Subject Review

Angiopoietin: A TIE(d) Balance in Tumor Angiogenesis

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Abstract

Angiopoietins (ANG-1 and ANG-2) and their TIE-2 receptor tyrosine kinase have wide-ranging effects on tumor malignancy that includes angiogenesis, inflammation, and vascular extravasation. These multifaceted pathways present a valuable opportunity in developing novel inhibition strategies for cancer treatment. However, the regulatory role of ANG-1 and ANG-2 in tumor angiogenesis remains controversial. There is a complex interplay between complementary yet conflicting roles of both the ANGs in shaping the outcome of angiogenesis. Embryonic vascular development suggests that ANG-1 is crucial in engaging interaction between endothelial and perivascular cells. However, recruitment of perivascular cells by ANG-1 has recently been implicated in its antiangiogenic effect on tumor growth. It is becoming clear that TIE-2 signaling may function in a paracrine and autocrine manner directly on tumor cells because the receptor has been increasingly found in tumor cells. In addition, $\alpha_5\beta_1$ and $\alpha_v\beta_5$ integrins were recently recognized as functional receptors for ANG-1 and ANG-2. Therefore, both the ligands may have wide-ranging functions in cellular activities that affect overall tumor development. Collectively, these TIE-2–dependent and TIE-2–independent activities may account for the conflicting findings of ANG-1 and ANG-2 in tumor angiogenesis. These uncertainties have impeded development of a clear strategy to target this important angiogenic pathway. A better understanding of the molecular basis of ANG-1 and ANG-2 activity in the pathophysiologic regulation of angiogenesis may set the stage for novel therapy targeting this pathway.

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Introduction

Angiogenesis as a rate-limiting step in tumor growth was first mooted more than 3 decades ago (1). Malignant cells transverse such limitations by accumulating mutations that stoke angiogenic response or by sequestrating circulating growth inhibitors (2). Intratumoral microvascular density is now recognized as an important and independent prognostic marker for metastasis and overall survival in patients with breast, cervical, colon, lung, renal, ovarian, and esophageal carcinomas (3-9).

Tumor ecosystem comprises malignant cells, endothelial cells, perivascular cells, fibroblasts, inflammatory cells, and their surrounding extracellular matrices. These constituents continuously partake in the evolution of the milieu by expressing a myriad of autocrine and paracrine factors that influence the outcome of the disease. Vascular endothelial growth factor (VEGF) and the angiopoietins (ANG) are among the most important growth factors in the ecosystem. Signaling primarily through their endothelial receptors, these factors are responsible for proliferation, migration, and survival of activated vascular endothelial cells. In addition, these signaling pathways are believed to be responsible for the integrity, maturation, and maintenance of the vascular network. Furthermore, recent findings also suggest that certain types of cancer cells may also be directly responsive to these factors (10-14), although their significance in disease progression remains largely undefined.

ANG-1 is critically important in the formation of vascular networks during developmental angiogenesis (15). Gene transfer of ANG-1 has been shown to promote robust angiogenesis in ischemic tissues (16-18). Surprisingly, ANG-1 has recently been implicated in the inhibition of pathologic vascular expansion via its effect on vessel maturation (19-21). This peculiar idiosyncratic effect of ANG-1 between physiologic and pathologic angiogenesis has profound implication in the development of strategy that targets this pathway for anticancer therapy. Similarly, the context-dependent activation of ANG-2 and inactivation of its cognate TIE-2 receptor also complicate the understanding of this signaling pathway.

Questions aimed at the molecular basis of ANG-1 and ANG-2 action in the angiogenic cycle may help to unravel the conundrum. Is ANG-2 an antagonist or agonist of TIE-2 signaling? Is there a transition phase between proangiogenic and antiangiogenic roles of ANG-1? What are the circumstances that determine the transition between these roles? What role does vessel maturation and vascular stability play in this reversal of function? Does extraendothelial TIE-2 signaling or TIE-2–independent signaling in endothelial cells affect the angiogenic outcome? How does the transition between TIE-2–dependent and TIE-2–independent activity of

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ANG-1 and ANG-2 affect disease outcome? In this review, we highlight the controversies surrounding this important pathway and attempt to elucidate this Jekyll and Hyde behavior of ANG-1 or ANG-2 to gain insights into the complex tumor ecosystem.

The ANG Family

The human ANG family comprises the ligands ANG-1, ANG-2, and ANG-4. Their cognate TIE-2 receptor (and a closely related orphan receptor, TIE-1) is mainly expressed in endothelial cells. They lack mitogenic activity toward endothelial cells [although conflicting data are emerging that showed otherwise (22, 23)] but affect distinct aspects of vascular development (24, 25). Transgenic mice lacking ANG-1 or overexpressing ANG-2 have defects attributed to disrupted interaction between endothelial and perivascular cells (15, 25). Mice lacking ANG-2 have defective lymphatic system that can be compensated by ANG-1 (26). This suggests a wide-ranging effect of ANGs on both vascular and lymphatic systems. Transgenic mice overexpressing ANG-1 produced enlarged vessels with highly regulated junctional complexes that resulted in leakage-resistant vessels (27). Consistently, hyperpermeable vessels in VEGF-overexpressing mice were restituted by ANG-1, whereby double transgenic mice of ANG-1/VEGF resulted in enhanced angiogenesis with leakage-resistant vessels (28). This suggests a complementary yet contradicting relationship between these important growth factors.

