothelial cell apoptosis via the Akt/survivin pathway, J. Biol. Chem. 275 (2000) 9102–9105.
[32] S. Babaei, K. Teichert-Kuliszewska, Q. Zhang, N. Jones, D.J. Dumont, D.J. Stewart, Angiogenic actions of angiopoietin-1 require endothelium-derived nitric oxide, Am. J. Pathol. 162 (2003) 1927–1936.
[33] R. Yabkowitz, S. Meyer, D. Yanagihara, D. Brankow, T. Staley, G. Elliott, S. Hu, B. Ratzkin, Regulation of tie receptor expression on human endothelial cells by protein kinase C-mediated release of soluble tie, Blood 90 (1997) 706–715.
[34] R. Yabkowitz, S. Meyer, T. Black, G. Elliott, L.A. Merewether, H.K. Yamane, Inflammatory cytokines and vascular endothelial growth factor stimulate the release of soluble tie receptor from human endothelial cells via metalloprotease activation, Blood 93 (1999) 1969–1979.
[35] M.B. Marron, D.P. Hughes, M.J. McCarthy, E.R. Beaumont, N.P. Brindle, Tie-1 receptor tyrosine kinase endodomain interaction with SHP2: potential signalling mechanisms and roles in angiogenesis, Adv. Exp. Med. Biol. 476 (2000) 35–46.
[36] L. Chen-Konak, Y. Guetta-Shubin, H. Yahav, A. Shay-Salit, M. Zilberman, O. Binah, N. Resnick, Transcriptional and post-translation regulation of the Tie1 receptor by fluid shear stress changes in vascular endothelial cells, FASEB J. 17 (2003) 2121–2123.
[37] A.C. Tsiamis, P.N. Morris, M.B. Marron, N.P. Brindle, Vascular endothelial growth factor modulates the Tie-2:Tie-1 receptor complex, Microvasc. Res. 63 (2002) 149–158.
[38] M.J. McCarthy, R. Burrows, S.C. Bell, G. Christie, P.R. Bell, N.P. Brindle, Potential roles of metalloprotease mediated ectodomain cleavage in signaling by the endothelial receptor tyrosine kinase Tie-1, Lab. Invest. 79 (1999) 889–895.
[39] M.B. Marron, D.P. Hughes, M.D. Edge, C.L. Forder, N.P. Brindle, Evidence for heterotypic interaction between the receptor tyrosine kinases TIE-1 and TIE-2, J. Biol. Chem. 275 (2000) 39741–39746.
[40] P. Saharinen, K. Kerkela, N. Ekman, M. Marron, N. Brindle, G.M. Lee, H. Augustin, G.Y. Koh, K. Alitalo, Multiple angiopoietin recombinant proteins activate the Tie1 receptor tyrosine kinase and promote its interaction with Tie2, J. Cell Biol. 169 (2005) 239–243.
[41] M. Vikkula, L.M. Boon, K. Carraway, J.T. Calvert, A.J. Diamonti, B. Goumnerov, K.A. Pasyk, D.A. Marchuk, M.L. Warman, L.C. Cantley, J.B. Mulliken, B.R. Olsen, Vascular dysmorphogenesis caused by an activating mutation in the receptor tyrosine kinase TIE2, Cell 87 (1996) 1181–1190.
[42] J.T. Calvert, T.J. Riney, C.D. Kontos, E.H. Cha, V.G. Prieto, C.R. Shea, J.N. Berg, N.C. Nevin, S.A. Simpson, K.A. Pasyk, M.C. Speer, K.G. Peters, D.A. Marchuk, Allelic and locus heterogeneity in inherited venous malformations, Hum. Mol. Genet. 8 (1999) 1279–1289.
[43] H. Wang, Y. Zhang, S. Toratani, T. Okamoto, Transformation of vascular endothelial cells by a point mutation in the Tie2 gene from human intramuscular haemangioma, Oncogene 23 (2004) 8700–8704.
[44] D. Voskas, N. Jones, P. Van Slyke, C. Sturk, W. Chang, A. Haninec, Y.O. Babichev, J. Tran, Z. Master, S. Chen, N. Ward, M. Cruz, J. Jones, R.S. Kerbel, S. Jothy, L. Dagnino, J. Arbiser, G. Klement, D.J. Dumont, A cyclosporine-sensitive psoriasis-like disease produced in Tie2 transgenic mice, Am. J. Pathol. 166 (2005) 843–855.
[45] L. Du, C.C. Sullivan, D. Chu, A.J. Cho, M. Kido, P.L. Wolf, J.X. Yuan, R. Deutsch, S.W. Jamieson, P.A. Thistlethwaite, Signaling molecules in nonfamilial pulmonary hypertension, N. Engl. J. Med. 348 (2003) 500–509.
[46] C.C. Sullivan, L. Du, D. Chu, A.J. Cho, M. Kido, P.L. Wolf, S.W. Jamieson, P.A. Thistlethwaite, Induction of pulmonary hypertension by an angiopoietin 1/TIE2/serotonin pathway, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 12331–12336.
