Wiring the angiogenic switch: Ras, Myc, and Thrombospondin-1

The formation of a blood supply is critical for tumor growth and metastasis; however, understanding the relationship of cellular transformation to tumor angiogenesis has been limited by the multifactorial nature of both processes. In this issue of *Cancer Cell*, Watnick and colleagues use a genetically defined tumor model system to determine the link between *ras*, *myc*, and angiogenesis and identify Thrombospondin-1 as being the critical regulator of angiogenesis in this system (Watnick et al., 2003).

In order for a tumor mass to get beyond a critical size, it must develop an associated vasculature. Over 30 years ago, Folkman proposed that targeting a tumor vasculature would limit tumor expansion and could be a useful cancer therapy (Folkman, 1971); however, the precise targets and means of inhibiting angiogenesis were poorly defined at that time. More recently, a variety of proangiogenic and antiangiogenic factors have been identified and have led to the concept of the "angiogenic switch," a process in which disruption of the normal ratio of angiogenic stimuli and inhibitors in a tumor mass allows for autonomous vascularization (Hanahan and Folkman, 1996). The angiogenic switch appears to be governed by the same genetic alterations that drive malignant conversion: the activation of oncogenes and the loss of tumor suppressor genes (Bouck, 1990). Thrombospondin-1 (TSP-1), an extracellular matrix glycoprotein, was the first naturally occurring inhibitor of angiogenesis to be identified (Good et al., 1990). TSP-1 is a potent inhibitor of in vivo neovascularization and tumorigenesis and has been best studied in a variety of mouse model systems. Overexpression of TSP-1 in tumor xenografts has been shown to inhibit tumor cell growth in a variety of tissue types, while mice with a genetic susceptibility to breast cancer show decreased tumor formation in the presence of a breast-specific TSP-1 transgene (Rodriguez-Manzaneque et al., 2001). In addition, mice null for both p53 and TSP-1 demonstrate an increased incidence of osteosarcoma versus mice lacking p53 alone (Lawler et al., 2001). Furthermore, tumor xenografts show increased vascular density and more rapid growth in mice null for TSP-1, suggesting that TSP-1 expression derived from either the tumor itself or the tumor matrix can influence angiogenesis and tumor growth (Lawler et al., 2001). More recently, the Id1 null mouse, which fails to support the growth of tumor xenografts, has been shown to express increased levels of TSP-1, and Id1 has been shown to function as a repressor of TSP-1 expression (Volpert et al., 2002). Data in human systems has been somewhat equivocal due to the multiple and varied genetic alterations occurring in human malignancies and the difficulties in assessing expression of an extracellular matrix protein in vivo.

The transcriptional regulation of thrombospondin-1 reflects the genetic regulation of tumor cell growth itself. Several oncogenes have been demonstrated to repress TSP-1, including oncogenic ras, c-*myc*, v-src, c-*jun*, and Id1 (Volpert et al., 2002), while the tumor suppressors p53 and PTEN have been shown to activate TSP-1 expression (reviewed in Lawler, 2002). While these studies have provided isolated glimpses into the ways in which TSP-1 can be regulated during tumor formation and the angiogenic switch, no studies have been able to put together the individual pieces of the puzzle to identify the critical pathways that regulate TSP-1 expression during the angiogenic switch until now.

In a series of elegant experiments from Robert Weinberg's laboratory, Watnick and colleagues use a genetically defined tumor model system to evaluate the molecular regulators of the angiogenic switch (reviewed in Hahn and

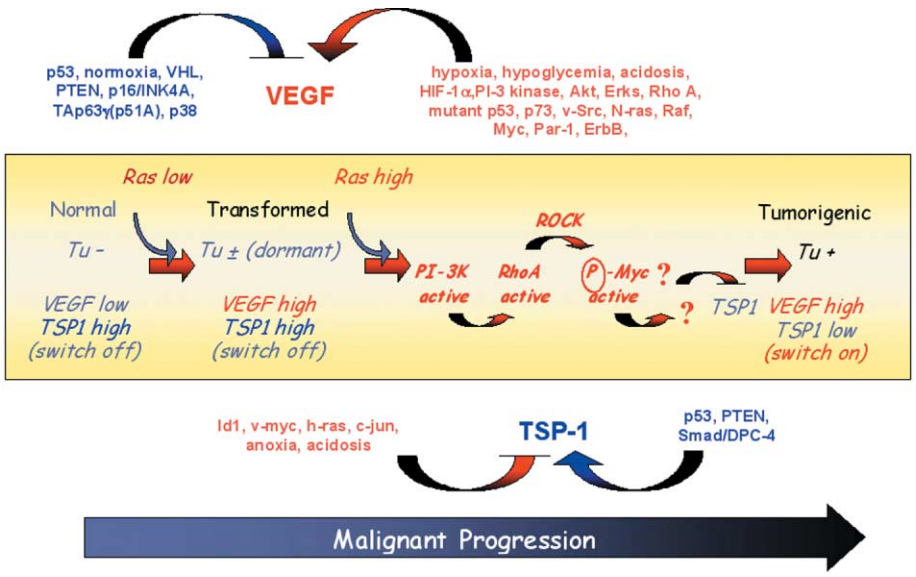

Figure 1. Effectors and mediators of the angiogenic switch driven by Ras as a rheostat