ANGs possess distinct structural domains with their receptor-binding site residing in the fibrinogen-like domain, whereas the coiled-coil region (Fig. 1) multimerizes the former into active multimeric ligands of ANG-1 or ANG-2 (29, 30). Paradoxically, dimeric form of ANG-1 has been found to inactivate TIE-2 receptor (30), and some isoforms of ANG-1 have been reported to negatively regulate TIE-2 activation (31). Interestingly, both ligands were recently reported to function in a TIE-2–independent manner whereby $\alpha_5\beta_1$ and $\alpha_v\beta_5$ integrins could act as functional receptors for ANG-1 and ANG-2 (Fig. 2; ref. 32). Interaction between isoforms of ANGs and integrin receptors may not be unexpected because differential binding of VEGF isoforms with family of integrins has been reported to induce distinct cellular responses (33). Therefore, activity of ANG-1 and ANG-2 is likely to have broad ramifications because integrins are expressed in multiple cell types.

ANG-1

Up-regulation of ANG-1 in high-grade gliomas (34, 35), non–small cell lung carcinoma (36), plasmacytomas (37), and ovarian (38), breast (39, 40), and gastric (41) carcinomas are strongly correlated with tumor malignancy. Furthermore, overexpression of ANG-1 in HeLa, GS9L, U87, U373, and U343 cell lines has been reported to increase tumor growth (see Table 1; refs. 13, 42, 43). Moreover, ANG-1–mobilized, bone marrow–derived endothelial cells have been linked to brain tumor angiogenesis (44) and myeloproliferative disorders (45). Surprisingly, overexpression of ANG-1 in MCF-7 breast cancer cells (19), HT29 colon cancer cells (21, 46), TA3 mammary cancer cells (47), Lewis lung carcinoma (47), and A431 squamous cell carcinoma (20) has been reported to show significant antitumor effect. Its inhibitory effect was linked to recruitment of perivascular cells by ANG-1 that restricts further expansion of tumor vasculature.

ANG-2

The role of ANG-2 in TIE-2 receptor activation is similarly controversial (see Table 2). Its peculiar context-dependent agonistic and antagonistic relationship with TIE-2 (25, 48-50) has further complicated the understanding of ANG-2 function in vascular development. Embryonic ANG-2 overexpression results in a major disruption of the developing vascular system, suggesting an antagonistic role in angiogenesis (25). Furthermore, it counteracts the angiogenic activity of VEGF and antagonizes the synergistic effect of VEGF with basic fibroblast growth factor in angiogenesis (51, 52). In addition, lung and mammary carcinomas that overexpressed ANG-2 and specific induction of ANG-2 in gliomas were found to retard tumor growth and metastasis (42, 47). In contrast, overexpression of ANG-2 in hepatomas, gliomas, and colorectal and gastric carcinomas was found to enhance angiogenesis and augment tumor malignancy (46, 53-56). Furthermore, strong correlation of ANG-2 with ANG-1 in neuroblastoma (57), gliomas (14, 35), breast and prostate carcinomas (58), hepatocellular carcinoma (59), non–small cell lung carcinomas (60, 61), and gastric adenocarcinoma (62) has been associated with aggressive tumor growth.

ANG-3/ANG-4

The other two members of ANG family, ANG-3 and ANG-4, are not well studied but are believed to be interspecies

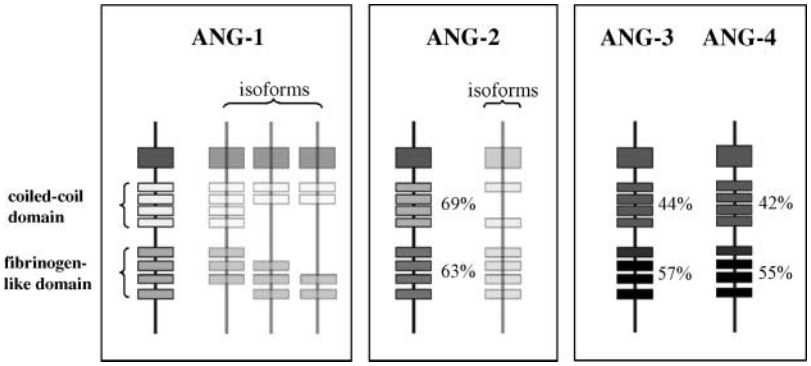

FIGURE 1. Structural organization of the ANG family. There are four ANG-1 isoforms with varying regulating activity on TIE-2 receptor (31). ANG-2 (25) and its isoform (125) are believed to be natural antagonists of the TIE-2 pathway. ANG-3 and ANG-4 are believed to be species orthologues in mouse and human, respectively (63), which regulate TIE-2 in a species-specific manner (65). The coiled-coil domain is involved in the multimerization of the ligands and the fibrinogen-like domain functions as the receptor-binding motif. The percentages in each box indicate identity to ANG-1 domains.

