Commentary

Tumor Plasticity Allows Vasculogenic Mimicry, a Novel Form of Angiogenic Switch

A Rose by Any Other Name?

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Tumors require a blood supply to sustain growth and to metastasize.¹ They initially co-opt existing vessels to survive and grow,² but the concept that they eventually send signals for new blood vessels to sprout (angiogenesis) is widely accepted as the mechanism by which most tumors metastasize. The literature on this subject is immense and growing. The purpose of the present commentary is not to review the literature on angiogenesis. Indeed, the findings presented by Maniotis and coworkers in this issue of *The American Journal of Pathology* are both novel and unexpected, and deal with a phenomenon that is best characterized as "vasculogenic mimicry."³ As such, the results may have implications beyond angiogenesis. These authors suggest a new mechanism by which some aggressive tumors may acquire a blood supply: the tumor cells themselves literally metamorphose into vessels that either carry blood or connect to the host's blood supply! The methods used by the investigators involve an unusual but necessary combination of patient data and biopsies, histology, electron microscopy, culture experiments under physiological conditions using both nonaggressive and highly aggressive melanoma cells, biomechanical assays, and cDNA microarray analysis. In essence, these findings add a new layer of complexity to the accepted paradigm for the generation of tumor microcirculation and should be taken seriously by the cancer research community as well as the oncologists who treat cancer patients. As with all other unexpected findings, it is of course necessary that the observations be repeated by other laboratories and the concepts confirmed.

In the University of Iowa's Departments of Pathology and Ophthalmology, Robert Folberg and his colleagues have intensively studied intratumoral patterns of microcirculatory vessels that they had first characterized in aggressive uveal (ocular) melanomas.⁴ Folberg and associates had reported a study of 234 patients whose eyes

had been removed for uveal melanoma, and had shown that the histologic detection of parallel vessels with cross-linking loops and networks significantly separated those patients who survived long-term from those who died of metastatic melanoma.⁵ A Cox proportional hazards model showed that the presence of periodic acid Schiff (PAS)-positive networks, observed histologically, had the strongest association with outcome of all tumor characteristics studied. In another study from the same group,⁶ PAS-positive patterns were studied in uveal nevi and the investigators reported that patients whose melanomas contained the nevus-like microcirculatory profile had a significantly more favorable outcome than patients whose tumors contained the invasive and metastasis-associated patterns. The observations linking the histological presence of loops and networks with death from metastatic melanoma have been confirmed by independent laboratories.⁵⁻⁷ Indeed, the histological detection of these patterns is highly reproducible among the different laboratories. These patterns have been identified in foci of metastatic melanoma regardless of the target organ.⁸

Maniotis, Folberg, Hendrix, and their colleagues had reasoned that because there are no lymphatics within the eye and because uveal melanomas must by necessity disseminate via blood,^{11,12} this tumor system might be useful in understanding the tumor angiogenic switch during malignancy by subtracting the influence of lymphatics from the process of angiogenesis. Unexpectedly, however, they found that endothelial cells (or fibroblasts) were not necessary to generate microvascular tubes in the aggressive melanoma cell cultures. Instead, they observed a striking correspondence between the patterned vessels generated by aggressive tumor cells in culture and the patterned microcirculation that Folberg and coworkers had previously associated with the clinical outcome of death from metastatic melanoma.⁵

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Maniotis et al demonstrate here³ that the microcirculation of highly aggressive uveal melanomas differs from classically described angiogenesis in two major respects. First, the patterned vascular channels are not lined by endothelium, nor are endothelial cells embedded in the channel-associated acellular matrix (Figure 2 of Ref. 3). Second, the melanoma microcirculation is highly patterned into loops and networks whereas angiogenic vessels are not patterned in two-dimensional histological sections (Figure 3, A and B, of Ref. 3). An examination of Figure 1A in their paper shows a whole mount of a tumor measuring at least 10 mm in diameter with no evidence of necrosis. Clearly, there had to be a blood supply to allow this tumor to grow and kill the patient, but the authors include histological evidence that classic tumor angiogenesis was absent in the interior of the aggressive uveal melanomas (Figure 3, C-E, of Ref. 3). Instead, the vascular channels were found to be lined externally by melanoma cells themselves as demonstrated by light microscopy (Figure 2D of Ref. 3), transmission electron microscopy (Figure 2, A-C, of Ref. 3), and immunohistochemistry (Figure 3L of Ref. 3). If these observations were restricted to uveal melanoma, a rather rare tumor, the results would be still exciting but there would be limited clinical application. However, the authors also illustrate these patterns histologically in human tissue samples of cutaneous melanoma and raise the possibility that tumor cell-generated patterned microcirculation may exist in tumors from other tissues as well (see below). The angiogenic switch therefore could be defined both by tumor cells' ability to turn on the host's blood vessels at a given juncture, as defined by Folkman,¹ but also by some other change in aggressive tumor cells that would allow them to turn themselves into vessels that could provide microcirculation, thus the term "vasculogenic mimicry."