[47] Y. Yu, J. Varughese, L. Brown, J.B. Mulliken, J. Bischoff, Increased Tie2 expression, enhanced response to angiopoietin-1, and dysregulated angiopoietin-2 expression in hemangioma-derived endothelial cells, Am. J. Pathol. 159 (2001) 2271–2280.
[48] C.R. Tait, P.F. Jones, Angiopoietins in tumours: the angiogenic switch, J. Pathol. 204 (2004) 1–10.
[49] W.N. Procopio, P.I. Pelavin, W.M. Lee, N.M. Yeilding, Angiopoietin-1 and -2 coiled coil domains mediate distinct homo-oligomerization patterns, but fibrinogen-like domains mediate ligand activity, J. Biol. Chem. 274 (1999) 30196–30201.
[50] S. Davis, N. Papadopoulos, T.H. Aldrich, P.C. Maisonpierre, T. Huang, L. Kovac, A. Xu, R. Leidich, E. Radziejewska, A. Rafique, J. Goldberg, V. Jain, K. Bailey, M. Karow, J. Fandl, S.J. Samuelsson, E. Ioffe, J.S. Rudge, T.J. Daly, C. Radziejewski, G.D. Yancopoulos, Angiopoietins have distinct modular domains essential for receptor binding, dimerization and superclustering, Nat. Struct. Biol. 10 (2003) 38–44.
[51] C.H. Cho, R.A. Kammerer, H.J. Lee, M.O. Steinmetz, Y.S. Ryu, S.H. Lee, K. Yasunaga, K.T. Kim, I. Kim, H.H. Choi, W. Kim, S.H. Kim, S.K. Park, G.M. Lee, G.Y. Koh, COMP-Ang1: a designed angiopoietin-1 variant with nonleaky angiogenic activity, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 5547–5552.
[52] U. Fiedler, T. Krissl, S. Koidl, C. Weiss, T. Koblizek, U. Deutsch, G. Martiny-Baron, D. Marme, H.G. Augustin, Angiopoietin-1 and angiopoietin-2 share the same binding domains in the Tie-2 receptor involving the first Ig-like loop and the epidermal growth factor-like repeats, J. Biol. Chem. 278 (2003) 1721–1727.
[53] Y. Xu, Q. Yu, Angiopoietin-1, unlike angiopoietin-2, is incorporated into the extracellular matrix via its linker peptide region, J. Biol. Chem. 276 (2001) 34990–34998.
[54] U. Fiedler, M. Scharpfenecker, S. Koidl, A. Hegen, V. Grunow, J.M. Schmidt, W. Kriz, G. Thurston, H.G. Augustin, The Tie-2 ligand angiopoietin-2 is stored in and rapidly released upon stimulation from endothelial cell Weibel-Palade bodies, Blood 103 (2004) 4150–4156.
[55] Y. Xu, Y.J. Liu, Q. Yu, Angiopoietin-3 is tethered on the cell surface via heparan sulfate proteoglycans, J. Biol. Chem. 279 (2004) 41179–41188.
[56] T.N. Sato, Y. Tozawa, U. Deutsch, K. Wolburg-Buchholz, Y. Fujiwara, M. Gendron-Maguire, T. Gridley, H. Wolburg, W. Risau, Y. Qin, Distinct roles of the receptor tyrosine kinases Tie-1 and Tie-2 in blood vessel formation, Nature 376 (1995) 70–74.
[57] D.J. Dumont, G. Gradwohl, G.H. Fong, M.C. Puri, M. Gertsenstein, A. Auerbach, M.L. Breitman, 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 (1994) 1897–1909.
[58] B. Witzenbichler, P.C. Maisonpierre, P. Jones, G.D. Yancopoulos, J.M. Isner, Chemotactic properties of angiopoietin-1 and -2, ligands for the endothelial-specific receptor tyrosine kinase Tie2, J. Biol. Chem. 273 (1998) 18514–18521.
[59] A.J. Hayes, W.Q. Huang, J. Mallah, D. Yang, M.E. Lippman, L.Y. Li, Angiopoietin-1 and its receptor Tie-2 participate in the regulation of capillary-like tubule formation and survival of endothelial cells, Microvasc. Res. 58 (1999) 224–237.
[60] I. Kim, H.G. Kim, S.O. Moon, S.W. Chae, J.N. So, K.N. Koh, B.C. Ahn, G.Y. Koh, Angiopoietin-1 induces endothelial cell sprouting through the activation of focal adhesion kinase and plasmin secretion, Circ. Res. 86 (2000) 952–959.
[61] H.J. Kwak, J.N. So, S.J. Lee, I. Kim, G.Y. Koh, Angiopoietin-1 is an apoptosis survival factor for endothelial cells, FEBS Lett. 448 (1999) 249–253.
[62] A. Papapetropoulos, G. Garcia-Cardena, T.J. Dengler, P.C. Maison-

