

LETTER TO THE EDITORS

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Oncogenes, p53, and tumor angiogenesis

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Sirs,

The development of newly formed blood vessels is a *conditio sine qua non* for malignant growth and metastasis (see Folkman 1995 for review). The sprouting out of new vessels is regulated by the balance between angiogenetic factors [i.e. vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF)] (Ferrara et al. 1989) and inhibitors of angiogenesis such as thrombospondin 1 (Rastinejad et al. 1989), angiostatin (Gateley et al. 1996) and the recently described endostatin (O'Reilly et al. 1997). The steps that lead to the angiogenetic phenotype are not yet fully understood and they presumably involve a shift in the balance between positive and negative regulators of angiogenesis. It has been proposed that oncogenes and tumor-suppressor genes are involved in the development of the malignant angiogenetic phenotype: dominant transforming oncogenes (such as *ras* and *raf*) might be regarded as pro-angiogenetic since they are associated with the overexpression of VEGF, whereas tumor-suppressor genes, such as *p53* “the guardian of the genome”, exert an opposite effect, down-regulating VEGF (Mukhopadhyay et al. 1995) and up-regulating thrombospondin 1 (Dameron et al. 1994).

These findings might be quite relevant in explaining the molecular mechanisms underlying malignant progression and metastasis. The key role of *p53* in the inhibition of tumor angiogenesis will be discussed and how this effect should be regarded as of primary importance among the other well-known antitumoral effects of *p53*. We also hypothesize how the antiangiogenetic properties of *p53* could open new horizons in the future of cancer therapy.

Dominant oncogenes as inducers of angiogenesis:
a necessary event for tumor growth

Until recently, research on oncogenes and research dealing with tumor angiogenesis were considered as two separate entities; however, it appears that these fields have tight relationships. Tumorigenic competence requires that transformed cells induce a vigorous and sustained angiogenetic response. This phenomenon is critical in solid tumors as they cannot grow more than few millimeters in diameter in the absence of efficient angiogenesis; consistently, failure to sustain angiogenesis results in a latent state of tumor growth that has been defined as “tumor dormancy” (Holmgren et al. 1995).

The first evidence that an activated oncogene contributes to the angiogenetic shift occurring during tumor progression was reported by Thompson et al. in 1989. These authors showed that an activated *v-ras* oncogene, transfected into cells that were subsequently grafted into the subrenal capsule, elicited a powerful angiogenetic response. Dominant oncogenes, such as *ras* and *raf*, could be considered as promoters of angiogenesis because they induce the expression of one of the most powerful angiogenetic factors, VEGF, also known as vascular permeability factor (Senger et al. 1987), a secreted heparin-binding protein. Activated endothelial cells in a growing vessel are the only cells known to express a significant level of the VEGF receptor (a tyrosine kinase receptor also described as FLT-1); because of this peculiarity, VEGF at present is considered the only specific growth factor for angiogenesis. VEGF also causes the accumulation of fibrin in and around blood vessels and this phenomenon strongly favors the sprouting out of new capillaries, thus amplifying the process (Dvorak et al. 1995). Consistent with this pivotal role in tumor growth, VEGF is the target of major dominant oncogenes: Grugel et al. (1995) showed that both *v-H-ras* and *v-raf* induce the expression of VEGF in NIH-3T3 fibroblasts. These authors also demonstrated that *v-raf*-transformed cells produce a significant amount of VEGF, which stimulates the proliferation of

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human endothelial cells. The role of the *ras* oncogene was further highlighted when the mutant K-*ras* gene was knocked out by genetic recombination techniques in transformed cell lines that contained a single copy of the oncogene; the ability to induce solid tumors in nude mice was irreversibly lost (Shirazawa et al. 1993). In addition, *ras* is not only an inducer of the angiogenetic factor VEGF, but also acts as a potent inhibitor of the expression of thrombospondin-1, one of the most efficient inhibitors of angiogenesis (Zabrenetsky et al. 1994).