The model established by Watnick et al. (2003) is shown in the center. Normal, nontumorigenic renal cells (Tu–) maintain angiogenic balance in favor of the inhibitory TSP-1. Low levels of ras expression induce transformation, but tumorigenicity of such cells is limited by threshold size of several millimeters (Tu±). Although the levels of proangiogenic VEGF are increased, they are insufficient to overcome antiangiogenic TSP-1 (angiogenic switch in the off position). Further increases in Ras expression have no effect on VEGF levels but instead trigger signaling events that involve PI-3 kinase, RhoA, and its target ROCK. The final known event in the cascade is Myc activation (via phosphorylation), leading to the repression of TSP-1, and the angiogenic switch is "turned on," allowing unlimited tumor expansion (Tu+). Shown outside the box are other factors, both genetic and epigenetic, that modulate the levels of secreted VEGF and TSP-1 and thus the on/off state of the angiogenic switch. Factors that enhance angiogenesis are shown in red, and factors that inhibit angiogenesis are shown in blue.

Weinberg, 2002). Using transformed human embryonic kidney cells and human mammary epithelial cells, these investigators note that the angiogenic switch in tumors derived from these cells is dependent on the level of expression of oncogenic Ras. The authors go on to identify TSP-1 as being the mediator of the angiogenic switch in these tumors and provide evidence for the specific inhibition of TSP-1 expression through a ras-mediated pathway. In order to further define the pathway from ras to TSP-1, the authors introduce a dominant-negative version of c-*myc* into transformed kidney cells with high ras expression and demonstrate that ras-dependent repression of TSP-1 in transformed kidney cells is dependent on c-*myc* function. The authors then go on to show that, in their tumor model system, myc function as a target of Ras signaling and repressor of TSP-1 is dependent upon phosphorylation of the Myc protein itself rather than the absolute expression level of Myc. Myc phosphorylation is then shown to be mediated through ras signaling via phosphatidyl inositol-3 kinase (PI3K). Unexpectedly, PI-3 kinase influences Myc activity not via its conventional target Akt/PKB, but via guanidine-exchange factor RhoA and its mediator ROCK (Figure 1).

The experiments by Watnick et al. in this issue of *Cancer Cell* show that relatively low levels of H-Ras in combination with SV40 early region and hTERT result in the transformed phenotype of primary human breast and kidney epithelial cells. While these cells are able to form tumors in nude mice, they are limited in size due to an inability to induce a vascular supply. Interestingly, these tumor cells expressed high levels of the potent angiogenic inducer VEGF; however, despite increased VEGF levels, these same cells formed only microscopic tumors mimicking dormancy that normally precedes the switch to autonomous tumor angiogenesis. This state of tumor dormancy could be overcome in two distinct ways: by adding exogenous VEGF to the system or by expressing higher levels of oncogenic *ras*. Remarkably, higher Ras levels had no significant effect on VEGF expression, but instead suppressed the production of inhibitory Thrombospondin-1. Although the concept of angiogenic switch has been widely accepted, careful attention has rarely been paid to both variables in the equation, pro- and antiangiogenic, since most investigators tend to focus on one particular pathway. Watnick and colleagues use a genetically defined tumor model to clearly delineate the nature of the angiogenic switch and its role in tumor progression. Taken as a whole, these studies reinforce the critical role for TSP-1 as a major effector of the angiogenic switch and place it squarely within the path of two major tumor promoters, ras and myc.

What are the potential clinical implications of this work? While it is clear that inhibition of angiogenic stimuli should be a major target for cancer therapeutics, this work implies that eliminating repressive effects on protective endogenous inhibitors of angiogenesis may be an alternative means of targeting the angiogenic switch. In addition, developing small molecules that mimic the antiangiogenic functions of TSP-1 or other endogenous inhibitors of angiogenesis may have potential as therapeutic agents. The utility of these agents as a whole is difficult to predict. Although all tumors require a blood supply to get beyond a particular mass, the precise molecules that are tipping the balance to initiate the angiogenic switch are likely to be varied and complex and dependent upon tumor cell type. Only once these therapeutics have been evaluated clinically will we be able to ascertain the relative contribution of regulators of the angiogenic switch to the growth of a particular human malignancy; however, genetically defined tumor model systems like the one used in the above studies provide useful paradigms from which we can identify therapeutic targets and develop therapeutic interventions.

Olga V. Volpert[1] and
Rhoda M. Alani[2,*]

[1]Department of Urology
R.H. Lurie Cancer Center
Northwestern University Medical School
Chicago, Illinois 60611
[2]The Sidney Kimmel Comprehensive
Cancer Center at Johns Hopkins
Johns Hopkins University School
of Medicine
Baltimore, Maryland 21231
[*]E-mail: ralani@jhmi.edu

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