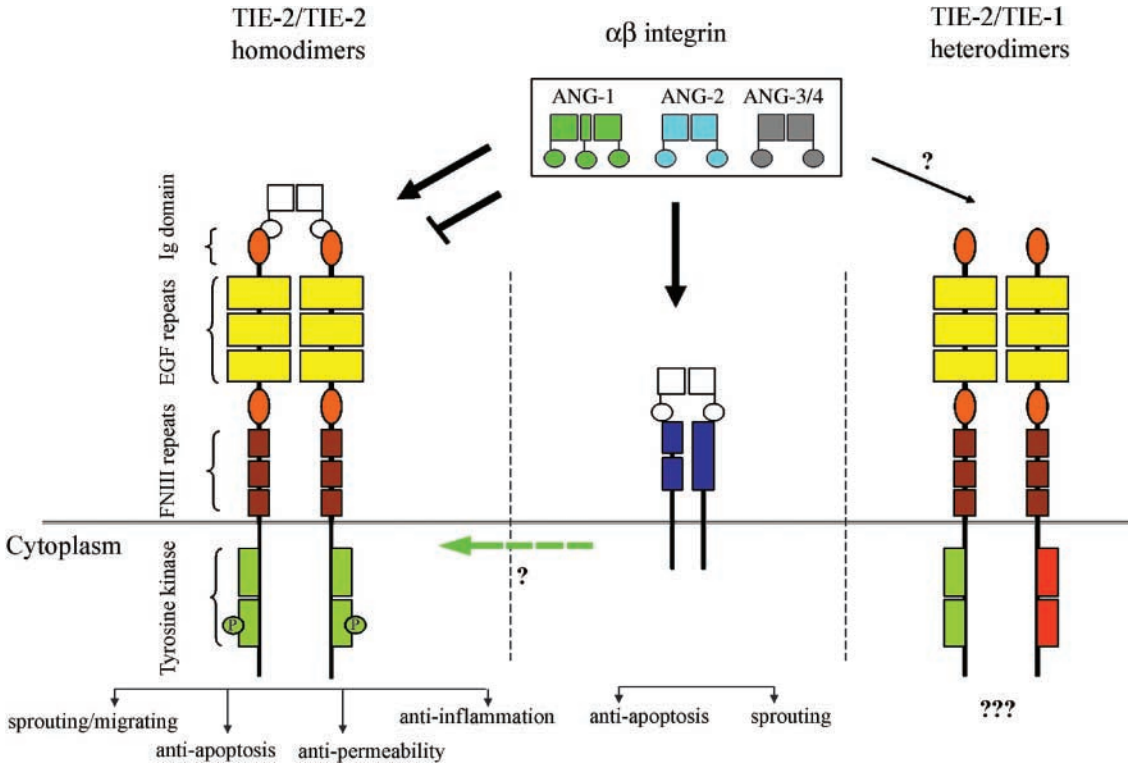

FIGURE 2. Structural organization of ANG-binding receptors. ANG-1 is known to form trimers and multimers to homodimerize and induce tyrosine phosphorylation of the TIE-2 receptor for intracellular signaling (29, 30). The other ANGs form dimers to bind to TIE-2 receptor (65). ANG-2 acts as antagonistic ligand for TIE-2 in low concentration but is able to activate TIE-2 in high concentration (49). TIE-2/TIE-1 heterodimerization is known to inhibit ANG-2 activation of the receptor (73). ANG-3 and ANG-4 are able to activate TIE-2 receptor in a species-specific manner (65). The integrin $\alpha_5\beta_1$ and $\alpha_v\beta_5$ receptors may transduce ANG-1 and ANG-2 signals independent of TIE-2 (32, 76, 77), but they may also work synergistically with TIE-2 receptor (131).

orthologues between mouse and human, respectively (63). The function of ANG-3 and ANG-4 in angiogenesis is equally controversial compared with the more established members of the family. ANG-3 has been reported to act as antagonist that interferes with ANG-1 activation of TIE-2 (63) and Akt in tumor growth (64). However, ANG-3 was recently found to strongly activate mouse TIE-2 receptor, but not its human counterpart, whereas ANG-4 displayed no such species selectivity in TIE-2 activation (65). This may have ramifications in the previously reported results of human ANG-1 and ANG-2 in mouse tumor models whereby the interfering effect of endogenous ANG-3 on TIE-2 binding by the ectopically expressed ligands may not have been properly accounted for.

Dr. Jekyll and Mr. Hyde in Tumor Angiogenesis

ANG-1 and ANG-2 are known to respond differentially to hypoxia with the latter often being up-regulated in hypoxic/ischemic tissues (66-69). ANG-1 is mainly produced by vascular mural cells, such as smooth muscle cells and pericytes, whereas endothelial cells are the main producers of ANG-2. Therefore, autocrine regulation of TIE-2 activity by ANG-2 may render the receptor less responsive to exogenous stimuli and presents a unique self-modulatory function to endothelial cells during angiogenesis (70). Furthermore, TIE-2 activity is autoinhibited by its COOH terminus (71, 72) and its ligand receptivity toward ANG-2 is reportedly modulated through TIE-1 heterodimerization (73), suggesting a tight regulatory control at the receptor level.

Intracellular signaling pathway of TIE-2 involves multiple cytosolic docking partners [for detail, see review (74)], suggesting that it may be regulated and coordinated in a dose- and spatiotemporal-dependent manner. This is evident by the unique agonistic and antagonistic relationship between ANG-1 and ANG-2 on TIE-2 phosphorylation in endothelial cells but not other cell types (27).