pierre, G.D. Yancopoulos, W.C. Sessa, Direct actions of angiopoietin-1 on human endothelium: evidence for network stabilization, cell survival, and interaction with other angiogenic growth factors, Lab. Invest. 79 (1999) 213–223.
[63] H.J. Kwak, S.J. Lee, Y.H. Lee, C.H. Ryu, K.N. Koh, H.Y. Choi, G.Y. Koh, Angiopoietin-1 inhibits irradiation- and mannitol-induced apoptosis in endothelial cells, Circulation 101 (2000) 2317–2324.
[64] J.R. Gamble, J. Drew, L. Trezise, A. Underwood, M. Parsons, L. Kasminkas, J. Rudge, G. Yancopoulos, M.A. Vadas, Angiopoietin-1 is an antipermeability and anti-inflammatory agent in vitro and targets cell junctions, Circ. Res. 87 (2000) 603–607.
[65] I. Kim, S.O. Moon, S.K. Park, S.W. Chae, G.Y. Koh, Angiopoietin-1 reduces VEGF-stimulated leukocyte adhesion to endothelial cells by reducing ICAM-1, VCAM-1, and E-selectin expression, Circ. Res. 89 (2001) 477–479.
[66] I. Kim, J.L. Oh, Y.S. Ryu, J.N. So, W.C. Sessa, K. Walsh, G.Y. Koh, Angiopoietin-1 negatively regulates expression and activity of tissue factor in endothelial cells, FASEB J. 16 (2002) 126–128.
[67] L. Pizurki, Z. Zhou, K. Glynos, C. Roussos, A. Papapetropoulos, Angiopoietin-1 inhibits endothelial permeability, neutrophil adherence and IL-8 production, Br. J. Pharmacol. 139 (2003) 329–336.
[68] A.M. Joussen, V. Poulaki, A. Tsujikawa, W. Qin, T. Qaum, Q. Xu, Y. Moromizato, S.E. Bursell, S.J. Wiegand, J. Rudge, E. Ioffe, G.D. Yancopoulos, A.P. Adamis, Suppression of diabetic retinopathy with angiopoietin-1, Am. J. Pathol. 160 (2002) 1683–1693.
[69] R. Harfouche, H.M. Hassessian, Y. Guo, V. Faivre, C.B. Srikant, G.D. Yancopoulos, S.N. Hussain, Mechanisms which mediate the antiapoptotic effects of angiopoietin-1 on endothelial cells, Microvasc. Res. 64 (2002) 135–147.
[70] C.H. Cho, R.A. Kammerer, H.J. Lee, K. Yasunaga, K.T. Kim, H.H. Choi, W. Kim, S.H. Kim, S.K. Park, G.M. Lee, G.Y. Koh, Designed angiopoietin-1 variant, COMP-Ang1, protects against radiation-induced endothelial cell apoptosis, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 5553–5558.
[71] S.R. Hubbard, J.H. Till, Protein tyrosine kinase structure and function, Annu. Rev. Biochem. 69 (2000) 373–398.
[72] N. Jones, D.J. Dumont, The Tek/Tie2 receptor signals through a novel Dok-related docking protein, Dok-R, Oncogene 17 (1998) 1097–1108.
[73] Z. Master, N. Jones, J. Tran, J. Jones, R.S. Kerbel, D.J. Dumont, Dok-R plays a pivotal role in angiopoietin-1-dependent cell migration through recruitment and activation of Pak, EMBO J 20 (2001) 5919–5928.
[74] N. Jones, S.H. Chen, C. Sturk, Z. Master, J. Tran, R.S. Kerbel, D.J. Dumont, A unique autophosphorylation site on Tie2/Tek mediates Dok-R phosphotyrosine binding domain binding and function, Mol. Cell. Biol. 23 (2003) 2658–2668.
[75] N. Jones, Z. Master, J. Jones, D. Bouchard, Y. Gunji, H. Sasaki, R. Daly, K. Alitalo, D.J. Dumont, Identification of Tek/Tie2 binding partners. Binding to a multifunctional docking site mediates cell survival and migration, J. Biol. Chem. 274 (1999) 30896–30905.
[76] L. Huang, C.W. Turck, P. Rao, K.G. Peters, GRB2 and SH-PTP2: potentially important endothelial signaling molecules downstream of the TEK/TIE2 receptor tyrosine kinase, Oncogene 11 (1995) 2097–2103.
[77] I. Kim, Y.S. Ryu, H.J. Kwak, S.Y. Ahn, J.L. Oh, G.D. Yancopoulos, N.W. Gale, G.Y. Koh, EphB ligand, ephrinB2, suppresses the VEGF- and angiopoietin 1-induced Ras/mitogen activated protein kinase pathway in venous endothelial cells, FASEB J. 16 (2002) 1126–1128.
[78] R. Tournaire, M.P. Simon, F. le Noble, A. Eichmann, P. England, J. Pouyssegur, A short synthetic peptide inhibits signal transduction, migration and angiogenesis mediated by Tie2 receptor, EMBO Rep. 5 (2004) 262–267.
[79] M.J. Yoon, C.H. Cho, C.S. Lee, I.H. Jang, S.H. Ryu, G.Y. Koh, Localization of Tie2 and phospholipase D in endothelial caveolae is involved in angiopoietin-1-induced MEK/ERK phosphorylation and migration in endothelial cells, Biochem. Biophys. Res. Commun. 308 (2003) 101–105.
