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Vasculogenic Mimicry: Role of melanoma differentiation associated gene-9/syntenin

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BACKGROUND

Malignant melanoma (MM) is the most aggressive skin cancer and the most frequent skin disorder in Caucasians. MM is associated with aggressive and progressive disease states, leading to major cancer-related morbidity and mortality. Recent investigations identify a new non-angiogenesis-dependent pathway vasculogenic mimicry (VM), which is considered a cancer hallmark that can independently facilitate tumor neovascularization by the formation of fluid-conducting and vascular endothelial cells. MM cells undergoing VM can dedifferentiate into numerous cellular phenotypes and acquire endothelial-like features, resulting in the formation of the *de novo* matrix-rich vascular-like network, such as plasma and red blood cells. The co-generation of endothelial cells, channels, laminar structures, and heparin sulfate proteoglycans are the main pathophysiological characteristics of VM in human melanoma patients. In highly aggressive melanoma cells downregulation of vascular endothelial cadherin and upregulation of ECM components promote the perfusion of the VM pathway. We investigated whether *mda-9/syntenin, a pro-metastatic gene,* affects VM in MM (Figure 1). The expression of mda-*9/syntenin* was modulated using gain-of-function and loss-of-function strategies to evaluate its potential role in VM. Downregulation of *mda-9/syntenin* in aggressive melanoma cells decreases VM, while over expressing *mda-9/syntenin* in immortalized human melanoma cells increases VM. These findings shed light on a novel role and molecular mechanism of action of *mda-9/syntenin* in VM, which may contribute significantly to the metastatic phenotype of these aggressive cancers.

METHODS

- Cell lines and culture conditions: FM-516 SV40 (referred to as FM-516) cells are normal human melanocytes immortalized using the SV40 T-antigen. WM-35 are early radial growth phase primary melanoma cells purchased from American Type Culture Collection (Manassas, VA). C8161.9 are metastatic melanoma cells (kindly provided by Dr. Danny R. Welch, University of Kansas Cancer Center).
- Matrigel assay formation: Experimental protocol was performed to observe VM formation- Preparation of tumor cells and microvascular endothelial cells, Matrigel preparation, tube formation, image and data analysis.
- Western Blotting: Cell lysates were collected from the treated cell-lines. Protein lysates were quantified and equal amounts of proteins were treated with specific antibodies.

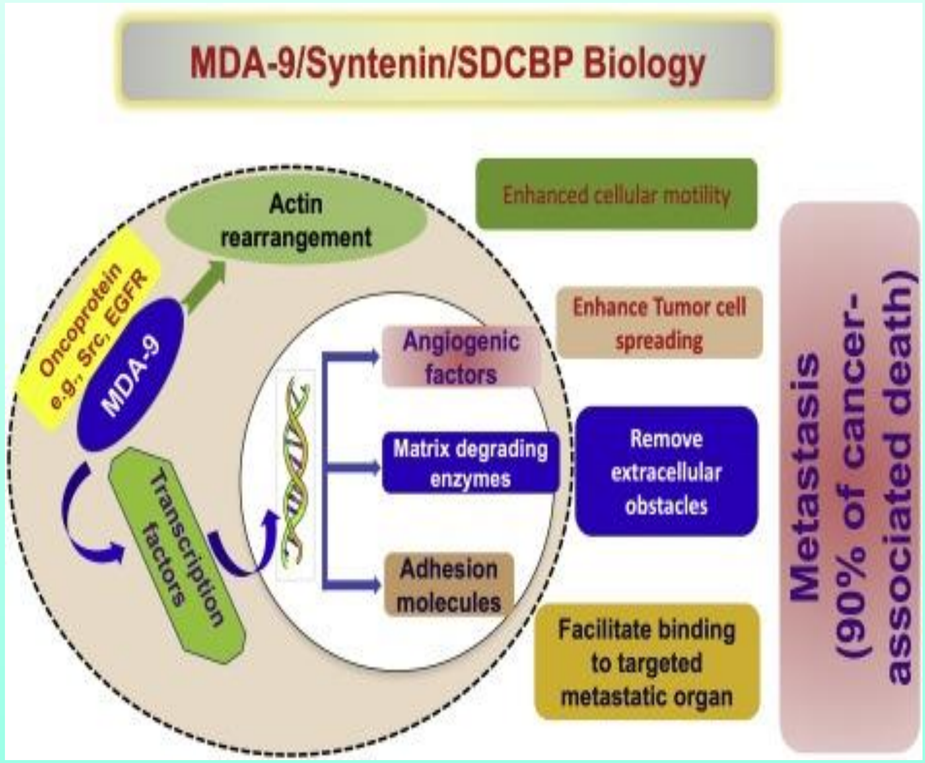

Figure 1. Role of MDA-9 in Cancer Biology.