The recent finding of extraendothelial TIE-2 receptor expression further complicates the understanding of ANG/TIE-2 system (see Table 3). It remains to be seen if nonendothelial TIE-2 receptors are functional or functionally similar to the endothelial-specific TIE-2 receptors. Nevertheless, TIE-2 receptors in trophoblasts have been found to mediate cellular migration and proliferation by interacting with ANG-1 and ANG-2, showing their direct effect on non-endothelial cells (75). Similar expression of VEGF receptors in various tumor cells has also been noted, but their implication in tumor angiogenesis remains largely undefined (12). Besides that, TIE-2–independent signaling of ANG-1 and ANG-2 is also increasingly recognized to have important functional roles in cellular behavior. The $\alpha_5\beta_1$ and $\alpha_v\beta_5$ integrins have recently been implicated in the differential cell spreading and migration activity of endothelial cells in response to ANG-1 and ANG-2 (32). Moreover, ANG-1 has been found to confer significant survival benefit to myocytes and affect neuronal patterning via β_1 integrin signaling (76, 77). Therefore, integrin-expressing tumor cells may respond to ANG-1 and ANG-2 independent of

TABLE 1. Effect of Stable Ectopic Expression of ANG-1 in Tumor Models

Tumor type	ANG-1	ANG-2	VEGF	TIE-2	Angiogenesis	Outcomes	Reference
Astrocytomas	↑	ND	=	—	↑	Inducible expression in U87-MG cell line Increase glomeruloid bodies Increase proliferating tumor cells and MVD	(135)
Breast cancer	↑	ND	ND	ND	↓	Stable expression in MCF-7 cell line Retard tumor growth in spite of the presence of FGF-2	(19)
Cervical cancer	↑	ND	=	ND	↑	Stable expression in HeLa cell line Increase MVD Decrease tumor cell apoptosis Tumor cell proliferation unaffected Increase vessel plasticity with fewer pericytes	(13)
Colorectal cancer	↑	+	ND	ND	↓	Stable expression in HT29 cell line Decrease MVD Reduced tumor cell proliferation	(46)
Colorectal cancer	↑	ND	ND	ND	↓	Stable transfected KM12L4 cell line Decrease MVD Decrease tumor cell proliferation and metastasis Decrease ascites formation	(136)
Glioblastoma	↑	=	=	=	↑	Inducible expression in GS9L cell line Highly branched vessels Mature vessels covered with pericytes Increase number of vessels <500 μm^2 Increase MVD	(42)
Glioblastoma	↑	ND	↑	ND	↑	Stable expression in U87, U373, and U343 cell lines Increase MVD only when VEGF is elevated	(43)
Hepatic colon cancer	↑	ND	ND	ND	↓	Stable expression in HT29 cell line Higher pericyte coverage in tumor vessels Decrease MVD Decrease proliferating tumor cells Decrease vascular leakage	(21)
Squamous cells carcinoma	↑	+	=	↑phos	↓	Stable expression in A431 cell line No change to MVD and VEGF Increase smooth muscle cell coverage of vessels	(20)
Lung carcinoma	↑	+	=	ND	—	Stable expression in Lewis lung carcinoma cell line No effect on tumor and endothelial cell apoptosis No effect on MVD or vessel maturation	(47)
Mammary carcinoma	↑	ND	=	ND	—	Stable expression in TA3 mammary carcinoma cell line No effect on tumor and endothelial cell apoptosis No effect on MVD or vessel maturation	(47)
Cervical cancer	↓	ND	=	ND	↓	Stable expression of antisense ANG-1 in HeLa cell line Decrease MVD Increase tumor cell apoptosis	(137)
Gastric carcinoma	↓	ND	ND	ND	↓	Stable expression of antisense ANG-1 in SGC7901 cell line Decrease MVD	(138)

NOTE: ↑ or ↓ indicates increased or decreased expression levels compared with control tumors; = indicates unchanged expression levels; — indicates no effect on the outcome; + indicates the factor was detectable but levels not quantified against control tumors.
Abbreviations: MVD, microvascular density; ND, not determined; phos, phosphorylated; FGF, fibroblast growth factor.

the vascular effects of these ligands. Although its implications in tumor development await further clarifications, such relationship between VEGF and integrins has been documented in endothelial cells and, notably, in tumor cells and tumor angiogenesis (78, 79).

In conclusion, the dynamic differential induction of ANG-1 and ANG-2 expression coupled with their paracrine/autocrine receptor-binding activity and the possibility of TIE-1 (73) and TIE-2 (80) cross-modulating their ligand-binding activity indicate a unique self-regulatory mechanism in endothelial responsiveness. Their expression and regulation are expected to have broad implications in the resulting angiogenesis or lack of it (Fig. 3). Such complex interrelationships between TIE-1/TIE-2 and TIE-2/integrin signaling in endothelial and non-endothelial cells may explain the seemingly contradicting outcomes in the ectopic expression of ANG-1 and ANG-2 in various tumor models (Tables 1 and 2).

Identity Crisis of ANG-1 and ANG-2: Angiogenic or Antiangiogenic?

Some slow growing and poorly angiogenic tumors with low VEGF expression have been shown to exhibit high microvascular permeability, suggesting that threshold levels of VEGF-induced vessel permeability are considerably lower than those needed for inducing angiogenesis (81, 82). Furthermore, microenvironmental concentration rather than the overall dose of VEGF has been found to be important in determining normal and pathologic angiogenic outcome (83). Such parallel observation is yet to be drawn on the diverse roles of ANG-1 in endothelial survival, sprouting, vessel maturation, and vascular permeability. This complex interplay between related, yet at times, conflicting roles of ANG-1 may be crucial in determining the outcome of angiogenesis. Central to this conflict is the dichotomy of functions and processes required for vascular sprouting and vessel maturation.