[80] C.D. Kontos, T.P. Stauffer, W.P. Yang, J.D. York, L. Huang, M.A. Blanar, T. Meyer, K.G. Peters, Tyrosine 1101 of Tie2 is the major site of association of p85 and is required for activation of phosphatidylinositol 3 kinase and Akt, Mol. Cell. Biol. 18 (1998) 4131–4140.
[81] I. Cascone, E. Audero, E. Giraudo, L. Napione, F. Maniero, M.R. Philips, J.G. Collard, G. Serini, F. Bussolino, Tie-2 dependent activation of RhoA and Rac1 participates in endothelial cell motility triggered by angiopoietin-1, Blood 102 (2003) 2482–2490.
[82] J.X. Chen, M.L. Lawrence, G. Cunningham, B.W. Christman, B. Meyrick, HSP90 and Akt modulate Ang 1-induced angiogenesis via NO in coronary artery endothelium, J. Appl. Physiol. 96 (2004) 612–620.
[83] E. Audero, I. Cascone, F. Maniero, L. Napione, M. Arese, L. Lanfrancone, F. Bussolino, Adaptor ShcA protein binds tyrosine kinase Tie2 receptor and regulates migration and sprouting but not survival of endothelial cells, J. Biol. Chem. 279 (2004) 13224–13233.
[84] D.P. Hughes, M.B. Marron, N.P. Brindle, The antiinflammatory endothelial tyrosine kinase Tie2 interacts with a novel nuclear factor kappaB inhibitor ABIN 2, Circ. Res. 92 (2003) 630–636.
[85] E.I. Korpelainen, M. Kärkkäinen, Y. Gunji, M. Vikkula, K. Alitalo, Endothelial receptor tyrosine kinases activate the STAT signaling pathway: mutant TIE 2 causing venous malformations signals a distinct STAT activation response., Oncogene 18 (1999) 1–8.
[86] J. Holash, P.C. Maisonpierre, D. Compton, P. Boland, C.R. Alexander, D. Zagzag, G.D. Yancopoulos, S.J. Wiegand, Vessel cooption, regression, and growth in tumors mediated by angiopoietins and VEGF, Science 284 (1999) 1994–1998.
[87] A. Stratmann, W. Risau, K.H. Plate, Cell type specific expression of angiopoietin-1 and angiopoietin-2 suggests a role in glioblastoma angiogenesis, ([In Process Citation])Am. J. Pathol. 153 (1998) 1459–1466.
[88] D. Zagzag, A. Hooper, D.R. Friedlander, W. Chan, J. Holash, S.J. Wiegand, G.D. Yancopoulos, M. Grumet, In situ expression of angiopoietins in astrocytomas identifies angiopoietin-2 as an early marker of tumor angiogenesis, Exp. Neurol. 159 (1999) 391–400.
[89] T. Asahara, D. Chen, T. Takahashi, K. Fujikawa, M. Kearney, M. Magner, G.D. Yancopoulos, J.M. Isner, Tie2 receptor ligands, angiopoietin-1 and angiopoietin-2, modulate VEGF-induced postnatal neovascularization [see comments], Circ. Res. 83 (1998) 233–240.
[90] I.B. Lobov, P.C. Brooks, R.A. Lang, Angiopoietin-2 displays VEGF-dependent modulation of capillary structure and endothelial cell survival in vivo, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 11205–11210.
[91] M. Scharpfenecker, U. Fiedler, Y. Reiss, H.G. Augustin, The Tie-2 ligand angiopoietin-2 destabilizes quiescent endothelium through an internal autocrine loop mechanism, J. Cell Sci. 118 (2005) 771–780.
[92] S.F. Hackett, S. Wiegand, G. Yancopoulos, P.A. Campochiaro, Angiopoietin-2 plays an important role in retinal angiogenesis, J. Cell. Physiol. 192 (2002) 182–187.
[93] J.E. Pitera, A.S. Woolf, N.W. Gale, G.D. Yancopoulos, H.T. Yuan, Dysmorphogenesis of kidney cortical peritubular capillaries in angiopoietin-2 deficient mice, Am. J. Pathol. 165 (2004) 1895–1906.
[94] S.A. Ahmad, W. Liu, Y.D. Jung, F. Fan, M. Wilson, N. Reinmuth, R.M. Shaheen, C.D. Bucana, L.M. Ellis, The effects of angiopoietin-1 and -2 on tumor growth and angiogenesis in human colon cancer, Cancer Res. 61 (2001) 1255–1259.
[95] S. Tanaka, M. Mori, Y. Sakamoto, M. Makuuchi, K. Sugimachi, J.R. Wands, Biologic significance of angiopoietin-2 expression in human hepatocellular carcinoma, J. Clin. Invest. 103 (1999) 341–345.
[96] J. Oliner, H. Min, J. Leal, D. Yu, S. Rao, E. You, X. Tang, H. Kim, S. Meyer, S.J. Han, N. Hawkins, R. Rosenfeld, E. Davy, K. Graham, F.

