The Angiopoietin Axis in Cancer
An Overview: The Angiopoietin Axis Plays an Essential Role in the Regulation of Tumor Angiogenesis

Growth of a tumor beyond a limiting size is dependent upon its ability to undergo angiogenesis. Angiogenesis is a complex process resulting from the input of multiple signaling pathways. One such pathway is the angiopoietin axis, which is a separate and distinct pathway from the vascular endothelial growth factor (VEGF) axis, and consists of the ligands angiopoietin-1 (Ang1) and angiopoietin-2 (Ang2) and their receptor, Tie2. These molecules control tumor angiogenesis by regulating endothelial cell survival, proliferation, and migration. Ang1 and Ang2 have distinct functions in the regulation of tumor angiogenesis. Tie2 signaling is activated by Ang1, resulting in stabilization and maintenance of vessel integrity. Ang2, on the other hand, is a natural antagonist of the Ang1/Tie2 interaction and prevents Ang1-mediated vessel normalization and remodeling, leading to new vessel sprouting. As the angiopoietins play a distinct role in modulating tumor angiogenesis, the angiopoietin axis may represent a potential pathway for therapeutic strategy in cancer.
Tumor Growth Requires a Dedicated Blood Supply Achieved Through the “Angiogenic Switch”

Tumors require a dedicated blood supply to provide oxygen and nutrients essential for tumor cell survival and growth. New tumor blood vessels form through angiogenesis, a complex process resulting from the concerted actions of many distinct signaling pathways. Angiogenesis is triggered in tumors via the “angiogenic switch,” which occurs when the actions of proangiogenic factors outweigh those of antiangiogenic factors. This is frequently achieved through altered expression of genes that are key regulators of angiogenesis, resulting in overproduction and secretion of proangiogenic molecules such as VEGF, basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), and Ang2, or conversely through the decreased expression of antiangiogenic molecules such as thrombospondin-1. Unlike normal blood vessels, tumor vasculature is structurally and functionally abnormal, exhibiting heterogeneity, irregularity, incomplete pericyte coverage, disorganized cell junctions, and nonuniform blood flow. These abnormalities produce leaky vessels, providing a pathway for tumor cells to gain access to the systemic circulation and to metastasize to distant tissue sites.
The Angiopoietin Axis Regulates Tumor Angiogenesis by Controlling Endothelial Cell Survival, Proliferation, and Migration

The angiopoietin axis consists of the ligands Ang1 and Ang2 and their receptor Tie2. Tie2 is expressed predominantly on endothelial cells and is activated by the binding of Ang1, which is secreted primarily by vascular smooth muscle cells and pericytes. Tie2 may also be bound by Ang2, which is secreted predominantly by endothelial cells and is generally found in tissues undergoing vascular remodeling. Activation of Tie2 stimulates receptor autophosphorylation, triggering downstream signal transduction cascades such as PI3K/Akt and Ras/ERK to regulate endothelial cell survival, motility, and proliferation.
The Angiopoietin Axis Controls a Wide Range of Endothelial Activities That Impact Tumor Vascular Architecture

Although Ang1 and Ang2 both bind the Tie2 receptor, they exhibit distinct functions in tumor vascular remodeling. Ang1 binding stimulates Tie2 signaling to normalize and maintain the integrity of blood vessels through organization of cell junctions, restoration of pericyte coverage, and the formation of linear, non-leaky vessels. Conversely, Ang2 has been described as a natural antagonist to the Ang1/Tie2 interaction; binding of Ang2 to Tie2 results in destabilization of blood vessels, increased vessel permeability, and stimulation of new vessel sprouting. However, in some context-dependent scenarios, Ang2 can act as a Tie2 agonist or functions independently of Tie2 by interacting with integrins, fibronectin, and laminins to promote endothelial cell proliferation, migration, and adhesion. The clinical relevance of this pathway in cancer is highlighted by the finding that Tie2 is upregulated during tumor angiogenesis and higher levels of Ang2 relative to Ang1 correlate with poor prognosis in various cancers, including breast, metastatic colorectal, hepatocellular, and ovarian.

