Tumor Endothelial Cells Join the Resistance

Commentary on Xiong et al. p. 4838

Andrew C. Dudley¹,² and Michael Klagsbrun¹,²,³

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 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 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.

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

**Fig. 1.** Xiong et al. used antibody-coupled immunomagnetic separation to isolate normal liver endothelial cells and tumor-specific endothelial cells from human hepatocellular carcinoma.
differential response in the endothelium from two angiogenesis-dependent diseases may be \textit{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 \textit{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.

\section*{References}

\section*{Disclosure of Potential Conflicts of Interest}
No potential conflicts of interest were disclosed.

\section*{Acknowledgments}
A.C. Dudley wishes to thank the American Cancer Society for funding his research with a post-doctoral fellowship.
Tumor Endothelial Cells Join the Resistance

Andrew C. Dudley and Michael Klagsbrun


Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-09-0902

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2009/09/03/1078-0432.CCR-09-0902.DC1

Cited articles
This article cites 12 articles, 3 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/15/15/4787.full#ref-list-1

Citing articles
This article has been cited by 3 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/15/15/4787.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.