Jacobsen, S. Stevenson, J. Ho, Q. Chen, T. Hartmann, M. Michaels, M. Kelley, L. Li, K. Sitney, F. Martin, J.R. Sun, N. Zhang, J. Lu, J. Estrada, R. Kumar, A. Coxon, S. Kaufman, J. Pretorius, S. Scully, R. Cattley, M. Payton, S. Coats, L. Nguyen, B. Desilva, A. Ndifor, I. Hayward, R. Radinsky, T. Boone, R. Kendall, Suppression of angiogenesis and tumor growth by selective inhibition of angiopoietin-2, Cancer Cells 6 (2004) 507–516.

[97] R.R. White, S. Shan, C.P. Rusconi, G. Shetty, M.W. Dewhirst, C.D. Kontos, B.A. Sullenger, Inhibition of rat corneal angiogenesis by a nuclease-resistant RNA aptamer specific for angiopoietin-2, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 5028–5033.

[98] T. Hawighorst, M. Skobe, M. Streit, Y.K. Hong, P. Velasco, L.F. Brown, L. Riccardi, B. Lange-Asschenfeldt, M. Detmar, Activation of the tie2 receptor by angiopoietin-1 enhances tumor vessel maturation and impairs squamous cell carcinoma growth, Am. J. Pathol. 160 (2002) 1381–1392.

