# Tumor Angiogenesis: Cause or Consequence of Cancer?

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## Abstract

**Both tumors and normal tissues need a blood supply for oxygen, nutrients, and waste removal. However, whereas normal vasculature is hierarchically assembled into efficient networks of arteries, capillaries, and veins, the blood vessels of tumors are a mess—chaotic, leaky, inefficient, and barely making do. Why the difference? Do tumor vessels lack the signals to mature or, instead, is their maturation actively suppressed? What triggers and maintains tumor vasculature? In a recent study using a switchable Myc-driven mouse tumor model, we addressed these fundamental questions. We identified the inflammatory cytokine interleukin-1β as an essential initiating trigger of vascular endothelial growth factor–dependent angiogenesis. Here, we consider how kinetic studies using regulatable forms of Myc or other oncogenes can shed new light on the way tumors initiate and maintain their aberrant blood supplies.** [Cancer Res 2007;67(15):7059–61]

## Introduction

Macroscopic expansion of tissues, both normal and neoplastic, requires the concomitant growth, infiltration, and elaboration of a supporting vasculature network to maintain oxygenation and provide nutrients. As normal tissues become established, their incipient vasculature matures into a highly structured, hierarchical network of arteries, capillaries, and veins that maintains efficient tissue perfusion. By contrast, tumor vasculature typically lacks hierarchy and retains the disordered, tortuous, and leaky characteristics of incipient vasculature. However, the relationship between normal and tumor vasculature remains uncertain. One possibility is that the maturation of vasculature in normal tissues is actively driven by signals that appear when tissues mature and stabilize, although the nature and source of such signals are unexplained. Alternatively, vascular maturation may be a default program that is actively suppressed by the regenerative programs that build normal tissues during ontogeny and repair, programs that are constitutively active in tumors. It is also possible that tumor vasculature is an aberrant monster assembled piecemeal from sporadic mutations in angiogenic pathways and with no direct mechanistic counterpart in normal tissue biology.

The key to understanding tumor angiogenesis is to identify the mechanisms that cause it. However, in this regard there is no clear consensus. One plausible candidate mechanism is hypoxia, a potent trigger of neovascularization in normal tissues that is an expected consequence of the relentless and untoward expansion of tumor cells (1). Hypoxia triggers a wide variety of adaptive responses that are coordinated transcriptionally by the hypoxia-inducible factors HIF1, HIF2, and HIF3, heterodimeric bHLH-PAS transcription factors (2, 3). Stability of the obligate HIF-α subunits is regulated by the pVHL E3-ubiquitin ligase according to oxygen availability (4). The HIFs have many target genes that govern diverse functions such as oxygen transport, glycolysis, glucose uptake, metabolism, inflammation, and angiogenesis. Prominent examples of the latter are vascular endothelial growth factor-A (*VEGFA*), a potent inducer of endothelial cell proliferation and remodeling, placental growth factor (*PIGF*), and basic fibroblast growth factor (*bFGF*; refs. 5–7). In other instances, however, evidence suggests that early-stage tumors are not competent for angiogenesis and acquire the capacity only sporadically, a transition dubbed "the angiogenic switch" that presumably involves accumulation of additional angiogenic mutations. More recently, evidence has accumulated that certain types of oncogenic mutation can directly instruct angiogenesis as well as stromal modeling in general. Indeed, Ras and Myc, whose activation is causally implicated in a significant minority of human cancers, are prominent examples of such angiogenic oncogenes, doubtless reflecting the way that evolution has interwoven the intracellular programs that drive cell growth and proliferation with extracellular programs that support the expansion of such proliferating cells. Oncogene-driven angiogenesis has two profound implications. First, tumors driven by such oncogenic mutations would presumably be angiogenic from the outset, potentially conferring a distinct pattern of tumor progression and prognosis. Second, maintenance of the vasculature of such tumors would presumably depend on the continued angiogenic activity of the initiating oncogenic mutation, suggesting an optimal strategy aimed at targeting the nodal oncogenic heart of the tumor rather than its various attendant attributes.