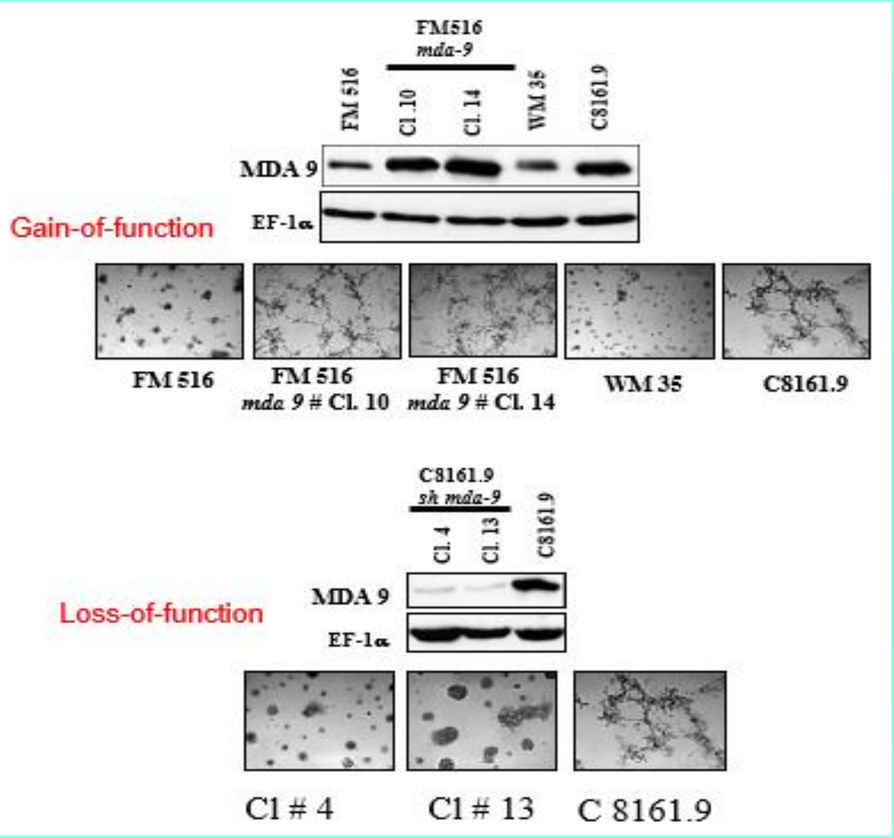

Figure 2. Modification in MDA-9 expression.

RESULTS

The expression of *mda-9/syntenin* in normal immortalized human melanocytes, FM-516, is less in comparison with WM-35 primary melanoma cells and C8161.9 metastatic melanoma cells (Figure 2). We determined the intrinsic ability of these cells to develop vasculogenic mimicry (VM) and observed that both FM-516 and WM-35 cells did not show VM abilities while metastatic C8161.9 cells displayed VM. We established clones of FM-516 cells stably overexpressing *mda-9/syntenin* and determined their ability to show VM. Stable overexpression of *mda-9/syntenin* in FM-516 cells resulted in an increase in VM. To further investigate the role of *mda-9/syntenin* in VM, we stably silenced *mda-9/syntenin* expression in C8161.9 cells and determined their ability to undergo VM. Stably silencing the expression of *mda-9/syntenin* in C8161.9 cells resulted in a decrease in VM, indicating an association between *mda-9/syntenin* expression and VM.

DISCUSSION

Experimental evidence from our laboratory confirms that specifically targeting pathways implicated in VM can inhibit tumor growth. Preclinical studies suggest that specific compounds affecting components of the described vascular, embryonic, or hypoxia pathways in tumor cells can inhibit VM formation in cancer models. VM can provide one of several sources for a tumor's blood supply that can directly or indirectly interact with additional vasculature. Each of the multiple pathways regulating VM formation warrant serious scrutiny as potential therapeutic targets and diagnostic indicator(s) of plasticity, drug resistance, and expression of an aggressive metastatic phenotype.

Questions to be addressed: Does VM play a significant role in metastasis? Do tumor cells form their own lymphatic vessels using processes analogous to VM? And finally, if tumors can self-vascularize, is an anti-angiogenic approach for shrinking solid tumors feasible?

Acknowledgments

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References

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