[99] O. Stoeltzing, S.A. Ahmad, W. Liu, M.F. McCarty, J.S. Wey, A.A. Parikh, F. Fan, N. Reinmuth, M. Kawaguchi, C.D. Bucana, L.M. Ellis, Angiopoietin-1 inhibits vascular permeability, angiogenesis, and growth of hepatic colon cancer tumors, Cancer Res. 63 (2003) 3370–3377.

[100] S.F. Hackett, H. Ozaki, R.W. Strauss, K. Wahlin, C. Suri, P. Maisonpierre, G. Yancopoulos, P.A. Campochiaro, Angiopoietin 2 expression in the retina: upregulation during physiologic and pathologic neovascularization, J. Cell. Physiol. 184 (2000) 275–284.

[101] S.J. Mandriota, M.S. Pepper, Regulation of angiopoietin-2 mRNA levels in bovine microvascular endothelial cells by cytokines and hypoxia, Circ. Res. 83 (1998) 852–859.

[102] Y. Xu, Y.J. Liu, Q. Yu, Angiopoietin-3 inhibits pulmonary metastasis by inhibiting tumor angiogenesis, Cancer Res. 64 (2004) 6119–6126.

[103] A.L. Wong, Z.A. Haroon, S. Werner, M.W. Dewhirst, C.S. Greenberg, K.G. Peters, Tie2 expression and phosphorylation in angiogenic and quiescent adult tissues, Circ. Res. 81 (4) (1997) 567–574.

[104] M.C. Puri, A. Bernstein, Requirement for the TIE family of receptor tyrosine kinases in adult but not fetal hematopoiesis, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 12753–12758.

[105] M.C. Puri, J. Partanen, J. Rossant, A. Bernstein, Interaction of the TEK and TIE receptor tyrosine kinases during cardiovascular development, Development 126 (1999) 4569–4580.

[106] P.N. Lin, P. Polverini, M. Dewhirst, S.Q. Shan, P.S. Rao, K. Peters, Inhibition of tumor angiogenesis using a soluble receptor establishes a role for Tie2 in pathologic vascular growth, J. Clin. Invest. 100 (8) (1997) 2072–2078.

[107] M. Hangai, Y.S. Moon, N. Kitaya, C.K. Chan, D.Y. Wu, K.G. Peters, S.J. Ryan, D.R. Hinton, Systemically expressed soluble Tie2 inhibits intraocular neovascularization, Hum. Gene Ther. 12 (2001) 1311–1321.

[108] G. Thurston, Q. Wang, F. Baffert, J. Rudge, N. Papadopoulos, D. Jean-Guillaume, S. Wiegand, G.D. Yancopoulos, D.M. McDonald, Angiopoietin 1 causes vessel enlargement, without angiogenic sprouting, during a critical developmental period, Development 132 (2005) 3317–3326.

[109] C.H. Cho, K.E. Kim, J. Byun, H.S. Jang, D.K. Kim, P. Baluk, F. Baffert, G.M. Lee, N. Mochizuki, J. Kim, B.H. Jeon, D.M. McDonald, G.Y. Koh, Long-term and sustained COMP-Ang1 induces long-lasting vascular enlargement and enhanced blood flow, Circ. Res. (2005) 86–94.

[110] F. Baffert, G. Thurston, M. Rochon-Duck, T. Le, R. Brekken, D.M. McDonald, Age-related changes in vascular endothelial growth factor dependency and angiopoietin-1-induced plasticity of adult blood vessels, Circ. Res. 94 (2004) 984–992.

[111] C. Suri, J. McClain, G. Thurston, D.M. McDonald, H. Zhou, E.H. Oldmixon, T.N. Sato, G.D. Yancopoulos, Increased vascularization in mice overexpressing angiopoietin-1, Science 282 (1998) 468–471.

[112] M.C. Puri, J. Rossant, K. Alitalo, A. Bernstein, J. Partanen, The receptor tyrosine kinase TIE is required for integrity and survival of vascular endothelial cells, EMBO J. 14 (1995) 5884–5891.

[113] S. Patan, TIE1 and TIE2 receptor tyrosine kinases inversely regulate embryonic angiogenesis by the mechanism of intussusceptive microvascular growth, Microvasc. Res. 56 (1998) 1–21.

[114] C.D. Kontos, E.H. Cha, J.D. York, K.G. Peters, The endothelial receptor tyrosine kinase Tie1 activates phosphatidylinositol 3-kinase and Akt to inhibit apoptosis, Mol. Cell. Biol. 22 (2002) 1704–1713.

