Endostatin's endpoints—Deciphering the endostatin antiangiogenic pathway

Up until now, the precise mechanism for endostatin's antiangiogenesis action was not known. In a recent report, Abdollahi et al. (2004) have taken advantage of gene array and proteomic analysis to map the antiangiogenic pathways turned on by endostatin. This study resolves some of the controversies surrounding endostatin's biology, and provides a new direction to help dissect the molecular pathways involved in endostatin's selective tumor antiangiogenic effects.

Groundbreaking science often has its ups and downs, especially when translating it for clinical application. We have seen before the so called "glitter-to-ashes" syndrome, where breakthrough science makes its way into the headlines amidst unreal expectations, there is a clinical disappointment, and the technology is perceived as being left in a smoldering heap of unfulfilled promises. But here is where the serious science just begins and the ultimate fate of the technology gets decided. Antisense technology is a good case in point. From being touted as the perfect therapy to being a useless dream, we now have about 40 antisense molecules in clinical trial and one molecule (Vitravene, Isis Pharmaceuticals) already FDA-approved, having been shown to be effective against CMV retinitis.

Perhaps never before, however, has any cancer therapy been as widely anticipated as the current wave of antiangiogenic therapies now making their way into the clinic. The concept is elegant in its simplicity: inhibit the growth of genetically stable endothelial cells, and most tumors should starve to death with little acquired resistance. Glitter to ashes, as we've come to expect, with early clinical stumbles. But now the resurrection begins with the recent approval of Genentech's Avastin, an antibody targeting VEGF-A and shown to extend the lives of patients with advanced colorectal carcinoma. A naturally occurring angiogenesis antagonist, endostatin, first shown to inhibit the growth of tumor xenografts with no acquired resistance (Boehm et al., 1997), has also had its ups and downs (Jouanneau et al., 2001). Now, in the latest issue of *Molecular Cell* (Abdollahi et al., 2004), Peter Huber and colleagues show that this protein alters the expression of a bevy of factors controlling angiogenesis, which may account for its antiangiogenic activity in vivo.

The precise mechanism for the antiangiogenic effect of endostatin has been elusive. Endostatin, a C-terminal fragment of collagen XVIII, has been shown to block endothelial cell proliferation, survival, and migration, in part through blocking VEGFR2 signaling, suppressing Wnt signaling by β catenin destabilization, or possibly altering β catenin/VE cadherin interactions in interendothelial cell junctions (for review see Dixelius et al., 2003). However, a unified model to describe the multifaceted antiangiogenic effect of endostatin has not been reported. In their study, Abdollahi et al. have taken advantage of gene profiling and protein phosphorylation studies to determine the global antiangiogenic effect of endostatin on primary cultured human endothelial cells. They provide evidence that endostatin induces its antiangiogenic effect through regulation or modification of a large cluster of genes known to affect endothelial growth and development.

There are several remarkable aspects of this detailed array analysis. Approximately 12% of the 74,834 genes on the chip have >2-fold alteration in expression, either up or down, in response to endostatin treatment. Of these, 71 or 0.8% are known mediators of angiogenesis. Strikingly, in the cases reported, known proangiogenic factors (such as Ids, HIF-1α, ephrins, and the STATs) are all downregulated, and antiangiogenic factors such as thrombospondin-1 (TSP-1), kininogen, and a precursor of vasostatin are all upregulated. The implication is that some of the genes affected that are not known to be angiogenesis regulators may in fact be, either directly or indirectly. Remarkably, in many cases, upstream regulators and downstream targets of these genes are affected in predicted ways. Using the Id analysis as an example, it is observed that one potential upstream regulatory pathway (namely Ets-1, which upregulates Egr1, which in

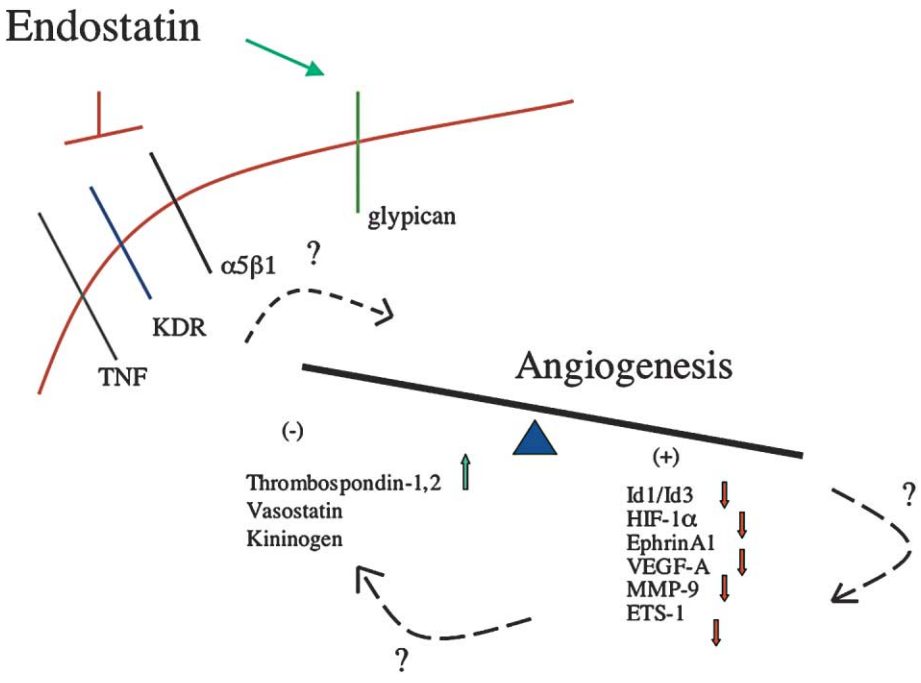

Figure 1. The angiogenic balance

Endostatin tips the scale toward angiogenesis inhibition by inhibiting the activity/expression of proangiogenic factors and stimulating antiangiogenic factors both at the cell surface and intracellularly. The arrows with question marks indicate our lack of understanding about signaling interdependencies versus parallel effects of endostatin.