<table>
<thead>
<tr>
<th>Function dependent upon binding to Tie2</th>
<th>Ang1</th>
<th>Ang2</th>
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<tbody>
<tr>
<td>Tie2 agonist</td>
<td>• Promotes stabilization of blood vessels through development of linear, non-leaky vessels, organized endothelial junctions, and pericyte coverage.</td>
<td>• Destabilizes quiescent vessels to prepare for new vessel sprouting. Tie2 agonist action: Ang1 is absent. Tie2 levels are elevated.</td>
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<tr>
<td>Tie2-independent function</td>
<td>None described</td>
<td>Interacts with integrins to promote endothelial cell proliferation and migration.</td>
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Ang2 blocks the vessel-stabilizing actions of Ang1 and induces rapid formation of new, abnormal vessels.

Higher levels of Ang2 relative to Ang1 correlate with poor prognosis in multiple cancer types including acute myeloid leukemia, breast, chronic lymphocytic leukemia, hepatocellular, metastatic colorectal, multiple myeloma, neuroendocrine, non-small cell lung, prostate, squamous cell, and ovarian cancers.
The Ability of Angiopoietins to Regulate Lymphangiogenesis May Also Contribute to Tumor Metastasis

In addition to its role in regulating blood vessel formation, the angiopoietin axis is required for normal lymphangiogenesis, as demonstrated by the dysfunctional lymphatic systems of mice harboring a genetic deletion of Ang2. Unlike their naturally opposing roles in angiogenesis, Ang1 and Ang2 both promote normal lymphatic vessel patterning and function, as evidenced by the ability of Ang1 to rescue the lymphatic vessel defects in Ang2-deficient mice. Compromising the integrity of the lymphatic vasculature can lead to leaky vessels that may facilitate tumor cell dissemination and metastasis to distant sites. This concept is supported by studies in preclinical tumor models, where Ang2 overexpression increased tumor lymphatic vessel density and enhanced invasive growth and the rate of lymphatic metastasis.
Regulation of Angiogenesis May Represent a Potential Therapeutic Strategy for Cancer

Angiogenesis is necessary for tumor growth and is a complex process involving multiple signaling cascades. Regulation of this process thus represents a rational therapeutic strategy against many tumor types, including ovarian, colon, renal, hepatocellular, and non-small cell lung cancers. The VEGF axis acts to stimulate endothelial cell growth in tumor angiogenesis and has therefore emerged as a potential pathway for intervention. However, evasive mechanisms may permit tumors to grow and progress despite aims to prevent VEGF signaling, reflecting the existence of alternative proangiogenic pathways beyond the VEGF axis. The angiopoietin axis represents a separate pathway from the VEGF axis and plays a distinct role in mediating tumor angiogenesis by interacting with Tie2 and affecting vascular remodeling. Thus, the role of alternative pathways involved in mediating tumor angiogenesis is of importance and is being actively investigated.
Vascular Remodeling in Tumors Is Influenced by the Balance Between Ang1 and Ang2

Preclinical studies have demonstrated that tumor vasculature and tumor growth are sensitive to the relative balance between Ang1 and Ang2 expression. If Ang2 expression predominates, the vasculature shows defects that typify tumor vessels, including poor contacts between endothelial cells, diffuse staining of endothelial cell junction proteins, and weakened association with pericytes whose numbers may be reduced.\(^{17-19}\) If Ang1 expression predominates, the vasculature is normalized, as reflected by tight contacts between endothelial cells, linear staining of endothelial cell junction proteins at the cell borders, and tight associations with pericytes.\(^{17,20,21}\) The clinical impact of tumor vessel normalization is currently unknown, and further investigation is warranted.
Summary: The Angiopoietin Axis in Cancer—A Key Regulator of Tumor Angiogenesis

Tumor angiogenesis is necessary for tumors to grow beyond a limiting size. Angiogenesis is initiated by the “angiogenic switch,” which involves a complex array of signaling molecules, such as VEGF, PDGF, and angiopoietins, acting on multiple cell types. Evasive mechanisms may permit tumors to grow and progress despite aims to prevent VEGF signaling, reflecting the existence of alternative proangiogenic pathways. The angiopoietin axis represents a separate pathway with distinct functions from the VEGF axis. Signaling via the angiopoietin axis, comprised of the ligands Ang1 and Ang2 and the Tie2 receptor, is essential for tumor angiogenesis, regulating a wide range of endothelial cell activities that impact tumor vascular architecture. Additionally, the ability of angiopoietins to regulate lymphangiogenesis may also contribute to tumor metastasis. Thus, angiopoietins play a distinct role in modulating tumor angiogenesis. As a result, the angiopoietin axis may represent a potential pathway for therapeutic strategy in cancer.