Vessel sprouts have been shown to loosen vascular integrity and intercellular contact among neighboring endothelial and smooth muscle cells in response to ANG-1 (84). This plastic state enables the endothelium to actively respond to angiogenic factors, such as VEGF, whereas mature vessels that are covered by smooth muscle cells are less responsive to stimulation of VEGF (83, 85). A similar observation has been reported between VEGF and ANG-2 whereby, in the presence of VEGF, ANG-2 promotes vascular sprouts whereas, in its absence, vascular regression accelerates (55, 86). Hence, this window period may enable fine tuning of the neovasculature to adapt to its microenvironment through a regulated process of pruning and remodeling because these plastic vessels are likely to be more susceptible to apoptosis (87, 88). Therefore, the major function of ANG-1 may lie with its antiapoptotic effect on endothelial cells during this plastic phase. It may possibly only have an indirect role in vessel maturation. In fact, conditions favoring vascular maturation and survival in different tissues have distinct consequences on functional outcome. For example, the disparity in the function of ANG-1 is evident in the conflicting conclusions proposed by Du et al. (89) and Zhao et al. (90) on the pathophysiology of pulmonary hypertension. Unregulated recruitment of perivascular cells to the vascular pulmonary networks by ANG-1 or excessive antiapoptosis signal from ANG-1 on terminal arterioles in the pulmonary vascular bed was separately attributed as the etiology of the disease.

The potent prosurvival effect of ANG-1 alone or in synergy with VEGF has been found to protect endothelial cells from apoptosis (91-96). ANG-1–mediated phosphatidylinositol 3-kinase–dependent activation of Akt and attachment to extracellular matrix are central to the survival of endothelial cells. This antiapoptotic effect is mediated through up-regulation of survivin (97) and suppression of caspase-3, caspase-7, and caspase-9 activity as well as inhibition of second mitochondrial-derived activator of caspase (Smac) release (98, 99). The protective role is evident in radiation, mannitol, and low-density lipoprotein-treated endothelial cells whereby apoptosis was ameliorated by addition of ANG-1 (92, 100, 101). In fact, vascular defects of disrupted endothelial and myocardial layers observed in the ANG-$1^{-/-}$ and TIE-$2^{-/-}$ mutant mice may be due to impaired survival of endothelium rather than deficiency in vessel maturation as previously thought (102, 103). Indeed, persistent perivascular cell recruitment in vessels composed of TIE-2–deficient endothelial cells that subsequently apoptosed strongly supports this contention (103, 104). Furthermore, overexpression of ANG-1 in the skin and lung failed to show evidence of enhanced recruitment of perivascular cells to the vessels (23, 27, 105). Dilated and pericyte-scarce vessels in

TABLE 2. Effect of Stable Ectopic Expression of ANG-2 in Tumor Models

Tumor Type	ANG-1	ANG-2	VEGF	TIE-2	Angiogenesis	Outcomes	Reference
Colorectal	ND	↑	ND	ND	↑	Stable expression in HT29 cell line Increase MVD Enhance tumor cell proliferation	(46)
Gastric	ND	↑	+	ND	↑	Stable expression in MKN-7 cell line Highly vascularized and metastatic tumor Decrease vessel maturation	(56)
Glioma	ND	↑	ND	ND	↑	Stable expression in U87MG cell line Highly invasive with up-regulated MMP-2 Increase angiogenesis	(139)
Glioma	ND	↑	ND	ND	↓	Stable expression in U87 cell line Increase tumor necrosis Decrease vascularization	(140)
Glioblastoma	=	↑	=	↑	↓	Inducible stable expression in GS9L cell line Aberrant vascular cords with aggregated endothelial cells with narrow lumens Less mature vessels with few pericytes Discontinuous basement membrane Decrease MVD Tumor apoptosis unaffected	(42)
Hepatomas	=	↑	ND	ND	↑	Stable expression in HuH-7 cell line Hemorrhagic tumors with hypervascular phenotypes	(53)
Hepatomas	ND	↑	ND	ND	↑	Stable expression in Morris hepatoma cell line Increase tumor perfusion and vascularization Up-regulate Flk-1 expression	(141)
Lung carcinoma	ND	↑	=	ND	↓	Stable expression in Lewis lung carcinoma cell line Increase tumor and endothelial cell apoptosis Decrease metastatic property Decrease vessel maturity	(47)
Mammary carcinoma	ND	↑	=	ND	↓	Stable expression in TA3 mammary carcinoma cell line Increase tumor and endothelial cell apoptosis Decrease metastatic activity Decrease vessel maturity	(47)
Squamous cell carcinoma	+	↑	=	=	—	Stable expression in A431 cell line No effect on MVD or vessel maturation	(20)

NOTE: ↑ or ↓ indicates increased or decreased expression levels compared with control tumors; + indicates the factor was detectable but levels not quantified against control tumors; = indicates unchanged expression levels; — indicates no effect on the outcome.
Abbreviation: MMP, matrix metalloproteinase.

TABLE 3. Physiology and Pathology of Extraendothelial Expression of TIE-2 Receptor

	Findings and Outcomes	Reference
Cancer cell types		
Inflammatory breast cancer cell line	Increase hematogenous metastases and correlated with poor prognosis	(39)
HeLa cervical cell line	Enhance survival of cervical tumor cells	(13)
Neoplastic glial cells	Associated with disease progression and matrix adhesion	(142)
Liver oval cells	Involve in preneoplastic to neoplastic conversion of hepatocytes	(143)
Thyroid tumor cells	Involve in cellular proliferation	(123)
Non–small cell lung carcinoma cells	Unknown function	(60)
Cancerous prostate cells	Unknown function	(124)
Glioma cell lines	Unknown function	(14)
Gastric carcinoma cells	Unknown function	(62)
Normal cell types		
Fetal trophoblasts	Involve in the proliferation, migration, and nitric oxide release	(75)
Ganglion cells	Promote neurite outgrowth when stimulated by ANG-1	(144)
Monocytes and mesenchymal cells	Promote paracrine angiogenic effect and tumor homing	(145)
Nerve cells	Phosphorylated by ANG-1 to prevent apoptosis of neuronal culture through phosphatidylinositol 3-kinase/Akt	(146)
Smooth muscle cells	To synchronize intercellular communication between endothelial and smooth muscle cells	(59)
Synovial cells	Correlate with cellular proliferation and possibly their pathologenesis	(147)
Synoviocytes and stromal fibroblasts	Unknown function	(148)
Synovial lining cells and macrophages	Unknown function	(149)
Choroidal neovascular membranes	Unknown function	(150)
Neuronal and Schwann cells	Unknown function	(151)
Glandular endometrial epithelial cells	Unknown function	(152)
Thyroid and granulose follicular cells	Unknown function	(153)
Granuloma-associated mesenchymal cells	Unknown function	(154)
Mesenchymal cells and osteoblasts	Unknown function	(155)