Because so little is known about the mechanism by which capillary tubules are maintained in a tumor microenvironment, the cell culture studies in this paper are a crucial adjunct to the findings *in vivo*. The studies demonstrate convincingly that highly aggressive uveal and cutaneous melanoma cells generate a patterned microcirculation in culture that is composed of tubes of acellular extracellular matrix (ECM) lined on the outside by the tumor cells. Furthermore, normal melanocytes, as well as poorly invasive melanomas, do not appear to be capable of generating these patterns even under the influence of soluble factors and hypoxic conditions that have been implicated indirectly in the pathogenesis of tumor angiogenesis. The authors state that although aggressive cells could generate tubes under appropriate culture conditions, non-aggressive cells were never observed to form such tubes or to constrict matrices under similar conditions. Thus, by the time the melanoma cells were able to phenotypically interconvert (defined as cells that express both mesenchymal and epithelial characteristics, a phenomenon extensively studied by Hendrix and her coworkers previously¹³⁻¹⁸), they were able to simulate a biomechanical behavior reminiscent of primitive endothelial cells.

Apropos of the above, in an ingenious study, Maniotis and his colleagues had established previously with endothelial cells that their shape requirements in the context of growth and limited cell detachment typical of endothelial cell differentiation may be mediated by the mechanical properties of a continuous cytomatrix. The latter would extend between the endothelial cells adhesion receptors and its nucleus and could determine the response of the cell to its changing architectural environment via a hard-wired signaling mechanism.¹⁹ This idea of an extended ECM/cytomatrix/nuclear matrix as a unit of structure and function, originally suggested in one form or the other by Coffey and his colleagues,²⁰ Bissell et al,²¹ and Ingber and Jamieson,²² has been explored now in many systems, and has been validated for a number of cell types including mammary epithelial cells²³⁻²⁵ and endothelial cells.²⁶⁻²⁸ Two points are worth noting, however: first, whereas the tubular structures formed in culture by highly invasive, interconverted primary and metastatic uveal melanoma cells have similarities to cords or tessellations generated by a variety of other cells on appropriate substrata, the mechanisms by which they are formed may be different. A number of cell types, including endothelial cells, can generate tractional forces on appropriate matrices, and cord-like tessellated structures are thought to form because of a limited degree of cell-ECM attachment and a high degree of cell-cell attachment, as postulated originally by Ingber and Folkman.²⁹ But the invasive and metastatic uveal melanoma cell-generated channels appear to consist of a tubular network embedded in the underlying monolayer of tumor cells rather than a tessellation raised above the monolayer (compare Figure 5, A and B with Figure 5C of Ref. 3); these channels appear to evolve dynamically, and anastomose within the monolayer over a 3-day to 3-week period. By contrast, tessellated networking cords made from normal endothelium form quickly after the first 12 to 18 hours of culturing on the surface of the monolayer or on beads. Second, although the aggressive cells also generate cords similar to endothelium within the first hours of culturing, the patterned tubes they generate appear to be completely acellular, can conduct dyes, and resemble with great fidelity the dimensions and patterning of networks observed in human tumors. Although it is known that cells can generate network patterns in a culture dish through specific biomechanical mechanisms involving cell traction, it has not been established before this study that the network patterns observed within aggressive human tumors *in vivo* may originate by such mechanisms. This continuous tumor-ECM interaction and traction consequently could contribute to tumor remodeling, metastatic potential, and vasculogenically patterned tumor-derived vessels in humans.

This study is timely because of some recent findings in other systems that are hard to interpret within the framework of the classical concepts of angiogenesis. In one of the best developed models of angiogenesis, that of the Rip-Tag2 mouse model developed by the combined talents of Hanahan and Folkman groups, there is some surprising behavior of tumor cells that may be reminiscent of the results and ideas presented here by Maniotis

et al. Bergers et al,³⁰ studying the pancreatic islet cell carcinoma metastasis, stated: "the observation that blood vessel density was not decreased [in the intervention and regression trials] (as shown in Figure 2 of their paper) supports the notion that some tumor cells can grow, or at least remain intact, in the face of treatment with angiogenesis inhibitors" That angiogenesis inhibitors, alone or in combination, did not prevent progression to the invasive carcinoma in these studies may support the notion that a tumor microcirculation not lined by endothelial cells could possibly play a physiological role in the maintenance and growth of other aggressive tumors in addition to melanomas. Although this possibility should be considered, it clearly would require much additional study to prove.