## Dissecting Cause and Effect in Tumorigenesis

No tumor cell is an island: tumors are aberrant tissues, not clonal monocultures, and whereas tumor cells may exhibit a measure of autonomy, they, like all vertebrate somatic cells, remain completely dependent on the multifarious cells within their local microenvironment for oxygen, nutrients, survival factors, mitogens, and structure. Indeed, the interdependence of tumor and microenvironment is so intimate that it begs the question of how it becomes established in the first place and, thenceforth, maintained. The debate over whether the tumor cell or the microenvironment is most critical in instigating and maintaining the tumor phenotype resembles a classic nature versus nurture argument—the two are integral parts of a whole and cannot be sensibly dissected one from the other.

By the time a tumor is established, countless interactions between tumor and stroma have already taken place, making it virtually impossible to dissect out the complex, reciprocal, and changing cause-and-effect relationships responsible for tumor evolution and maintenance. Recently, this problem has been approached through use of novel reversibly switchable *in vivo* mouse tumor models that allow synchronous activation, and subsequent deactivation, of defined oncogenic lesions within specific orthotopic somatic compartments. In this way, the cause-and-effect chain of processes triggered by acute oncogene activation *in vivo* can be elucidated and, where and when

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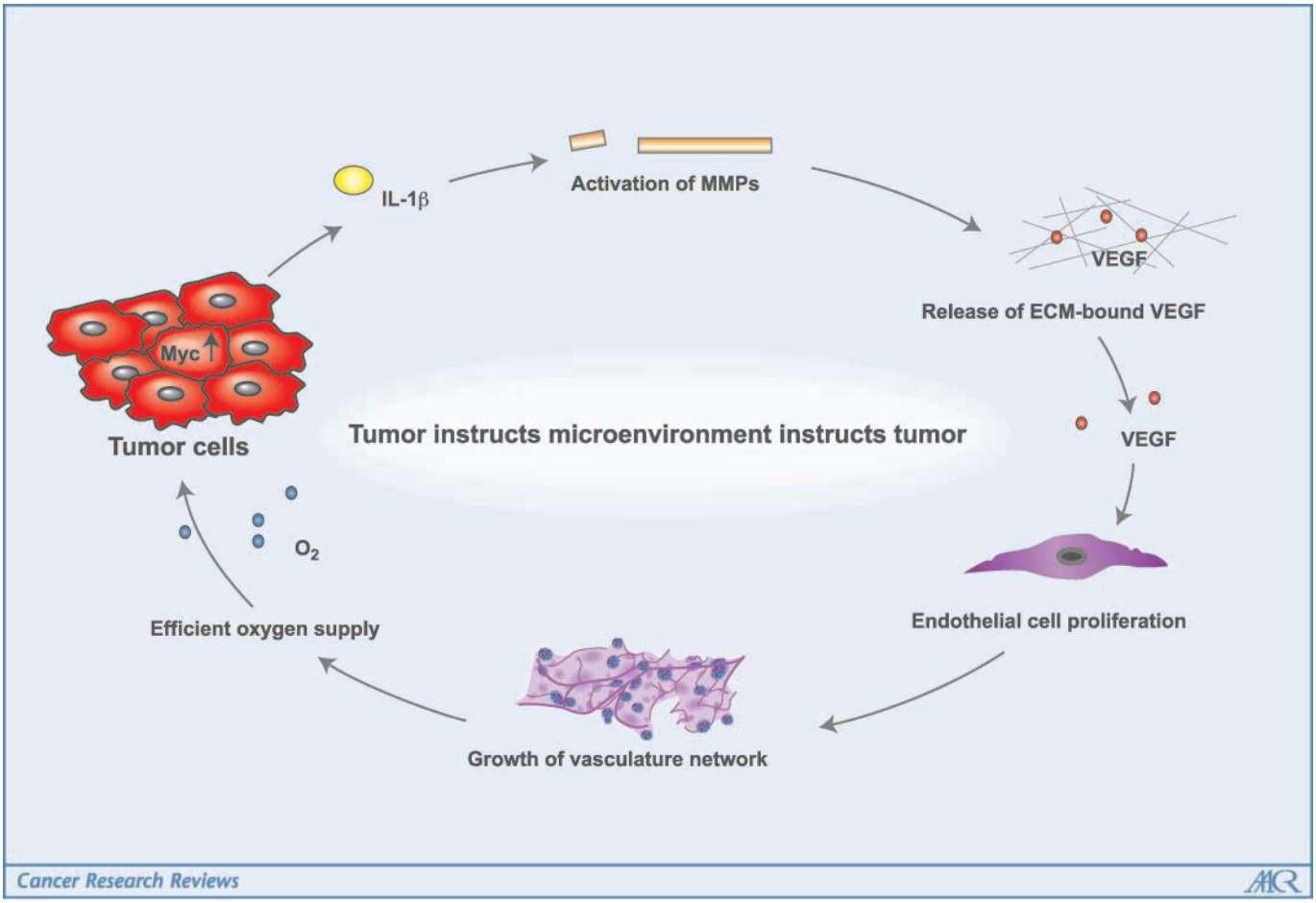