venous malformation that are associated with excessive activation of TIE-2 receptor (106) do not support a vessel maturation role for ANG-1. Furthermore, inhibition of TIE-2 function in retinal vasculature failed to affect pericyte recruitment (107). In addition, ANG-1 restored hierarchical structure of vascular network and rescued retinal edema and hemorrhage in the complete absence of smooth muscle cells (108).

The previous observations that ANG-1 restricted tumor growth by promoting vessel integrity via pericyte recruitment (19-21) is difficult to reconcile with the current contradicting findings. It is unclear how vessel maturity may have played a significant role in retarding the tumor angiogenesis. In fact, maturation of vessels and normalization of microcirculation by smooth muscle cell coverage have been linked to a more aggressive tumor growth, possibly due to better nutrient exchange in the previously dysfunction vasculature (42). The reported tumor-inhibiting effect of ANG-1 may be related to its anti-inflammation action (109, 110) because inflammation was recognized as a key trigger for pathologic angiogenesis mediated by VEGF (111).

It is noteworthy that neovascularized tumors exhibit temporal angiogenic phenotype because not all parts of the tumor vessels are concurrently participating in angiogenesis. For example, TIE-2 expression was reported to be restricted to stromal vessels rather than intratumoral vessels in human mammary carcinomas (112). Furthermore, hypoxia-inducing factor-1 modulates the expression of ANG-1 and ANG-2 in a cell type–specific manner, whereby ANG-2 expression was induced in endothelial cells but suppressed in smooth muscle cells, whereas ANG-1 levels were unaffected in both cell types (113). Moreover, only a subset of endothelial cells is responsive to hypoxia induction of ANG-2 (114). Therefore, failure of signal transduction from TIE-2 receptors in different populations of endothelial cells may account for the observed discrepancies in the action of ANG-1 and ANG-2 (48, 115).

The present evidence suggests that ANG-1 predominantly functions as a survival factor leading to angiogenic sprouting rather than a vessel maturation agent that restricts tumor expansion. There may be a more complex controlling mechanism for vessel maturity whereby ANG-1 may act only indirectly, perhaps, in cooperation with other major mediators, such as platelet-derived growth factor (87), ephrin (116), transforming growth factor-β, and sphingosine-1-phosphate (117).

Angiogenic Cycle Mediated through TIE-2 Pathway

Induction and up-regulation of TIE-2 and ANG-2 expression in endothelial cells are regulated by hypoxia and proinflammatory cytokines, such as tumor necrosis factor-α and interleukin-1β (68, 118-122). Conversely, such stimuli down-regulate the expression of ANG-1 (66, 67), suggesting a delicate inverse relationship between ANG-1 and ANG-2 in the regulation of TIE-2 signaling. Therefore, spatiotemporal changes of these unique relationships among ANG-1, ANG-2, and TIE-2 may be one of the most crucial aspects in determining the outcome of vascular angiogenesis. The initial quiescent endothelium goes through cyclical phases of (*a*) basal quiescent, (*b*) plastic, (*c*) angiogenic, and (*d*) stable maturing stage (Fig. 4) to complete the angiogenic cycle for neovascularization. The transitions are likely to be influenced by tissue milieu, whereby phase changes may occur depending on the presence of specific growth factors and inhibitors.

In the basal quiescent phase, constitutive expression of ANG-1 from perivascular cells couples with minimal expression of ANG-2 and uniform expression of TIE-2 in the endothelial cells would be expected. Together with basal levels

of platelet-derived growth factor and ephrin, this maintains the endothelium in a stable differentiated state by reciprocal interactions between endothelial and perivascular cells. On stimulation, the ratio of ANG-1 to ANG-2 may shift in favor of the latter to promote transient vessel plasticity by dissociation of endothelial cells with perivascular cells. This loosens the tight association between neighboring endothelial cells as well as extracellular matrix during the initial plastic phase. Furthermore, induction of TIE-2 expression during this period may significantly favor increased binding of ANG-2 or increased numbers of unbound, therefore presumably, unphosphorylated TIE-2 receptor, thereby reverting vessels to a more plastic state that are more responsive to angiogenic stimuli.