turn controls Id1 gene expression in response to serum [Ruzinova and Benezra, 2003]) is downregulated upon endostatin treatment concomitant with Id1 loss of expression. In addition, some known downstream targets of Id1 loss (namely HIF-1α reduction [Ruzinova et al., 2003]) and elevation of TSP-1 [Volpert et al., 2002]) are regulated as though Id1 loss is the primary event. Caution in the interpretation of direct effects of Id loss versus parallel effects of endostatin must be exercised: loss of Id1 has been shown to affect TSP-1 levels in Id1 knockout fibroblasts, but no effects are observed in tumor endothelial cells (Ruzinova et al., 2003), suggesting parallel effects of endostatin signaling in the current study. Other examples of this type of potential collaborative networking exist in the analysis: downregulation of the TNFR1 leading perhaps to downregulation of Ephrin A1 via attenuation of jnk and p38 signaling, and NfkB signaling inhibited by reduced expression and dephosphorylation and downregulation of its targets, including the antiapoptotic c-*myc*, iNOS, and Cox2. More detailed analyses will be required to distinguish between different possible regulatory schemes (Figure 1).

There are other unresolved issues. Endostatin may exert its antiangiogenic effect through its binding to $\alpha5\beta1$ integrin and the consequent inhibition of matrix adhesion and signaling (Wickstrom et al., 2002), or low affinity binding to glycopican-1 and glycopican-4 or as yet unidentified receptors (Karumanchi et al., 2001). It is not obvious which endostatin receptor conveys the antiangiogenic signals described, but interestingly, many of the genes upregulated by $\alpha5\beta1$ engagement and NfκB activation (Klein et al., 2002) are downregulated by endostatin treatment. A further complication is the study referred to above showing that endostatin interferes with VEGFR2 (KDR, Flk-1), the tyrosine kinase receptor that is absolutely essential for the proliferation, survival, and migration of endothelial cells. The gene profiling study by Abdollahi et al. was performed with cultured primary human dermal microvascular endothelial (HDMVEC) cells that were maintained in 5% serum supplemented with endothelial growth factors VEGF-A and FGF-2. As serum contains antiangiogenic agents as well (i.e., TSP-1 or TGF-β), it is possible that endostatin may not directly induce the expression of these antiangiogenic agents delineated in this study, but rather remove the trophic effect of proangiogenic factors (i.e., VEGF-A), thereby shifting the balance toward endothelial cell apoptosis. Thus, the molecular resetting toward endothelial cell death may be induced by removal of proangiogenic factors rather than by a direct induction of antiangiogenic pathways.

Accumulating evidence also suggests that tumor endothelial cells may represent a phenotypically and functionally different type of endothelia. Abdollahi et al. used primary HDMVEC treated for 4 hr with 200 ng/ml of endostatin. In contrast to other primary human endothelial cells, only particular organ-specific vessels, including brain and HDMVEC at early passages, express CD36 (GPIV), which is the cognate receptor for the antiangiogenic factor TSP-1 (Bornstein, 2001; Lawler, 2002). Therefore, it remains to be determined whether endostatin has similar effects on other types of tumor-derived endothelial cells or organ-specific microvascular endothelial cells.

One other as of yet unexplained but remarkable phenomenon is that endostatin has no major effect on angiogenesis during wound healing, pregnancy, or tissue reparative processes. As wound healing is dependent on the recruitment of dermal microvascular endothelial cells, it remains to be determined why endostatin does not target HDMVECs during this process. It is conceivable that unique proangiogenic growth factors other than VEGF-A or FGF-2 may override the antiangiogenic effect of endostatin.

One approach to validate the data generated by Abdollahi et al. would be to isolate tumor endothelial cells from implanted tumors immediately after endostatin treatment. This will help identify genes that are activated de novo selectively in tumor endothelial cells during endostatin treatment. Comparison of this gene profiling data with endostatin-resistant endothelial cells present during wound healing may provide an explanation for the divergent effects of endostatin observed. In addition, evaluating the effects of endostatin on spontaneous tumor endothelium seems warranted given the dramatic differences in effects of antiangiogenic stress between xenografts and genetic models of cancer (Ruzinova et al., 2003).

Despite these considerations, the report by Abdollahi et al. provides instructive road maps for future studies to understand the mechanism whereby endostatin, or other antiangiogenic factors such as tumstatin, exert their potent antiangiogenic effects under certain pathophysiological conditions. There is no doubt that endostatin targets selective vascular niches dictated by the expression of its known and as of yet unidentified receptors. Tumor vascular microenvironment may be conducive to endostatin's antiangiogenic effects, as these endothelial cells express functional endostatin receptors or are dependent on angiogenic growth factors whose receptors are targeted by endostatin. This study by Huber and colleagues has undoubtedly opened up a new chapter in the intriguing antiangiogenic biology of endostatin.

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Selected reading

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