**Figure 1.** Tumor instructs microenvironment instructs tumor. Oncogenic activation of Myc in tumor cells triggers expression and release of the inflammatory cytokine IL-1β. IL-1β, in turn, activates metalloproteinases that release extracellular matrix (*ECM*)–bound VEGF, flicking the angiogenic switch. The elaborating vasculature promotes further tumor growth and survival, effectively engaging a mutually supportive reciprocal interaction that, nonetheless, requires continuous Myc activity for its maintenance.

necessary, perturbed, providing an indication of the extent to which the tumor cell instructs its microenvironment versus the microenvironment instructing the tumor. Such models are, of course, highly simplified and accelerated representations of the true complexity of human cancers: nonetheless, because they use relevant oncogenic mutations driving autocthonous tumors within tissues *in vivo*, they offer many unique insights into tumor biology and evolution. We have used one such model, based around reversible activation of Myc in pancreatic β cells, to establish how and why Myc drives tumorigenesis (8, 9).

Multiple studies attest to the potent oncogenic potential of Myc. Myc is a bHLH-Zip transcriptional modulator that plays an important role in both the normal vertebrate biology and the pathology of cancer. Aberrantly high and/or deregulated activity of Myc is implicated causally in the majority of cancers and often associated with more aggressive, poorly differentiated, and angiogenic tumors. Through its widespread actions on many target genes, Myc engages and coordinates multiple intracellular proliferative programs that raise metabolic activity, promote growth, and drive proliferation. Recently, it has become clear that Myc also equips proliferating cells with the means to expand within their somatic compartment by engaging extracellular programs such as angiogenesis. Myc exerts its angiogenic activity in diverse ways: through down-regulation of endogenous inhibitors of angiogenesis (thrombospondin-1; refs. 10, 11) and up-regulation of vasculature growth promoting factors—both those that act as direct endothelial mitogens (e.g., VEGFA, PIGF, and FGF; refs. 12–14) and those that act indirectly by modulating supply, distribution, and accessibility of endothelial growth factors such as proteases and their activators. Such oncogene-dependent signals seem to be independent from, but may run parallel to, other angiogenic mechanisms in tumors such as hypoxia or sporadic secondary proangiogenic mutations.

To address the kinetics and mechanism of Myc-mediated angiogenesis in cancer, we used $MycER^{TAM}$, a fusion protein of Myc with a modified hormone binding domain from the estrogen receptor that renders Myc function dependent on provision of the synthetic steroid 4-hydroxytamoxifen. Acute activation of Myc in pancreatic β cells precipitates a consistent and defined cascade of events that, over time, establish the various aspects of tumorigenesis necessary for macroscopic tumor growth and spread. At any time, $MycER^{TAM}$ function can be quelled and the dependence (or not) of such functions on Myc activity established. In this way, both the sequence of events responsible for tumor formation and their reliance on sustained Myc activity can be ascertained.

Acute activation of Myc in β cells triggers synchronous entry of β cells into cycle. However, such β-cell proliferation is quickly overwhelmed by apoptosis, leading to rapid involution of all islets throughout the pancreas. However, when Myc-induced β-cell apoptosis is blocked by coexpression of the apoptosis inhibitor Bcl-$x_L$, Myc activation triggers immediate and progressive expansion of β cells that rapidly evolve into highly invasive,

dysplastic, and highly vascularized β-cell carcinomas (8). The dramatic and sustained angiogenesis in this model is surprising given that Myc is not expressed in the vascular compartment, only in β cells. Hence, Myc activation in β cells triggers their release of factors that recruit the adjacent endothelial cells into cycle and drive their progressive elaboration into the leaky and tortuous vessels we observe. Kinetic analysis of angiogenic factors present in islets following Myc activation indicated dramatic up-regulation of soluble VEGFA by 72 h, consistent with the onset of angiogenesis we observed. However, such VEGFA is not synthesized *de novo* in response to Myc activation but, instead, mobilized from preexisting reserves bound to extracellular matrix, most probably through the action of extracellular proteases such as matrix metalloproteinase MMP-9 (15).