The mechanisms underlying embryonic pluripotentiality are unknown. In this study, the collaborative work with Meltzer and Trent at the Cancer Genetics Branch, National Human Genome Research Institute (NHGRI) at the NIH revealed interesting data derived from microarray analysis which links the deregulated gene expression and the interconverted phenotype of uveal melanoma cells. Examples of some of the unexpected genes expressed by these tumor cells include tyrosine kinase with immunoglobulin EGF factor homology domains-1 (TIE-1), an endothelial receptor kinase thought to be involved in endothelial vessel formation and maturation, urokinase type plasminogen activator, epithelial cell kinase, and keratin 8, an epithelial intermediate filament marker. The presence of these genes collectively support the notion that the highly invasive melanoma cells express a deregulated yet pluripotent phenotype. It is also interesting to note that these same cells simultaneously expressed a series of genes capable of generating biological molecules necessary to form microvascular channels, including connective tissue growth factor and ECM-associated fibrillin, collagens I and VI, and fibronectin. Furthermore, the deregulated, aggressive cells underexpressed myosin light chain kinase, which is an important regulator of actomyosin interactions. This finding was validated in culture by the reversible inhibition of gel contraction with cytochalasin D. The involvement of the actin cytoskeleton suggests that perhaps the matrix-remodeling potential of these aggressive tumor cells is not only mediated by degradative enzymes, but also depends in part on a tension-generating machinery within these cells, mechanisms that Maniotis and his colleagues had studied previously.^{19,31,32}

The phenotypic versatility of the aggressive melanomas in this study is reminiscent of recent findings in our laboratory wherein malignant human breast epithelial tumor cells, which otherwise form a disorganized mass in a three-dimensional basement membrane assay, are shown to undergo phenotypic reversion to form acini resembling normal breast. Reversion can occur simply by manipulating their surface receptors in the 3-D assay.^{33,34} In the studies reported by Maniotis et al, the tumor cells masquerade as a different tissue to gain their end: that of the demise of the host. In our studies, the tumor cells stop growing and appear normal, a process that may play a role in tumor dormancy. Both processes

speak to the clever ploys tumor cells can employ to escape elimination by conventional chemotherapy. But these results also point to the enormous potential of both normal (witness Dolly, the cloned sheep) and malignant cells to reorganizing their genome to create new forms and tissues if appropriate microenvironmental controls are present. There is now ample evidence to suggest that terminal differentiation may indeed be a perception rather than a reality, a possibility raised almost two decades ago.³⁵

The clinical implications of the work by Maniotis et al³ are far-reaching for the practice of surgical pathology, the development of non-invasive imaging of tumors, and cancer therapy. Many pathologists who study human neoplasms have attempted to quantify the degree of angiogenesis by recording the density of microvessels within tumors and to relate this index to tumor progression.³⁶ There is much debate in the pathology literature about the best way to demonstrate tumor microcirculation in tissue sections. However, this entire issue needs reexamination if the tumor microcirculation is not always lined by endothelium. It is of paramount importance to the clinical oncology community to consider that if tumor cells, rather than endothelial cells, form and maintain intratumoral vessels in aggressive melanomas, then cytotoxic or cytostatic agents directed specifically toward endothelial cells at these clinically detectable stages of tumor progression would not inhibit the growth and progression of these tumors, nor would they block the growth of metastases. On the other hand, if tumor cells mimic endothelial cells, it may be possible to find common inhibitors to combat both the angiogenic switch and the vasculogenic mimicry.

The high association between the presence of the patterned tumor cell-generated microcirculation and clinical outcome makes it possible to design diagnostic tests that may allow physicians to assign a biological grade to the tumors without sampling the tissue. The design of a non-invasive substitute for biopsy is, of course, critical for a tumor such as uveal melanoma that develops within the eye, because it is difficult to remove tumor tissue for biopsy without risking loss of the patient's vision.^{11,12} Maniotis et al show a striking correlation between angiographic demonstration of microcirculatory networks and the corresponding histology from the same area of the tumor (Figure 1, G and H, of Ref. 3). The generation of patterned microvascular channels by aggressive tumor cells may therefore provide new biological markers for demonstrating tumor progression both in biopsy samples and by *in vivo* imaging. As the authors point out, there is significant progress in the detection of tumor remodeling associated with tumor cell-generated patterned microcirculation by the non-invasive ultrasound parameter imaging technique.³⁷ We may also hope for design of additional therapeutic interventions using these novel end points.

In summary, these novel biological observations shed light on the long-standing and detailed studies of the pathology of human melanoma performed by Folberg and colleagues,^{4-6,10,11,38-47} the studies of interconverted melanoma phenotype of Hendrix and cowork-

ers,^{13–18} and the biomechanical properties of cells studied by Maniotis et al.^{19,31,32,48–50} Furthermore, the human melanoma vascular channel formation work sheds new light on our understanding of tumor perfusion. Folkman is correct: even when lymphatics are removed from the picture, tumors require a microcirculation for sustained growth, invasion, and metastasis, and, as originally proposed, the complex angiogenic switch may depend more on tumor-ECM interactions than on soluble factor stimulation.²⁹ However, in aggressive melanomas and perhaps in other tumors, there may yet be a different mechanism that provides a microvascular supply to the tumors.

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Note added in proof

It has been brought to my attention recently that ATCC has discovered that a cell line ECV-304, which was presumed to be an endothelial cell line (it expresses factor VIII, forms tubules on Matrigel and contains Weibel-Palade bodies), is indeed a derivative of a human bladder tumor cell line, T24. This may be yet another example of the phenomenon described by Maniotis et al.³

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