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Commentary

Whence Thrombospondin?

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Commentary to:

Inhibition of Tie-2 Signaling Induces Endothelial Cell Apoptosis, Decreases Akt Signaling and Induces Endothelial Cell Expression of the Endogenous Anti-Angiogenic Molecule, Thrombospondin-1

Qi Niu, Carole Perruzi, Daniel Voskas, Jack Lawler, Daniel Dumont and Laura E. Benjamin

The field of experimental cancer research burgeoned ever since oncogenes and tumor suppressors were first identified in late 1970's to early 1980's. These genes affect, in a positive and a negative fashion respectively, the ability of neoplastic cells to expand in the absence of appropriate intrinsic and extrinsic cues. Consequently, such cells might have become refractory to senescence, hypoxia, genotoxic stress-induced death, or chemotherapeutic agents. These devious tumor phenotypes often can be traced back to discrete gain-of-function mutations in oncogenes and loss-of-function alterations of tumor suppressors.

However, each tumor cell, no matter how self-reliant in the matters of procreation and death, exists in the rich extracellular milieu and participates in local invasion, neovascularization, metastatic spread, and the avoidance of immune surveillance. The attribution of such non-cell-autonomous processes to distinct molecular events is proving to be much more difficult. The analysis of tumor neovascularization is a case in point. While more and more proteins are reported to be either pro- or anti-angiogenic, placing them in a proper cellular context remains a daunting task. Consider thrombospondin-1, a secreted glycoprotein abundantly expressed in most tissues and plasma. In purified or recombinant form, it readily inhibits sprouting of new blood vessels (angiogenesis) in a variety of surrogate in vitro and in vivo assays.[1] But modeling its anti-angiogenic activity in chicken chorioallantoic membranes or mouse corneal implants is a far cry from catching thrombospondin red-handed, at the exact time and place of action.

Perhaps the earliest attempt to that end was documented by Noel Bouck and her colleagues in the 1989 *Cell* paper inconspicuously entitled "Regulation of the activity of a new inhibitor of angiogenesis by a cancer suppressor gene."[2] An inhibitor subsequently turned out to be thrombospondin, and a cancer suppressor—the p53 tumor suppressor. The pairing with p53 proved fortunate for thrombospondin which starred in a series of high profile papers, including the 1994 article "Control of angiogenesis in fibroblasts by p53 regulation of thrombospondin-1."[3] In it, sharp declines in thrombospondin levels following homozygous loss of p53 were shown to be sufficient to trigger the angiogenic switch. Thus, it has been suggested that while p53 acts primarily as a guardian against unwarranted entry into the cell cycle, it also has a non-cell-autonomous function: to maintain high levels of thrombospondin-1. By doing so, it could stave off unwarranted vascularization of normal mesenchymal tissues. Subsequent work has demonstrated that many oncoproteins (e.g., Src,[4] Jun,[5] Myc,[6-8] and Ras[9]) act in a predictably opposite fashion and suppress thrombospondin levels. Thus, one might suspect that anti-angiogenic thrombospondin would be silenced, one way or the other, in all naturally occurring neoplasms. This assumption proved to be incorrect: thrombospondin is downregulated in many but by no means all human neoplasms, invasive ductal breast carcinoma being a conspicuous counterexample.[10]

To explain this paradox, one might argue that perhaps tumor vascularization is dictated by levels of thrombospondin in the non-neoplastic stroma, an abundant component of most solid malignancies. From there, thrombospondin would function as a "landscaper", by limiting ingrowth of blood vessels.[11] Indeed, engrafted tumors were observed to grow much faster in thrombospondin-null hosts, due at least in part to an increase in microvascular density.[11] In a more natural setting, formation of mammary tumors induced by the MMTV-*neu* transgene was also accelerated in the thrombospondin-null background.[12] However, contrary to many expectations, thrombospondin-1-null mice do not exhibit a clear vascular phenotype on their own,[13] and in human neoplasms both upregulation and downregulation of stromal thrombospondin have been observed.[14] In addition, there is ample evidence that thrombospondin is subject to degradation by extracellular proteases, and whether its proteolytic products stimulate or suppress angiogenesis remains to be seen (Lee N, Iruela-Arispe ML; manuscript in preparation). To compound the matter further, thrombospondin and its proteolytic fragments possess adhesive and potentially pro-metastatic properties.[15,16] Collectively, these results raise the question of just how diligent a landscaper

thrombospondin is. Perhaps its famed anti-angiogenic behavior requires some serious prodding.

Several papers published in the last year suggest that thrombospondin-mediated anti-angiogenesis indeed can be elicited by seemingly irrelevant therapeutic interventions. The group in Toronto, led by Robert Kerbel, modeled in the mouse the so-called metronomic (low-dose/high-frequency) chemotherapy. The underlying idea was that administering sub-maximal tolerated doses on accelerated schedules might prevent tumor vasculature from recovering during intervals between treatments.[17,18] Indeed, protracted low-dose treatments with cyclophosphamide proved to be exquisitely cytotoxic for endothelial cells comprising blood vessels.[19] This cytotoxicity was accompanied by a rapid increase in the thrombospondin production by endothelial cells and could be partially alleviated by neutralizing antibodies to thrombospondin. Remarkably, in vivo beneficial effects of low-dose cyclophosphamide were apparent in wild-type mice, but completely abolished in thrombospondin-null hosts. This suggested that thrombospondin is a key mediator of endothelial cell sensitivity to metronomic therapy.

In this issue of *Cancer Biology & Therapy*, Niu et al. report that endothelial cells can also be killed, at least in vitro, by the targeting of Tie-2, the receptor for endothelial cell survival factor Ang-1.[20] The Boston group successfully employed both the RNAi oligonucleotides against Tie-2 and the dominant-negative Tie-2 mutant.[21] The ensuing down-regulation of Tie-2, although relatively modest, resulted in a dramatic suppression of the Akt survival pathway and massive death of endothelial cell. Provocatively, there was an accompanying increase in thrombospondin levels. Could there be a cause-and-effect connection? In support of this idea, the authors demonstrate that thrombospondin-derived peptides have the same disastrous effects on endothelial cell survival as the Tie-2 blockade. This finding, of course, doesn't prove the role of endogenous thrombospondin in endothelial cell killing beyond a reasonable doubt—for this targeting of the tsp1 gene itself would be necessary. But it certainly contributes to the preponderance of evidence implicating thrombospondin activation as the crucial mediator of anti-angiogenic interventions, be that metronomic chemotherapy or interference with survival pathways. One corollary of this model is that therapies employing thrombospondin mimetics[22] could be a perfect complement, not substitute, for other types of anti-angiogenic treatments since endothelial cells would be bombarded with thrombospondin from both sides of the plasma membrane. As we all know, sometimes more is more!

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