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Antiangiogenic Therapy and *p53*

Antiangiogenic therapy is based upon the fact that tumor vasculature forms from genetically stable endothelial cells that grow into the genetically unstable tumor. Endothelial cells represent attractive targets for therapy, because tumor expansion depends on angiogenesis and endothelial cells are less likely to develop drug resistance than the tumor cells that surround them (1, 2). The findings of Yu *et al.* (3) claiming that the status of the *p53* tumor suppressor gene in the cancer can limit the efficacy of antiangiogenic therapy are thus alarming.

The Yu *et al.* finding was based solely on work with the HCT116 *p53*^{+/+} and *p53*^{-/-} cell lines. Although these lines have found extensive use in many elegant genetic studies (4), we question their use in this one. Yu *et al.* showed that, before antiangiogenic treatment, tumors derived from either cell line grew at similar rates [figure 1A of (3)]; however, if hypoxia-induced apoptosis were indeed more significant in the *p53*^{+/+} cell line than in *p53*^{-/-} cells, as they suggest, tumors, derived from the *p53*^{+/+} line would be expected to grow slower than the *p53*^{-/-} tumors, irrespective of treatment status. Previous work (5) has shown that HCT116 cells do not exhibit differences in apoptosis between oxic and hypoxic regions in transplanted tumors. The main conclusions of the work reported by Yu *et al.* are based on the data shown in figures 1A and 2 in (3), yet the error bars shown suggest that the findings are, perhaps, not as significant as the authors claim. The HCT 116 matched pair has also been used to demonstrate that the hypoxia-inducible factor-1 α (HIF-1 α) response, including induction of vascular endothelial growth factor (VEGF), is amplified in *p53*^{-/-} cells, which suggests that the vasculature in tumors derived from these cells may be more extensive than in tumors derived from *p53*^{+/+} cells (6).

At present, a variety of clinical studies have already been initiated or are planned that incorporate either antibody therapy targeting the VEGF receptor (VEGFR) or small-molecule inhibitors of VEGFR in the treatment of solid tumors. The findings of Yu *et al.* could have significant impact on the way these trials are designed and developed as well as on the analysis of the results. We would suggest that, before that occurs, the phenomenon described by Yu *et al.* requires further validation. Other matched cell lines for *p53* or means of inducing *p53* into a *p53*-null background exist. The findings of Yu *et al.* may indeed be significant, but more data are needed before their relevance to antiangiogenic therapy can be considered.

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Yu *et al.* (1) have provided an insightful report—but unfortunately, although the data are clear, they have been widely misinterpreted to imply that antiangiogenic therapy will eventually fail because of acquired resistance. How could such straightforward results be so profoundly misinterpreted?

The answer appears to lie in the fact that a reduced tumor response to antiangiogenic therapy is not the same as resistance to conventional chemotherapy. For example, although *p53*^{+/+} tumors responded well to antiangiogenic therapy, the growth of *p53*^{-/-} tumors was also clearly inhibited compared to untreated controls [figure 1 of (1)]; that is, both types of tumors did indeed respond to antiangiogenic therapy. That the *p53*^{-/-} tumor cells were less responsive to antiangiogenic therapy does not mean they were resistant. Indeed, the result presented by Yu *et al.* is consistent with numerous observations, described at length in the literature, that different animal tumors have different sensitivities to antiangiogenic agents.

Why would the responses to antiangiogenic therapy differ among tumors? First, the tumor's angiogenic output is the net balance of positive and negative regulators of neovascularization, a balance that differs among tumors and that may change as a tumor mutates or progresses. Some human breast cancers, for example, when first diagnosed release only VEGF, while subsequent recurrences commonly release additional pro-angiogenic proteins such as basic fibroblast growth factor (bFGF) and transforming growth factor- β (TGF- β) (2). Second, wild-type *p53* normally suppresses tumor angiogenesis by at least four known mechanisms: (i) up-regulation of thrombospondin-1 (3); (ii) degradation of HIF-1 α (4); (iii) transcriptional suppression of VEGF expression (5); and (iv) down-regulation of bFGF-binding protein expression (6).