Inspection of the kinetics of events after Myc activation identified the prominent induction and release of the inflammatory cytokine interleukin-1β (IL-1β), its expression appearing within only 1 to 2 h of Myc activation. Such rapidity most likely precedes any release of significant *trans*-active cytokines or signals from the β cells and occurs long before infiltration of the islets by inflammatory or other ectopic cells. Thus, it is most likely that β cells themselves are the immediate source of IL-1β, as has been documented (16). Intriguingly, although Myc induces IL-1β expression, secretion of the mature cytokine requires processing of the 31- to 33-kDa pro-ILβ precursor polypeptide into its 15- to 17-kDa secreted active form by the inflammasome, a process whose mechanism remains unclear. Systemic administration of IL-1β–blocking antibodies over the relevant 0- to 72-h time window following Myc activation profoundly delayed and reduced Myc-induced VEGFA release and islet tumor angiogenesis, confirming a causal role for IL-1β as the pivotal toggle for the Myc-dependent angiogenic switch. Moreover, exposure of isolated islets to recombinant IL-1β proved competent to trigger mobilization and release of islet VEGF, confirming that IL-1β is sufficient to trigger angiogenesis and that all other components of the IL-1β–dependent angiogenic switch preexist within each islet (15). During classic inflammation, IL-1β is known to activate MMPs through both cyclooxygenase-2 and p38 mitogen-activated protein kinase, suggesting that this same inflammatory pathway is commandeered during islet tumorigenesis (17, 18). A schematic depiction of how Myc activation in β cells initiates this reciprocal interplay between tumor cell and microenvironment is shown in Fig. 1.

Is the same IL-1β–dependent pathway likely to operate during Myc-induced tumorigenesis in other tissue types? At present, this is unclear. Although Myc seems to be potently angiogenic in many different tissue types, there are nonetheless reasons to suspect that Myc-dependent angiogenesis may operate by discrete mechanisms in differing tissues. Most tissues do not harbor appreciable levels of extracellular matrix–bound VEGFA; thus, release of preexisting VEGF reserves cannot be the mechanism of angiogenesis in such tissues. As already discussed, down-regulation of thrombospondin-1, perhaps via the Myc-specific miR-19 microRNA, seems to play a critical angiogenic role in several other tissues (11) whereas studies in skin using an analogous reversibly switchable Myc oncogenesis model indicate that Myc-induced epithelial papilloma angiogenesis is mediated by induction of *VEGF* gene expression together with onset of tissue hypoxia (19). Moreover, such angiogenesis is spatially restricted to underlying dermis adjacent to the tumors, consistent with the normal pattern of angiogenesis in that tissue. It seems that activated Myc drives angiogenesis by engaging whatever inherent angiogenic programs reside within each tissue type.

Given the extensive differences in architecture, dynamics, regenerative capacity and risk of neoplasia, damage, and infection between different tissues, it makes sense that each would evolve a different response to the generalized proliferative impetus of Myc. This is certainly consistent with the vast number of genes that Myc has been shown to regulate, genes that share surprisingly little overlap between different cell and tissue types. We may have to face the reality that different tissues are, well, different—and that promiscuous oncogenes like Myc exert their oncogenic influence by subverting the inherent regenerative program peculiar to each tissue type rather than imposing their own oncogenic will on their prey. Rather than one mechanism of Myc-induced oncogenesis, there may be as many mechanisms as there are distinct cell types.

However, even this cloud has a silver lining. If each cell type has its own idiosyncratic configuration of susceptibilities to oncogenesis, there may be more opportunities for therapies that are tissue and tumor specific. Systematic analysis of how individual oncogenes drive tumorigenesis in distinct tissues should indicate whether our cup is empty, full, or runneth over.

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