[115] R.M. Porat, M. Grunewald, A. Globerman, A. Itin, G. Barshtein, L. Alhonen, K. Alitalo, E. Keshet, Specific induction of tie1 promoter by disturbed flow in atherosclerosis-prone vascular niches and flow-obstructing pathologies, Circ. Res. 94 (2004) 394–401.

[116] E. Kriehuber, S. Breiteneder-Geleff, M. Groeger, A. Soleiman, S.F. Schoppmann, G. Stingl, D. Kerjaschki, D. Maurer, Isolation and characterization of dermal lymphatic and blood endothelial cells reveal stable and functionally specialized cell lineages, J. Exp. Med. 194 (2001) 797–808.

[117] T. Tammela, A. Saaristo, M. Lohela, T. Morisada, J. Tornberg, C. Norrmen, Y. Oike, K. Pajusola, G. Thurston, T. Suda, S. Yla-Herttuala, K. Alitalo, Angiopoietin-1 promotes lymphatic sprouting and hyperplasia, Blood 105 (2005) 4642–4648.

[118] T. Morisada, Y. Oike, Y. Yamada, T. Urano, M. Akao, Y. Kubota, H. Maekawa, Y. Kimura, M. Ohmura, T. Miyamoto, S. Nozawa, G.Y. Koh, K. Alitalo, T. Suda, Angiopoietin-1 promotes LYVE-1-positive lymphatic vessel formation, Blood 105 (2005) 4649–4656.

[119] E. Kukk, U. Wartiovaara, Y. Gunji, J. Kaukonen, H.J. Buhring, I. Rappold, M.T. Matikainen, P. Vihko, J. Partanen, A. Palotie, K. Alitalo, R. Alitalo, Analysis of Tie receptor tyrosine kinase in haemopoietic progenitor and leukaemia cells, Br. J. Haematol. 98 (1997) 195–203.

[120] A. Iwama, I. Hamaguchi, M. Hashiyama, Y. Murayama, K. Yasunaga, T. Suda, Molecular cloning and characterization of mouse TIE and TEK receptor tyrosine kinase genes and their expression in hematopoietic stem cells, Biochem. Biophys. Res. Commun. 195 (1993) 301–309.

[121] N. Takakura, X.L. Huang, T. Naruse, I. Hamaguchi, D.J. Dumont, G.D. Yancopoulos, T. Suda, Critical role of the TIE2 endothelial cell receptor in the development of definitive hematopoiesis, Immunity 9 (1998) 677–686.

[122] F. Arai, A. Hirao, M. Ohmura, H. Sato, S. Matsuoka, K. Takubo, K. Ito, G.Y. Koh, T. Suda, Tie2/angiopoietin-1 signaling regulates hematopoietic stem cell quiescence in the bone marrow niche, Cell 118 (2004) 149–161.

[123] J. Partanen, M.C. Puri, L. Schwartz, K.D. Fischer, A. Bernstein, J. Rossant, Cell autonomous functions of the receptor tyrosine kinase TIE in a late phase of angiogenic capillary growth and endothelial cell survival during murine development, Development 122 (1996) 3013–3021.

[124] J. Folkman, P.A. D'Amore, Blood vessel formation: what is its molecular basis? Cell 87 (1996) 1153–1155.

[125] E. Iivanainen, L. Nelimarkka, V. Elenius, S.M. Heikkinen, T.T. Junttila, L. Sihombing, M. Sundvall, J.A. Maatta, V.J. Laine, S. Yla-Herttuala, S. Higashiyama, K. Alitalo, K. Elenius, Angiopoietin-regulated recruitment of vascular smooth muscle cells by endothelial-derived heparin binding EGF-like growth factor, FASEB J. 17 (2003) 1609–1621.

[126] T. Nishishita, P.C. Lin, Angiopoietin 1, PDGF-B, and TGF-beta gene regulation in endothelial cell and smooth muscle cell interaction, J. Cell. Biochem. 91 (2004) 584–593.

[127] L.J. Metheny-Barlow, S. Tian, A.J. Hayes, L.Y. Li, Direct chemotactic action of angiopoietin-1 on mesenchymal cells in the presence of VEGF, Microvasc. Res. 68 (2004) 221–230.

