Tumor Endothelial Cells Join the Resistance

Commentary on Xiong et al. p. 4838

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The field of antiangiogenesis research has been met with some surprises, including the realization that tumor blood vessels are more complex and labile than expected. In this issue of Clinical Cancer Research, Xiong and colleagues show that tumor-specific endothelial cells are less sensitive to cytotoxic and antiangiogenic drugs compared to their normal counterparts.

Perspective

In this issue of Clinical Cancer Research, Xiong and colleagues report that isolated tumor-specific endothelial cells (TECs) from human hepatocellular carcinoma (HCC) are less sensitive to cytotoxic and antiangiogenic drugs when compared to their normal counterparts in vitro (1). The results of this study are in good accord with the growing body of in vivo evidence and clinical data suggesting that, in contrast to dogma, TECs may acquire drug resistance or be less sensitive to antiangiogenic strategies compared to normal endothelial cells (NECs).

Due to genomic instability and a high mutation rate, tumor cells are mutable and may become drug resistant over time. It was therefore proposed over 30 years ago by Folkman that targeting the tumor-associated endothelium, which provides blood and nutrients to growing tumor cells, could be an alternative strategy for eliminating solid tumors (2). Almost a half century of angiogenesis research has produced several ground-breaking antiangiogenic therapies that are now used for treating some cancers and other angiogenesis-dependent diseases, including age-related macular degeneration (3). As the prototype of successful bench-to-bedside investigation, the anti-VEGF (vascular endothelial growth factor) monoclonal antibody Bevacizumab (Avastin) is approved by the FDA for treating colon, breast, and lung cancers in combination with chemotherapy.

When Folkman postulated that tumors could be shrunk by targeting the blood vessels feeding them, it was assumed that the tumor endothelium was “normal” and was unlikely to evade antiangiogenic therapies. However, a number of studies have now documented changes at the morphologic and molecular levels in TECs from a variety of tumors (4), and clinical studies seem to support the possibility that TECs may become refractory to antiangiogenic therapy over time (particularly to anti-VEGF therapies) (ref. 5). Perhaps more disconcerting, some antiangiogenic therapies have recently been reported to unexpectedly facilitate metastasis in preclinical studies (6, 7). Why are antiangiogenic therapies not producing the sustained anti-tumor benefit as hoped? A two-tiered model of resistance to antiangiogenic therapies was recently put forth (8). First, TECs may develop “evasive” resistance by adapting to a specific angiogenesis inhibitor, for example by upregulating compensatory cellular survival pathways in response to anti-VEGF treatment. Second, inherent differences in TECs compared to their normal counterparts might impinge on the effectiveness of antiangiogenic therapies. These inherent differences may come about due to acquired alterations, perhaps as TECs evolve in the face of microenvironmental stress created by the growing mass of tumor cells.

It has been challenging to address specific questions about TEC biology because these cells are difficult to isolate and culture. But as Xiong and colleagues have done, one approach is to use antibody-coupled magnetic beads to isolate ECs from collagenase-digested tumors and counterpart tissues (Fig. 1). Using this technique, Xiong and colleagues identified inherent differences in TECs from HCC compared to their counterparts from normal liver. The authors determined that compared to NECs, TECs isolated from HCC were less sensitive to adriamycin, 5-fluoruracil, and Sorafenib (an inhibitor of VEGFR-2, PDGFR, and c-Kit) when cultured and treated with each drug ex vivo. Because drug resistance usually implies activation of compensatory pathways after inhibition of a specific pathway, these findings do not necessarily mean that TECs have developed drug resistance by the textbook definition; instead, the author’s results suggest that TECs are inherently less sensitive to these drugs even without prior treatment. Therefore, whatever changes in TECs that resulted in their decreased sensitivity were already present. Our laboratory (9) and others (10) have shown similar differences in drug sensitivity when TECs and other tumor stromal cells were compared to their normal counterparts in vitro. It may be that common pathways (e.g., p53) known to mediate cellular responses to chemotherapies are defective both in the tumor stromal cells and in the tumor cells themselves (11). Sorafenib is currently approved by the FDA for the treatment of HCC, and, if feasible, it would be informative if the authors were to isolate TECs from patients with HCC after Sorafenib treatment. In that way, whether TECs from these patients acquire “evasive” resistance, perhaps by upregulating compensatory cellular survival pathways, could be determined.
An advantage of isolating and obtaining pure cultures of TECs is that cellular signaling pathways can be analyzed and functional assays can be carried out in vitro. This is contrasted with gene-expression studies using only the RNA extracted from TECs that were never cultured (12). Xiong and colleagues put their cultured TECs to good use and compared the functional differences between NECs and TECs by using several “standard” in vitro angiogenesis assays. For example, the authors report that TECs show increased migration and proliferation with serum as well as decreased apoptosis without serum compared to NECs. Furthermore, in contrast to NECs, Sorafenib-treated TECs persistently formed tubes in matrigel and “sprouts” when cultured as spheroids. To elucidate which intracellular pathways could account for TECs’ decreased sensitivity to Sorafenib, the authors used western blotting to probe for proteins that might be downstream, including the phosphorylated forms of STAT3, Akt, and MAPK. Although there were subtle differences in the phosphorylation of these proteins when comparing NECs and TECs, it is unclear what specific role these factors might play in mediating decreased sensitivity to Sorafenib. Because the authors have already isolated TECs in culture, it should be relatively straightforward to use a siRNA approach to knock down these specific factors and then ask questions about the role of each factor in mediating decreased sensitivity to Sorafenib, or any other antiangiogenic or cytotoxic therapy.

The possibility that TECs might be refractory to antiangiogenic therapies is a pressing clinical question. The attractiveness of an antiangiogenesis approach in cancer was that tumors could be shrunk or maintained in a dormant state without the possibility of acquired drug resistance and without the toxic side effects of conventional chemotherapies. On one hand, antiangiogenic therapies such as Lucentis (a Fab fragment derived from the same parent molecule as Bevacizumab) have produced “miraculous” results in patients with macular degeneration, a disease also characterized by pathologic angiogenesis (13). On the other hand, Bevacizumab has produced mixed results in patients with solid tumors, with some indication that tumors may ultimately rebound or not respond at all. This
differential response in the endothelium from two angiogenesis-dependent diseases may be a priori evidence that TEC biology is more complex than previously thought. Taking advantage of cell separation methodologies to isolate and obtain pure cultures of TECs that can be characterized in vitro should go a long way toward a better understanding of how and why these cells might be less sensitive or even resistant to antiangiogenic strategies.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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