Angiopoietins and Their Role in Colon Cancer Angiogenesis

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Tumor angiogenesis is a complicated process that is regulated by numerous factors simultaneously and in a coordinated fashion.

Angiogenesis, a process of blood vessel formation whereby new vessels sprout and mature from the preexisting vasculature,[1] is required for a variety of physiologic processes, including pregnancy, wound healing, tissue repair, and organ regeneration. Angiogenesis also contributes to the development of pathologic conditions such as cancer progression and metastasis, diabetic retinopathy, psoriasis, atherosclerosis, and rheumatoid arthritis. [2,3]

Angiogenesis is a complicated process that is regulated by numerous factors that occur simultaneously and in a coordinated fashion. The process of angiogenesis requires that endothelial cells (ECs) detach from pericytes and the extracellular matrix (ECM), proliferate, migrate, and form capillary tubes that connect to other newly developed vascular tubes.[4] The result is a primitive yet functional vascular network. Recent evidence indicates that the presence of specific cytokines mediates EC "stability/instability." [5] These factors either protect EC from undergoing apoptosis or facilitate EC "instability" that allows the response to mitogenic factors. The angiopoietins are a family of proteins that mediate EC stability and survival. A better understanding of the biologic effects of angiopoietins in the angiogenic process may contribute to the development of novel therapeutic strategies.

Angiopoietins and Their Tyrosine Kinase Receptor, Tie-2

The angiopoietins are a family of growth factors identified as being specific for the vascular endothelium. The specificity of the angiopoietins for the vascular endothelium results from the restricted distribution of the angiopoietin tyrosine kinase receptor Tie-2 (also known as TEK [6]) to endothelial cells. Four different angiopoietins—Ang-1 through Ang-4—have been described.[7-9] The best characterized of these are Ang-1 and Ang-2. Ang-1 exerts its biologic effect by binding to Tie-2, inducing phosphorylation of Tie-2.[10,11] Ang-1 also may control the ability of ECs to stabilize the structure and modulate the function of blood vessels. In vivo analysis by targeted gene inactivation revealed that Ang-1 recruits and sustains periendothelial support cells.[12] Ang-2 is antagonistic to Ang-1 and also binds to Tie-2, but it does not typically induce phosphorylation. However, at supraphysiologic doses, Ang-2 also may initiate EC signaling and survival.[13] Tie-1 is an orphan receptor, but its ligand has not been identified.

Several investigators have demonstrated in vitro that Ang-1 serves as a survival factor for ECs. Kwak and associates [14] examined the effect of Ang-1 on apoptosis in human umbilical vein ECs (HUVECs). Ang-1 dose-dependently inhibited apoptosis under serum-deprived conditions, with significant inhibition occurring with Ang-1 doses as low as 50 ng/mL. Furthermore, the addition of 20 ng/mL VEGF (a potent angiogenic factor known also to be an EC survival factor) to 200 ng/mL Ang-1 augmented the antiapoptotic effects of Ang-1, suggesting that Ang-1 acted in conjunction with VEGF. Similar results were obtained by Papapetropoulos et al,[15] who also demonstrated dose-dependent stabilization of HUVEC network organization by Ang-1. In addition, these authors demonstrated that this response was indeed dependent on Tie-2 activation, as addition of a soluble form of Tie-2, but not Tie-1, completely blocked the effects of Ang-1. It was also demonstrated that the signaling pathway by which Ang-1 protects ECs from apoptosis is likely through phosphorylation of the survival serine-threonine kinase, Akt. [16,17] This finding occurred in association with the up-regulation of the apoptosis inhibitor, survivin, in ECs and protection of endothelium from apoptosis.[16] In addition, transfection of a dominant-negative survivin construct abrogated the ability of Ang-1 to protect cells from undergoing apoptosis. These data suggest that the activation of antiapoptotic pathways mediated by Akt and survivin in ECs may contribute to Ang-1 stabilization of vascular structures during angiogenesis.[16]
It is likely that Ang-1 works in conjunction with VEGF to help stabilize vascular networks. Ang-1 appears to recruit periendothelial support cells,[12] and this interaction may be required for EC survival. This is supported by evidence showing that Ang-1 knockout embryos are able to undergo VEGF-dependent angiogenesis, but ECs are unable subsequently to interact with periendothelial support cells.[12] This deficit leads to vascular regression. Ang-2 is antagonistic to Ang-1 and thus leads to EC destabilization. In the presence of VEGF, this destabilization leads to robust angiogenesis.[18]

Inhibition of the activity of the Angs by soluble Tie-2 has been investigated as a method of inhibiting angiogenesis. Lin and colleagues[19,20] constructed an adenoviral vector containing the mouse extracellular domain coding region of Tie-2, which can systemically deliver recombinant soluble Tie-2 (AdExTek) capable of blocking Tie-2 activation. Administration of soluble Tie-2 (AdExTek) inhibited the growth in both primary and metastatic tumors in mice. However, it is unclear whether the observed effects are due to sequestration of Ang-1 or Ang-2.

Figure 1 depicts the roles of Ang-1 and Ang-2 in relation to Tie-2 expression and activity.

