Commentary

Mig-7 Linked to Vasculogenic Mimicry

Gavin P. Robertson

From the Departments of Pharmacology, Pathology, and Dermatology, The Pennsylvania State University College of Medicine, Hershey; and The Foreman Foundation for Melanoma Research and the Penn State Melanoma Therapeutics Program, Hershey, Pennsylvania

Solid tumor development consists of a series of stages during which cells undergo genetic and phenotypic alterations that allow cancer cells to evade normal regulation and to finally colonize distant sites in the body. In this issue of *The American Journal of Pathology*, Petty et al report that expression of a relatively understudied protein called migration-inducing protein 7 (MIG7 or Mig-7) facilitates tumor cell dissemination by increasing invasion and promoting the process of vasculogenic mimicry.¹ Vessels that form during tumorigenesis are usually lined by an endothelium, but abnormal vessel development in which cancer cells directly line vessels or blood channels within tumors have been described and termed vasculogenic mimicry.^{2–5} One of the earliest descriptions of these tumor-lined vasculogenic channels was by Willis, who stated in 1948 that “in rapidly growing tumors, [vessels] consist of little more than irregular channels lined by endothelium only or by naked tumor cells.”² These structures have also been noted by vascular biologists as a category of blood vessel or “blood channels lacking an endothelial lining” in which “blood percolates around and between tumor cell chords” that occur in tumors.³ Renewed interest in these structures occurred after publication of a manuscript in 1999 by a group led by Mary Hendrix, which reported tumor lined-blood channels in areas of looping extracellular matrix in uveal and cutaneous melanomas.⁴ Since then, the slow process of unraveling the genes, signaling pathways, and pathophysiological significance of these structures has been underway.^{6–10} This remains a very important area of research with significant implication for therapeutic development.^{11,12}

Tumor growth is limited by how rapidly the vasculature can develop to provide the growing cancer cell mass with adequate nutrition and removal of toxic waste products.¹³ Angiogenesis occurs early during tumorigenesis to fulfill these needs, but as tumors become larger and

enter the exponential phase of growth, angiogenesis alone may be insufficient to meet the increasing vascular needs.¹³ These tumors could then be forced to develop strategies to enhance the development of a vasculature capable of supporting continued and rapid tumor growth that include the secretion of factors to enhance endothelial cell growth or additional proteinases to increase degradation of the extracellular matrix.¹³ The development of an adjunct vascular system to enhance the tumor blood network, such as one lined directly by tumor cells that connects and communicates with the endothelial-lined vasculature, is one possibility. There is no doubt that tumor cell-lined vessels or channels exist and that the genes as well as signaling pathways regulating development of these structures are slowly being unraveled.^{1,6–10} Petty et al provide significant insight into this process by demonstrating that the Mig-7 protein plays an important role in regulating vasculogenic mimicry and facilitating metastatic spread through increased invasion.¹ However, the complete pathophysiological significance of these structures in the tumorigenic process remains to be fully elucidated in animal models.¹⁴ It is predicted that these structures may serve as an adjunct to the existing vasculature system, thereby aiding tumor growth as well as contributing to the metastatic process; however, this possibility remains controversial.^{14,15}

Mig-7, which is the focus of the Petty et al article, is a cysteine-rich protein found in cell membranes and cytoplasm of carcinoma cells.^{16,17} Previous studies have already shown that tumor tissue and blood from over 200 cancer patients express Mig-7, regardless of tissue origin.¹⁶ In contrast, it was not detectable in tissues or blood from 25 normal subjects.¹⁶ Thus, Mig-7 seems to be a promising cancer cell marker for detection, diagnosis, and disease progression. This possibility is further supported by immunohistochemical and reverse transcription-polymerase chain reaction assays that have de-

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Address reprint requests to Gavin P. Robertson, Department of Pharmacology–H078, Pennsylvania State University College of Medicine, 500 University Drive, Hershey, PA 17033. E-mail: gprobertson@psu.edu.

tected Mig-7 in circulating tumor cells, demonstrating that it has significant potential as an early marker of migrating and circulating carcinoma cells.¹⁶

Petty et al elegantly link up-regulation of Mig-7 expression in embryonic cytotrophoblasts to acquisition of an invasive phenotype capable of pseudovasculogenesis and in carcinoma cells to an ability to undergo vasculogenic mimicry.¹ Similarities between embryonic cytotrophoblast cells and cancer cells have been described for years¹⁸; specifically, in terms of invasiveness, where embryonic cytotrophoblasts invade maternal tissues from the site of embryo implantation during placental development and remodeling of the maternal vasculature.¹⁸ In this study, the authors show that Mig-7 levels are highest when human cytotrophoblasts invade maternal decidua and vasculature during early placental development with peak invasion and vascular remodeling occurring between 14 and 22 weeks of gestation and greatly reduced by term (Figure 1).¹ In a similar fashion, Petty et al show that Mig-7 expression is elevated in invading carcinoma cells (Figures 4 and 5),¹ suggesting that this protein plays an important role in promoting plasticity of both cell types enabling mimicry of endothelial cell-like traits.

