Matrix reloaded to circulation hits the tumor target

Proteolytic fragments of various components of the extracellular matrix exhibit antiangiogenic activity via interaction with endothelial cell surface integrins. Kalluri and coworkers (Hamano et al., 2003; this issue of *Cancer Cell*) use gene-targeted mice to show a physiological role for a carboxy-terminal fragment of collagen IV in the regulation of tumor angiogenesis.

Tumor tissue, like normal tissue, is dependent on functional vasculature for sufficient supply of nutrients and oxygen. An expanding tumor becomes rapidly hypoxic, resulting in the activation of hypoxia-inducible genes such as the gene encoding vascular endothelial growth factor (VEGF), which is secreted from the cells and induces angiogenesis, the formation of new capillaries from preexisting vessels (Ferrara, 2002). At the cellular level, angiogenesis can be dissected into events starting from endothelial cell activation, subsequent degradation of the underlying basement membrane, migration of the cells toward the direction of a chemotactic stimulus, and finally reassembly and maturation of vessel structures. The shift from a quiescent to an angiogenic phenotype has been described as a biological switch: an increase in proangiogenic stimuli with simultaneous downregulation of inhibitory signals (Bergers and Benjamin, 2003). The inhibitory signals are commonly cryptic fragments derived from the extracellular matrix by proteolytic cleavage. These endogenous angiogenesis inhibitors include a fragment of type XVIII collagen C-terminal (NC1) domain termed endostatin and the NC1 domain of the α3 chain of type IV collagen termed tumstatin, as well as others, such as canstatin, arresten, and anastellin (Kerbel and Folkman, 2002). All of these have been shown to inhibit tumor angiogenesis when delivered as recombinant proteins or via viral transduction, but their physiological roles have remained elusive. In this issue of *Cancer Cell*, Hamano and coworkers describe enhanced tumor angiogenesis, but normal developmental angiogenesis, in mice lacking the α3 chain of type IV collagen containing the tumstatin fragment (Hamano et al., 2003). Interestingly, tumstatin is generated from type IV collagen by matrix metalloprotease-9 (MMP-9), a protease previously described as a trigger of the angiogenic switch (Bergers and Benjamin, 2003). Furthermore, its inhibitory signals are transmitted to the tumor endothelium via integrin $\alpha_v\beta_3$, a molecule shown to regulate tumor angiogenesis both positively and negatively (Stupack and Cheresh, 2002; Reynolds et al., 2002). In one sense, this endogenous angiogenesis inhibitor, which floats around the body fluids, would then function as tumor suppressor.

The dual role of integrins

Integrins are heterodimeric transmembrane receptors that bind to ligands in the extracellular matrix or counter-receptors on adjacent cells and regulate multiple aspects of cell behavior (Hynes, 2002). During the process of angiogenesis, integrins act in coordination with growth factors and their receptors to promote cell migration and adhesion (Stupack and Cheresh, 2002). Their downstream targets consist of a number of signaling cascades involving molecules such as Src, focal adhesion kinase, Ras, MAP kinase, the Rho family of GTPases, and PI3 kinase (Hynes, 2002).

Endothelial cells express multiple integrins, but endothelial cell integrins $\alpha_v\beta_3$, $\alpha_v\beta_5$, and $\alpha_5\beta_1$ are considered central to the regulation of angiogenesis. $\alpha_v\beta_3$ and $\alpha_v\beta_5$ become dramatically induced in activated endothelial cells in the tumor vasculature. In addition, inhibitors of $\alpha_v\beta_3$, and $\alpha_v\beta_5$ disrupt pathological angiogenesis in various model systems (Stupack and Cheresh, 2002). $\alpha_5\beta_1$ integrin is upregulated on endothelial cells in response to angiogenic growth factors, and antibodies binding to this integrin have been shown to inhibit angiogenesis (Kim et al., 2000). However, recent data using gene-targeted mice revealed that the picture is not so straightforward. Mice lacking β_3 integrins have no defects in developmental angiogenesis, but instead display enhanced tumor angiogenesis (Reynolds et al., 2002), suggesting that β_3 integrins can also function as negative regulators of angiogenesis. The new results from Hamano and coworkers now suggest that physiological levels of circulating tumstatin suppress tumor angiogenesis through interaction with the $\alpha_v\beta_3$ integrin expressed in the tumor endothelium (Hamano et al., 2003; Figure 1). This observation might in part explain the unexpected observation of increased tumor angiogenesis in mice lacking the β_3 integrin (Reynolds et al., 2002). Absence of this integrin with concomitant loss of the inhibitory effects of tumstatin would shift the balance of pro- and antiangiogenic factors to favor the former.