These findings show that loss of *p53* results in increased tumor neovascularization. This is not resistance. The increased angiogenic effect

of mutated *p53* could presumably be offset by administering higher doses of antiangiogenic therapy or combinations of angiogenesis inhibitors. For example, in a *p53*-mutated human pancreatic cancer (7) in mice, there was dose-dependent response to a single angiogenesis inhibitor, varying from 33% inhibition to 97% inhibition of tumor growth with regression (8). Efficacy with angiogenesis inhibitors against RIP-Tag pancreatic islet carcinomas in which *p53* is functionally inactivated has also been reported (9). Because wild-type *p53* suppresses the expression of bFGF-binding protein (6), the DC101 antibody used by Yu *et al.* (1), which blocks only VEGFR-2, would not effectively target the increased bFGF signalling induced by loss of *p53*. Indeed, Klement *et al.* have already demonstrated the responsiveness of tumors lacking functional *p53* (10) to this antiangiogenic therapy (vinblastine plus DC101) (11).

The field of antiangiogenic therapy, for all the excitement it has generated, remains in its infancy. As the Yu *et al.* study indicates, tumors have different sensitivities to antiangiogenic agents, and these differences merit further investigation. Does this mean that antiangiogenic agents will ultimately run up against an insurmountable wall of resistance? This is unlikely, for two important reasons. First, as originally pointed out by Kerbel (12), genetically stable endothelial cells, in contrast to mutating tumor cells, retain their normal cellular controls. This important difference between normal and cancerous cells is illustrated by the clinical observation that, unlike tumors, normal tissues such as bone marrow and gastrointestinal mucosa never become resistant to chemotherapy (13). Second, as the Yu *et al.* study demonstrated (1), although tumors may vary in their sensitivity to hypoxia, their growth and progression remain angiogenesis-dependent despite their genetic instability, and they cannot survive in anoxic conditions. In other words, some tumors may be able to hold their breath longer than others, but none can hold their breath indefinitely.

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Response: Although we appreciate some of their comments, Hammond and Giaccia seem to have missed the major point of our study: that genetic mutations in cancer cells can have a significant impact on response to antiangiogenic therapies, even though the target of such drugs is the genetically stable host endothelial cell. In the model we used, *p53* inactivation was the genetic change implicated. Does that mean that tumors with *p53* mutations will always be less responsive or “resistant” to antiangiogenic therapy? That is highly unlikely. As noted in the comment by Browder *et al.*, the *p53*^{-/-} mutant tumor cells still responded significantly to the antiangiogenic therapy we used—though clearly not as well as their otherwise genetically identical *p53*^{+/+} counterparts (1). That may have been due to increased angiogenic proficiency in the *p53*^{-/-} cells, but that does not negate the main point and conclusion of our study. Moreover, although it is well known that *p53* status can lead to chemotherapy resistance, that relationship is quite variable and inconsistent (2) because of confounding genetic and epigenetic factors in the background of the tumor cells—which is exactly why we used the genetically matched *p53*^{+/+}/*p53*^{-/-} HCT116 system—and because of various microenvironment and host factors. These variables make it extraordinarily difficult to exploit *p53* status as a reliable predictor of chemotherapy response; the same would be true for antiangiogenic therapy. Hence, it is indeed quite premature to consider incorporating screens of *p53* status into the design of antiangiogenic drug clinical trials.

As for some of the technical concerns raised by Hammond and Giaccia, we note the following: (i) The differences we observed in the growth of the cell lines and their response to therapy in SCID mice were significant and reproducible. (ii) A close examination of the results of the Ravi *et al.* study cited by Hammond and Giaccia

shows that relatively small numbers of cells were injected in their studies— 2.5×10^4 to 2.5×10^5 cells [figure 1B in (3)], which is close to the threshold of tumor take—whereas we used much larger cell numbers. It was solely this difference in tumor take, or latency, that was responsible for the increased growth of the *p53*^{-/-} cells in the study of Ravi *et al.* Subsequent tumor growth rates (i.e., slopes) were identical, consistent with our own results (1). (iii) Our results showing colocalization of hypoxia, apoptosis, and *p53* are consistent with previously published studies from Giaccia’s lab using transplantable mouse systems (4), but are inconsistent with this group’s more recent results using the HCT116 human tumor xenograft system (5). We cannot at present explain the inconsistency of our results with their human tumor results, but the differences could stem from technical differences such as different mouse strains and tumor sizes.