[128] M. Iurlaro, M. Scatena, W.H. Zhu, E. Fogel, S.L. Wieting, R.F. Nicosia, Rat aorta-derived mural precursor cells express the Tie2 receptor and respond directly to stimulation by angiopoietins, J. Cell Sci. 116 (2003) 3635–3643.

[129] S. Tian, A.J. Hayes, L.J. Metheny-Barlow, L.Y. Li, Stabilization of

breast cancer xenograft tumour neovasculature by angiopoietin-1, Br. J. Cancer 86 (2002) 645–651.
[130] Y.S. Park, N.H. Kim, I. Jo, Hypoxia and vascular endothelial growth factor acutely up-regulate angiopoietin-1 and Tie2 mRNA in bovine retinal pericytes, Microvasc. Res. 65 (2003) 125–131.
[131] S. Poncet, J.M. Gasc, R.C. Janzer, S. Meyer, L. Juillerat-Jeanneret, Expression of Tie-2 in human peripheral and autonomic nervous system, Neuropathol. Appl. Neurobiol. 29 (2003) 361–369.
[132] S. Valable, A. Bellail, S. Lesne, G. Liot, E.T. Mackenzie, D. Vivien, M. Bernaudin, E. Petit, Angiopoietin-1-induced PI3-kinase activation prevents neuronal apoptosis, FASEB J. 17 (2003) 443–445.
[133] J. Kosacka, M. Figiel, J. Engele, H. Hilbig, M. Majewski, K. Spanel-Borowski, Angiopoietin-1 promotes neurite outgrowth from dorsal root ganglion cells positive for Tie-2 receptor, Cell Tissue Res. 320 (2005) 11–19.
[134] N.L. Ward, T. Putoczki, K. Mearow, T.L. Ivanco, D.J. Dumont, Vascular-specific growth factor angiopoietin 1 is involved in the organization of neuronal processes, J. Comp. Neurol. 482 (2005) 244–256.
[135] T.R. Carlson, Y. Feng, P.C. Maisonpierre, G.D. Yancopoulos, M. Mrksich, A.O. Morla, Direct cell adhesion to the angiopoietins mediated by integrins, J. Biol. Chem. 9 (2001) 9.
[136] S.M. Dallabrida, N. Ismail, J.R. Oberle, B.E. Himes, M.A. Rupnick, Angiopoietin-1 promotes cardiac and skeletal myocyte survival through integrins, Circ. Res. 96 (2005) e8–e24.
[137] C.C. Weber, H. Cai, M. Ehrbar, H. Kubota, G. Martiny-Baron, W. Weber, V. Djonov, E. Weber, A.S. Mallik, M. Fussenegger, K. Frei, J.A. Hubbell, A.H. Zisch, Effects of protein and gene transfer of the angiopoietin-1 fibrinogen-like receptor-binding domain on endothelial and vessel organization, J. Biol. Chem. 280 (2005) 22445–22453.
[138] G. Fachinger, U. Deutsch, W. Risau, Functional interaction of vascular endothelial–protein–tyrosine phosphatase with the angiopoietin receptor Tie-2, Oncogene 18 (1999) 5948–5953.
[139] C. Daly, V. Wong, E. Burova, Y. Wei, S. Zabski, J. Griffiths, K.M. Lai, H.C. Lin, E. Ioffe, G.D. Yancopoulos, J.S. Rudge, Angiopoietin-1 modulates endothelial cell function and gene expression via the transcription factor FKHR (FOXO1), Genes Dev. 18 (2004) 1060–1071.
[140] S. Hori, S. Ohtsuki, K. Hosoya, E. Nakashima, T. Terasaki, A pericyte-derived angiopoietin-1 multimeric complex induces occludin gene expression in brain capillary endothelial cells through Tie-2 activation in vitro, J. Neurochem. 89 (2004) 503–513.
[141] X. Li, C.N. Hahn, M. Parsons, J. Drew, M.A. Vadas, J.R. Gamble, Role of protein kinase Czeta in thrombin-induced endothelial permeability changes: inhibition by angiopoietin-1, Blood 104 (2004) 1716–1724.
[142] Y. Wang, S. Pampou, K. Fujikawa, L. Varticovski, Opposing effect of angiopoietin-1 on VEGF-mediated disruption of endothelial cell–cell interactions requires activation of PKC beta, J. Cell. Physiol. 198 (2004) 53–61.