MMP-9 in angiogenesis: A switch on and off

MMP levels are increased in the majority of human cancers, correlating positively with advanced tumor stage, invasiveness, and poor prognosis. In various mouse models, loss of MMP-9 has been observed to result in decreased tumorigenesis and metastasis (Egeblad and Werb, 2002). In the RIP1-Tag2 transgenic mouse model of pancreatic islet carcinomas, MMP-9 has been shown to induce the transition of benign carcinoma in situ lesions into angiogenic carcinomas via an increase in the bioavailabilty of vascular endothelial growth factor VEGF (Bergers and Benjamin, 2003). Surprisingly, Hamano et al. saw no difference in tumor growth between MMP-9-deficient and wild-type mice when analyzing tumors smaller than 500 m^3 in size, but when the tumors exceeded this size, accelerated tumor growth occurred in mice lacking MMP-9. This was associated with reduced levels of plasma tumstatin, and by returning to physiological levels of plasma tumstatin by delivery of recombinant protein, tumor growth decreased to the level of the wild-type mice (Hamano et al., 2003). MMP-9-deficient mice still have relatively high concentrations of tumstatin in their circulation (141 ± 21 versus 350 ± 24 ng/ml in wild-type mice), but the growth rate of the tumors in these mice did not differ from that of the tumstatin-deficient mice. The reason for such a threshold in tumstatin activity is unclear. Taken together, the current data suggest that MMP-9 acts on both sides of the angiogenic switch: via release of VEGF, it triggers the onset of angiogenesis in tumors, thus accelerating tumor growth, but in later stages of tumor development, the MMP-9-mediated turnover of basement membranes releases matrix fragments with antiangiogenic activity, resulting in the suppression of tumor growth.

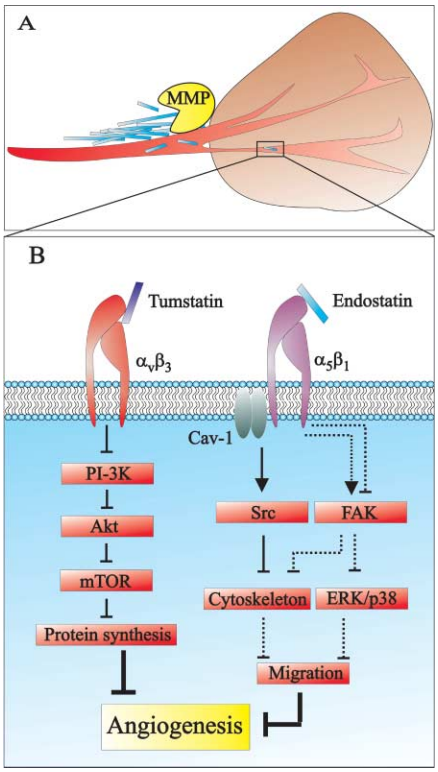

Figure 1. Putative integrin-mediated signals generated by extracellular matrix-derived fragments tumstatin and endostatin in the tumor endothelium

A: Tumor growth is associated with accelerated turnover of extracellular matrix and basement membranes. Increased secretion of MMPs leads to the release of extracellular matrix protein fragments such as tumstatin and endostatin (Egeblad and Werb, 2002). These fragments are delivered via the circulation to the tumor vasculature.

B: Tumor endothelial cells express increased levels of $\alpha_v\beta_3$ and $\alpha_5\beta_1$, which facilitate the targeting of tumstatin and endostatin selectively to the developing tumor vasculature (Kim et al., 2000; Hamano et al., 2003). Tumstatin binds $\alpha_v\beta_3$ and inhibits endothelial cell cap-dependent protein synthesis via inhibition of PI3 kinase and mTOR activity (Maeshima et al., 2002). Endostatin in turn binds to $\alpha_5\beta_1$ and inhibits endothelial cell migration possibly via inhibition of FAK and ERK/p38 activity and through caveolin-1-mediated activation of Src and subsequent inhibition of actin cytoskeleton turnover (Rehn et al., 2001; Sudhakar et al., 2003; Wickström et al., 2002). A pathway in which FAK is activated by endostatin followed by disassembly of the actin cytoskeleton has also been described (Dixelius et al., 2002). The findings of Hamano et al. suggest that tumstatin released into the circulation by MMP-9 is an important physiological suppressor of tumor angiogenesis.

Recent advances in understanding the complex mechanisms of tumor angiogenesis and the dual roles of key molecules involved in these processes may help to explain some of the disappointing results from clinical trials using angiogenesis inhibitors that target integrins or MMPs. At the same time, they underline the importance of therapeutic attempts that combine two or more agents, targeting more than one process or several cell types. In light of the results of Hamano et al., the use of MMP inhibitors together with matrix-derived angiogenesis inhibitors could result in synergistic effects. Emerging knowledge on the apparently opposing functions of certain regulatory matrix proteins and their integrin receptors during different stages of carcinogenesis prompts the need for detailed studies to define the optimal time points for therapeutic intervention with these agents.

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