Up-regulation of ANG-1, ANG-2, and TIE-2 in the active angiogenic phase would promote vessel differentiation by migrating/sprouting and antiapoptotic effect of TIE-2 signaling. There are increasing numbers of tumor cells reported to express TIE-2 receptor (13, 14, 39, 60, 123, 124). However, the significance of TIE-2–expressing tumor cells during this period is largely unknown, but these extraendothelial TIE-2 receptors may serve to sequestrate the availability of ANG-1 from the vulnerable endothelial cells. Furthermore, it is unclear if the ANG-1–binding integrins on the endothelial and tumor cells may synergize or counteract ANG-1 action through TIE-2 receptor. Nevertheless, the lack of ANG-1 activity in this vulnerable state may render the endothelium more susceptible to apoptosis. The loss of this antiapoptotic signal may significantly affect the outcome of tumor angiogenesis. These may be the missing pieces that determine the transition between proangiogenic and antiangiogenic roles of ANG-1 reported in the literature. In the final stage, the cycle reenters quiescent phase after the expression of growth factors returns to basal levels. This tenuous equilibrium between vascular supply and tumor demand may favor a stabilization and maturation of the nascent vasculature. However, this equilibrium is likely to be transient because tumor vasculature is hyperpermeable and lacking in pericyte coverage. This cyclical pathway may explain the governing dynamism in angiogenesis and provide a rational interventional window to strategically target each phase of vascular development in the evolving tumor ecosystem.

Conclusions

The varied role of TIE-2 signaling pathway in endothelial survival, vessel growth, and vascular maturation may be intrinsic to different types of tumors. However, emerging evidence suggests that additional signaling of ANG-1 and ANG-2 through integrin receptors may be important in their diverse contribution toward tumor growth. The existence of various ligands (ANG-1, ANG-2, and ANG-4), their isoforms

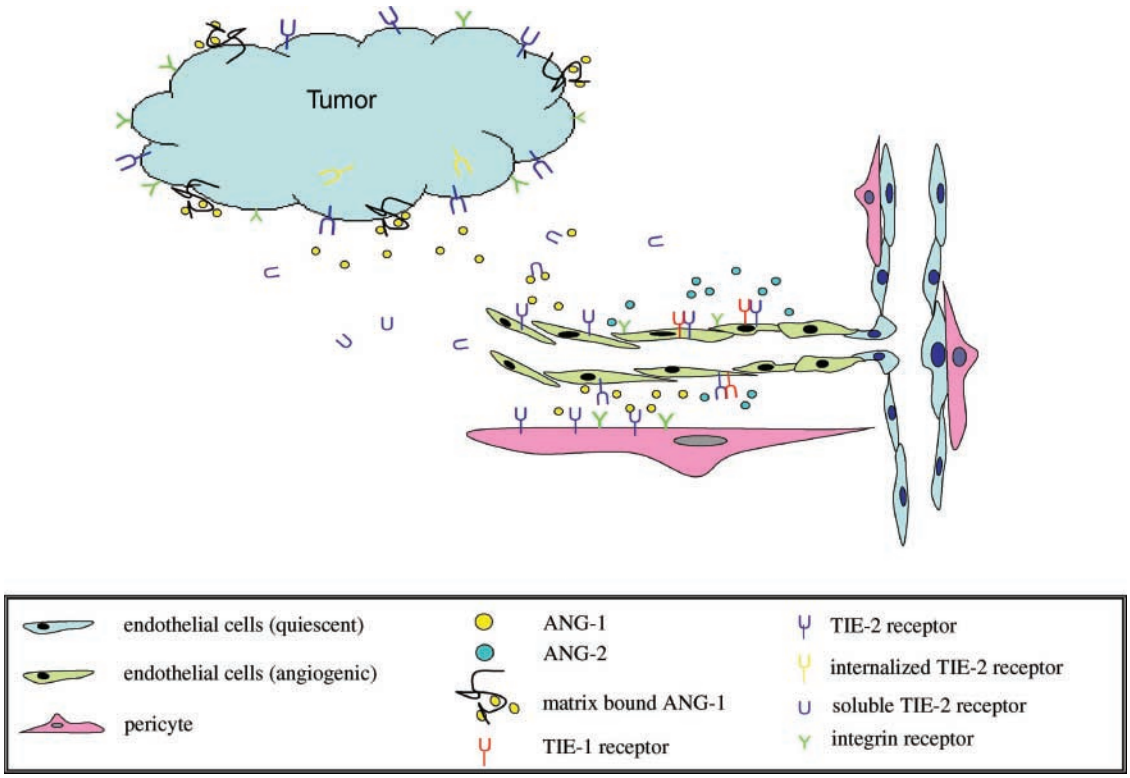

FIGURE 3. TIE-2–dependent and TIE-2–independent signaling of ANGs in endothelial and nonendothelial cells. Majority of the ANG-1 is secreted by tumor cells and pericytes, whereas ANG-2 is mainly produced by endothelial cells. The matrix-bound ANG-1 (132) and Weibel-Palade body-stored ANG-2 (133) are likely to act as a readily releasable reservoir in tumor and endothelial cells, respectively. Differential ANG-1 gradient attracts endothelial cell migration toward tumor, whereas ANG-2 maintains a "pericyte-free" endothelium. The internalization of TIE-2 following receptor activation releases the ANG-1 and ANG-2 back into the active pool of ligands (134). Soluble TIE-2 (127) may act as a decoy for ligand binding to regulate the membrane-bound TIE-2, whereas heterodimerization between TIE-1 and TIE-2 may modulate their ligand receptivity (73, 80). Integrin receptors may act as primary and secondary binding partner of the ligands (32, 76, 77).