Vasculogenic mimicry has been described in multiple tumor types and associated with a poor prognosis for the patient.^{4,12,19} Tumor-lined structures are capable of being perfused with blood, and erythrocytes have been observed in channels in patient tumors, suggesting that these structures serve as conduits for erythrocytes, which are structures similar to those shown in Figure 4 of the Petty et al manuscript.^{1,4,20} Petty et al show that Mig-7 protein expression was found in aggressively invasive melanoma cells capable of vasculogenic mimicry (C918, C8161, MUM2B) but not in poorly invasive that do not form the structures (A375P, MUM2C) (Figures 4 and 5).¹ Petty et al further show that carcinoma invasiveness and vascular mimicry pseudovasculogenesis are similar processes initiated by common signaling events. Specifically, receptor tyrosine kinase ligands, such as hepatocyte growth factor or epidermal growth factor, in concert with cognate receptors and $\alpha v\beta 5$ integrin have previously been reported to induce expression of Mig-7 in carcinoma cells.^{16,17} Petty et al confirm these earlier reports demonstrating that plating carcinoma cells or cytotrophoblasts with epidermal growth factor or hepatocyte growth factor induces Mig-7 expression (Figures 2 and 3).¹ Previous reports have also shown that blocking antibodies to the $\alpha v\beta 5$ integrin can inhibit hepatocyte growth factor induction of Mig-7, and Mig-7-specific antisense-mediated inhibition can inhibit cell scattering.¹⁷ The authors also show that expression of Mig-7 affected the invasiveness of carcinoma cells and cleavage of laminin 5 $\gamma 2$ chain fragments, which was dependent on the extracellular matrix, laminin attachments, and growth factors (Figure 2).¹ Laminin 5 $\gamma 2$ chain fragmentation is required for carcinoma cell invasion and for vasculogenic mimicry.⁹ Mig-7 expression leads to formation of laminin 5 $\gamma 2$ promigratory fragments that are important for vasculogenic mimicry.⁹ Laminin 5 is the only laminin that contains the $\gamma 2$ chain, which following cleavage into promigratory fragments, the domain III region, causes increased levels

of metalloproteinase-2.⁹ Metalloproteinase-2 and membrane-type 1 metalloproteinase cooperate to cleave $\gamma 2$ chain into fragments that promote melanoma cell invasion and vasculogenic mimicry (Figures 2 and 3).^{1,9}

Whereas the above studies are based on cultured systems, Petty et al use an elegant animal model to show that Mig-7 protein localized to cells, forming vessel-like structures in lymph nodes where human carcinoma cells had spread following injection into nude mice (Figure 7).¹ Staining of serial histological sections of these areas of carcinoma cell invasion showed colocalized Mig-7 and Willebrand factor (factor VIII) in irregular vessel-like structures commonly observed during vasculogenic mimicry.⁴ Staining spread away from the vessels, suggesting that these may be leaky vessels typical of those observed in tumors and spiral arteries seen in the resulting cytotrophoblast-mediated remodeling. Structures appeared similar to those described by Hashizume et al that were adjacent to one another but not similar to normal vessels, had small diameters, and contained cytoplasmic projections or membranous extensions in the lumen.⁵ Furthermore, Mig-7 protein expression also colocalizes with vasculogenic mimicry markers vascular endothelial-cadherin and laminin 5 $\gamma 2$ chain domain III fragments in this lymph node metastases model, leading to the conclusion that these carcinoma cells were masquerading as endothelial cells (Figure 7).^{1,8,9} Similar results were observed using *in vitro* models of vasculogenic mimicry. Specifically, Mig-7 expression induced invasion and formation of vessel-like structures in two-dimensional and three-dimensional Matrigel cultures (Figure 4).¹ Thus, Mig-7 expression enables cells to sense the environment-promoting invasion and vasculogenic mimicry through a novel relationship with laminin 5 $\gamma 2$ chain domain III fragments (Figure 2).^{1,9}

Unraveling the genes, signaling pathways, and pathophysiological significance of vasculogenic mimicry are important scientific questions. Petty et al contribute to this research arena by discovering that Mig-7 is an important regulator of vasculogenic mimicry. Because Mig-7 is not detectable in normal tissues, it is possible it could be used as a specific marker for vasculogenic mimicry, poor patient prognosis associated with vasculogenic mimicry, and/or for targeted delivery of therapeutics. Under these circumstances, tumor-lined channels, with development mediated by Mig-7, could provide a route for viral and nonviral gene therapy targeting Mig-7 expressed in the cells. The potential therapeutic utility for blood flowing past exposed tumor cells expressing Mig-7 is significant since it might be used to deliver Mig-7-inhibitory agents into those cells. Furthermore, targeting Mig-7 in combination with current therapies might increase the efficacy of conventional anti-cancer drugs. Combinatorial therapeutics targeting Mig-7 expressing cancer cells in addition to endothelial cell-targeted angiogenesis inhibitors might prove more effective than either treatment alone. Mig-7 also provides a specific marker expressed by tumor cells that invade or mimic endothelial cells. The presence of tumor-lined channels mediated by Mig-7 signaling may also predispose cancer patients to the blood-borne spread of tumor cells; therefore, targeting

Mig-7 might decrease the metastatic spread of cancer and thereby prolong the life or quality of life for cancer patients. In conclusion, the study by Petty et al contributes to a better understanding of tumor cell invasion and vasculogenic mimicry, which in the long term, could contribute significantly to the development of improved detection of invasive cancer and novel therapeutic strategies targeting vasculogenic mimicry.

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