In sum, our studies showed that cancer cell genetic mutations can, in principle, diminish responsiveness to antiangiogenic therapy. This could be one of several factors explaining the limited benefits obtained thus far in antiangiogenic therapy-based clinical trials undertaken in advanced-stage incurable cancer patients, in contrast to the striking results in treating large, life-threatening, or disfiguring (but benign) tumors such as hemangiomas and giant tumors of the mandible (6, 7), although that remains to be proven. The results also suggest a number of ways to improve and prolong the efficacy of antiangiogenic therapies—which we consider reason for optimism, not alarm.

We share the concern of Browder *et al.* that some aspects of the Yu *et al.* study have been misinterpreted. They correctly highlight the good response achieved when the *p53*-inactivated tumor cells we tested were exposed to an antiangiogenic therapy. Indeed, the effect we observed was rather typical of many antiangiogenic compounds in tumor-bearing-mice—growth delay, but not stabilization or regression of tumors. Thus, the results should not be interpreted to claim that *p53*-mutant tumors will be intrinsically “resistant” to antiangiogenic therapy.

The results do suggest, in our opinion, that such tumors (or tumors with other kinds of genetic mutation) may be able to adapt more quickly to such a therapy and become less responsive, or perhaps even nonresponsive, more quickly over time. But it should be pointed out that, just as *p53* mutations are known to contribute in a general way to resistance to chemotherapeutic drugs (2), there are many exceptions, some of which undoubtedly stem from the genetic background of the tumor cell pop-

ulation (2), as discussed above. Thus, as with chemotherapy, we should not expect that tumors with *p53* mutations will always be less responsive to antiangiogenic therapies. In some cases, as Browder *et al.* note, such tumors may even be hyperresponsive. Our results simply showed that tumor cell genetic instabilities or mutations, which in some cases may include *p53*, can have a significant negative impact on response over time to an antiangiogenic therapy—which is contrary to current dogma. If the hypothesis we put forward about selection of less vascular-dependent tumor cells that are hypoxia resistant is correct (8), our results should also apply to direct angiogenesis inhibitors as well as to indirect inhibitors. Supporting or refuting that hypothesis, however, will require additional experiments.

Perhaps in contrast to Browder *et al.*, however, we still find it legitimate to use the word “resistance” in the context of our results, especially in light of the selection/overgrowth results that we presented [figure 2 in (1)]. When the tumors of patients stop responding to a particular chemotherapeutic drug, they may respond again to another chemotherapeutic drug (second-line therapy). Does that mean that the term “resistance” should not be used in conjunction with the first line therapy used? If it were more readily appreciated that resistance is not necessarily absolute, that it can be delayed or even reversed—as shown so elegantly by Browder *et al.* in a recent preclinical study involving cyclophosphamide (9)—perhaps the term “resistance” would not be so frightening or evocative. Notwithstanding the work of Yu *et al.* (1), we still hold to the view that antiangiogenic therapy has the greatest promise to deal with the problem of drug resistance—but that does not mean it will be completely “resistance free.”

Cancer cells possess an awesome arsenal of genetic mutation-driven mechanisms to evade the effects of cancer cell-directed therapeutics, from drug efflux pumps to enhanced DNA repair to hypoxia and apoptosis resistance. We believe that the spectrum of such mechanisms will be much narrower for antiangiogenic drugs (especially direct-acting inhibitors) and that this will result in much longer term responses and, hence, greater prolongation of survival. Time will tell whether this view is correct.

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