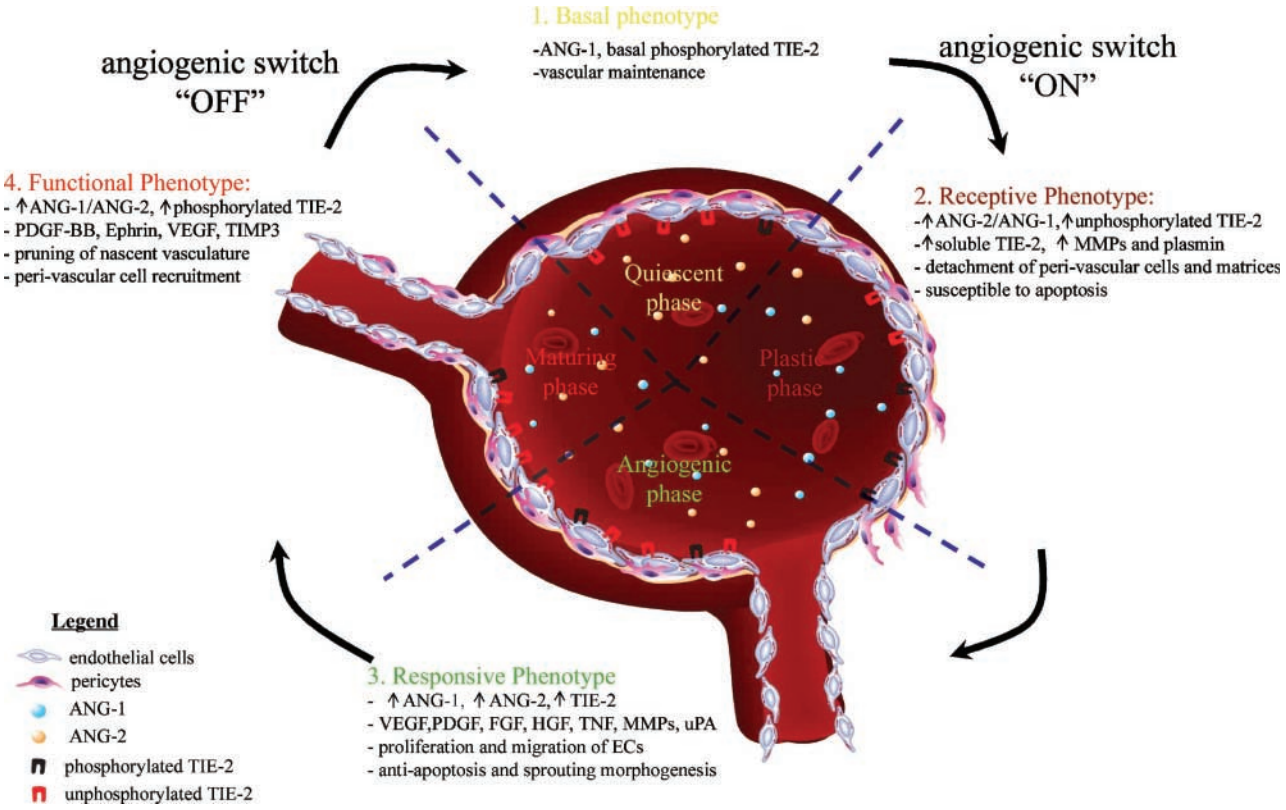

FIGURE 4. Angiogenic cycle of vascular developments. 1, angiogenic switch is triggered by hypoxia, inflammation, or genetic mutations that disrupt the spatiotemporal balance between angiogenic promoters and inhibitors. 2, the up-regulation of ANG-2 in response to the stimuli destabilizes the endothelium by decreasing the phosphorylation status of TIE-2 receptor and resulting in increased vessel plasticity with a more responsive endothelial cells. 3, the activated matrix proteases promote pericyte detachment and facilitate endothelial chemotaxis and sprouting in response to VEGF, ANG-1, fibroblast growth factor (*FGF*), platelet-derived growth factor (*PDGF*), and hepatocyte growth factor (*HGF*). 4, the cyclical supply and demand reaches an equilibrium (even if transiently) after waning effect of the stimuli and tapering ANG-2 expression that enable reinvestment of basement membrane, extracellular matrices, and pericyte recruitment to the nascent vasculature. MMPs, matrix metalloproteinases; TIMP3, tissue inhibitor of metalloproteinase 3; TNF, tumor necrosis factor; uPA, urokinase-type plasminogen activator.

[four ANG-1 isoforms (31) and two ANG-2 isoforms (125)], and soluble forms of TIE-1 and TIE-2 receptors (126, 127) and TIE-2/TIE-1 heterodimerization in modulating receptivity (73) and differential induction of both receptors and ligands (119, 128, 129) compounded the difficulty of precise definition of the role of the ANGs during angiogenesis.

The concept of molecular balance between ANG-1/ANG-2 as a trigger between active angiogenesis and vascular regression is an oversimplification of the inherently complicated process. Together with autocrine or paracrine interactions with its ligands in various cell types, TIE-2 signaling pathway may not be totally restricted to endothelial cells during angiogenesis and the pathway may have wide-ranging functions in other cellular activities. The aberrant vascularization resulted from imbalance between ANG-1 and ANG-2 together with VEGF may explain one aspect of the controversies of TIE-2 signaling in tumor angiogenesis [for detail, see review (130)]. Increasingly, TIE-2–expressing tumor cells and ANG-1–binding integrins may be the emerging puzzles that help in the understanding of the multiple roles of ANG-1 and ANG-2, be it endothelial TIE-2 dependent or independent, in the tumor ecosystem.

It is plausible that an antiangiogenesis approach targeting TIE-2 pathway may be applicable to both endothelial cells as well as tumor cells. If we could control Dr. Jekyll and Mr. Hyde in the ANGs, a similar strategy targeting the overall tumor ecosystem by controlling the survival pathway of angiogenesis may prove to be more effective in containing malignancy and restricting tumor progression.

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