Angiopoietins in Gastrointestinal Cancer

Few studies have examined the role of angiopoietins in gastrointestinal cancer progression and metastasis. In our laboratory, we investigated whether angiopoietins are expressed by human colon carcinoma.[21] Using reverse transcriptase-polymerase chain reaction (RT-PCR), Ang-1 and Ang-2 expression was measured in normal colonic mucosa, colon cancer specimens, and colon cancer cell lines. Results showed a relatively equal frequency of expression of Ang-1 and Ang-2 in normal colonic mucosa. However, 11 of 11 colon cancer specimens expressed Ang-2, while only 6 of 11 (54%) expressed Ang-1 (P = .04). Similarly, the majority of colon carcinoma cell lines expressed Ang-2 (14/18) (78%), whereas fewer (7/18) (39%) expressed Ang-1 (P = .04). These preliminary studies suggest that an imbalance of activity of Ang-2 over Ang-1 may play a role in colon cancer angiogenesis, whereas angiopoietins are expressed with relatively equal frequency in normal tissues. We hypothesized that the balance of angiopoietin expression in normal tissues likely promotes homeostasis, whereas the imbalance of Ang-2 over Ang-1 in malignant tissues leads to the initiation of angiogenesis.

To examine further the role of angiopoietins in human colon cancer, we used immunohistochemical techniques to assess Ang-1 and Ang-2 expression in 20 primary colon cancer and 5 liver metastasis specimens. Results demonstrated that Ang-1 and Ang-2 both were present in all normal colonic mucosa specimens studied. However, Ang-1 was not expressed in any of the colon cancer specimens, while Ang-2 was expressed in all of them. To determine the cell of origin of the angiopoietins, we performed immunofluorescent double staining with antibodies that bind to either Ang-1 or Ang-2 and CD-31 (endothelial cell marker). Results demonstrated coexpression of the angiopoietins in the vasculature of normal colonic mucosa. However, angiopoietins also were expressed in surrounding tissues. The same staining pattern was noted for Ang-2 in both normal colonic mucosa and tumor tissue. To confirm that the angiopoietins were expressed in the surrounding epithelium (both normal and malignant), double staining with cytokeratin-22 (CK-22, an antibody that recognizes epithelial cells) and antibodies to the angiopoietins was performed.

Results showed colocalization of Ang-1 and Ang-2 in colonic epithelial cells in normal colonic mucosa. Ang-2 colocalized with malignant colonic (CK-22) epithelium, but, as in the above studies, there was no evidence of Ang-1 expression in malignant colonic epithelium. The same staining pattern held true for colon cancer liver metastasis specimens. The above data suggest that the imbalance of activity of Ang-2 over Ang-1 may be associated with the initiation of angiogenesis in malignant tissues.[21]

To investigate this hypothesis further, we performed in vivo studies with cell lines stably transfected with full length cDNA constructs for Ang-1 or Ang-2.[22] The HT-29 human colon cancer cell line was transfected with the empty vector (pcDNA.3) as a control of the Ang-1 and Ang-2 constructs. Stable clones then were injected subcutaneously in nude mice. In the initial study, mice implanted with tumors overexpressing Ang-2 had a tremendous increase in tumor growth (Figure 2). Due to the large tumor volumes, all of the mice were sacrificed at one time point. Tumor weight was significantly decreased in the Ang-1 group and was increased in Ang-2-overexpressing tumors. Analogous to this finding, vessel counts were decreased in tumors grown from Ang-1-overexpressing cells and were increased in tumor cells overexpressing Ang-2 (Figure 3). This pattern held true for tumor cell proliferation as demonstrated by proliferating cell nuclear antigen (PCNA) staining.

To determine if the decrease in vessel count in Ang-1-overexpressing tumors could lead to a
potential decrease in tumor growth as compared with control groups, the study was repeated with mice sacrificed at a later time point. Ang-2-overexpressing tumors again grew rapidly; all mice in this group had to be sacrificed due to large tumor burden. However, over a longer time period, the growth curves between the control cells and Ang-1-overexpressing cells diverged; tumor growth was relatively dormant in Ang-1-overexpressing cells while it continued to be exponential in control cells. These data suggest that Ang-1 overexpression possibly stabilized ECs, preventing their release from pericytes and the basement membrane, thus inhibiting initiation of EC proliferation and neovascularization.

Other investigators recently reported results of similar studies in various tumor types. Hayes and colleagues demonstrated that overexpression of Ang-1 in the human breast cancer cell line MCF-7 decreased tumor growth threefold.[23] Furthermore, Etoh and colleagues conducted a comprehensive study of the role of Ang-2 in gastric carcinoma. [24] These authors, using 85 gastric cancer specimens, demonstrated that high levels of tumor Ang-2 expression were associated with more frequent vascular involvement and more advanced disease stages as compared with low levels of tumor Ang-2 expression. Patients whose tumors had high Ang-2 mRNA expression had poorer survival than did those with relatively low Ang-2 expression.

As in our studies, these authors found that Ang-2 was expressed predominantly in malignant tissues as compared with normal tissues. In in vivo studies, gastric cancer cells transfected with an Ang-2 construct developed highly metastatic tumors that were hypervascular when compared with control cells. Further studies also demonstrated that the production of Ang-2 led to the upregulation of proteases such as matrix metalloproteases-1 and -9 and urokinase-type plasminogen activator in ECs in the presence of VEGF.[24]

Conclusions

Results from the studies described herein support the hypothesis that the imbalance of Ang-2 activity over Ang-1 activity is important in the angiogenic process and, in fact, may be an initiating factor in angiogenesis.[18] In contrast to approaches that target the activity of the angiogenic tyrosine kinase receptor, a novel anti-angiogenic approach may be one that actually increases the activity of the agonist Ang-1, thus potentially stabilizing ECs such that they cannot respond to mitogenic signals in the microenvironment. This strategy potentially would lead to tumor dormancy and would be unlikely to have dose-limiting toxicities. Thus, this strategy represents the paradigm of converting a rapidly growing tumor ("acute disease") into a slowly growing or dormant tumor ("chronic disease").

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