The VEGF receptor is a transmembrane protein with intrinsic tyrosine kinase activity. Similarly to the platelet-derived growth factor receptor, it has an extracellular immunoglobulin-like domain and a cytoplasmic interkinase domain interspersed between two tyrosine kinase domains. Following binding of the specific ligand, two VEGF receptors dimerize and transphosphorylate at tyrosine residues (Waltherberger et al. 1994). This event is instrumental in recruiting SH₂-domain-containing signalling proteins, such as pp60^{src}, GAP-p21^{ras}, phosphatidylinositol 3-kinase, phospholipase C γ , and p74^{raf}. SH₂ domains have a strong affinity for phosphotyrosine and the interaction between SH₂-domain-containing proteins and proteins phosphorylated at tyrosine is a well-characterized mechanism for molecular recognition. Thus, VEGF causes the tyrosine phosphorylation of phospholipase C γ and of GAP-p21^{ras}. These events lead to activation of mitosis-associated protein (MAP) kinases, which, in turn, leads to the activation of the nuclear transcription factor AP1 (of the *fos-jun* complex) (Burgess and Mamciag 1989) with consequent DNA duplication and cell division and, only in endothelial cells, to the expression of the VEGF gene with further VEGF production. It was demonstrated that the signalling pathway of VEGF is specific for endothelial cells; thus, the product of the *ras* oncogene shows a peculiar dual role uniquely in endothelial cells. It further amplifies the angiogenetic response by inducing VEGF gene expression, at the same time inhibiting the expression of the powerful inhibitor of angiogenesis, thrombospondin 1 (Zabrenetzky et al. 1994).

The oncosuppressor gene *p53* as a negative regulator of tumor angiogenesis: induction of inhibitors and repression of stimulators

Inhibition of angiogenesis has to be considered among the antitumoral properties of the "master tumor-suppressor gene" *p53*. In fibroblasts from Li-Fraumeni patients, which are typically devoid of the wild-type *p53* alleles, the loss of *p53* occurred concomitantly with the loss of expression of thrombospondin 1, a powerful angiogenesis inhibitor, and these two events corresponded to the shift toward the angiogenetic phenotype (Dameron et al. 1994). The link between wild-type *p53* and thrombospondin 1 has to be found in the up-regu-

lation of the thrombospondin 1 gene by *p53*. In Li-Fraumeni fibroblasts, reintroduction of wild-type *p53* restored both thrombospondin 1 gene expression and the antiangiogenetic phenotype. Thrombospondin 1 gene expression and the antiangiogenetic phenotype could also be restored by the stable transfection of a temperature-sensitive mutant of *p53*. Furthermore, the antiangiogenetic phenotype was abolished both in vivo and in vitro by neutralizing antibodies directed against thrombospondin 1. *p53* works through direct transactivation of the thrombospondin 1 gene: consistently, when a reporter gene linked to the human thrombospondin 1 gene promoter at the first intron was cotransfected with temperature-sensitive *p53*, it was expressed only when *p53* was in the wild-type configuration (Volpert et al. 1997).

Beside *p53*, other tumor-suppressor genes contrast the mechanism of angiogenesis. The Von Hippel Lindau tumor-suppressor gene in renal cell carcinoma also inhibits VEGF and produces a similar effect in hemangiomas (Wizigmann-Voos et al. 1995). Thus, as a general rule, dominant transforming oncogenes favor angiogenesis (possibly through direct induction of angiogenetic growth factors), whereas tumor-suppressor genes inhibit angiogenesis either by inducing inhibitors of angiogenesis (such as thrombospondin 1) or by down-regulating the expression of angiogenetic growth factors (Mukohpadhyay et al. 1995).

In addition to thrombospondin 1 and to *p53*-dependent antiangiogenetic factors, other recently described peptides have powerful antiangiogenetic properties; however, it is still unclear whether they are under *p53* control. Some of these peptides are formed by proteolytic cleavage of proteins of the perivascular district, such as angiostatin, which is produced through the action of a serine protease on the plasminogen molecule (Gatley et al. 1996). Another member of this class of peptides is endostatin, a C-terminal product deriving from collagen XVIII; this type of collagen belongs to a group of proteins, referred to as multiplexins, that are found around blood vessels (O'Reilly et al. 1997). Some recent studies point to endostatin as a molecule that could be used in antineoplastic therapy because of its antiangiogenetic properties; in experiments in mice, endostatin considerably reduced the solid neoplastic mass without any appreciable toxic side-effect and, what is more important, without causing the onset of any resistance to the treatment (Boehm et al. 1997). Toxic side-effects and the appearance of drug-resistant clones are the two major limiting factors in conventional anti-neoplastic chemotherapy; thus, the antiangiogenetic-antineoplastic effect of endostatin could lead to the development of a novel generation of drugs "resistant to resistance".

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