**Abstract**

Cancer is a progressive disease that, in many instances, if untreated, can culminate in metastatic spread of primary tumor cells to distant sites in the body. Metastasis frequently confers virulence and therapy resistance to cancer cells, and defining the molecular events that control metastasis will be mandatory to develop rational, targeted therapies for effective intervention, prevention of recurrence, and the "holy grail" of engendering a cure. Adapter proteins are physiologically pertinent molecules that, through interactions with key regulatory proteins via specific conserved domains, control important cellular events. Melanoma differentiation associated gene-9 (mda-9), also known as syntenin, is a PDZ domain-containing adapter protein that is involved in organization of protein complexes in the plasma membranes, regulation of B-cell development, intracellular trafficking and cell-surface targeting, synaptic transmission, and axonal outgrowth. Recent studies now define a seminal role for mda-9/syntenin in cancer metastasis. The present review provides a current perspective of our understanding of this important aspect of mda-9/syntenin, suggesting that this gene and its encoded protein and interacting protein partners may provide viable targets for intervening in the final and invariably the most lethal stage of cancer progression, namely, cancer metastasis. [Cancer Res 2008;68(9):3087–93]

**Introduction**

Adapter proteins play an essential role in modulating signal transduction from the extracellular environment to the intracellular milieu by virtue of their association with key regulatory molecules (1). PDZ domain-containing molecules are a family of proteins that control diverse and central physiologic processes (2). The term PDZ is an acronym representing three proteins, postsynaptic density protein PSD95/SAP90, drosophila tumor suppressor DLGA, and tight junction protein ZO-1, in which this conserved sequence element was first identified. PDZ domains are ubiquitous signaling domains with more than 400 distinct copies in the human genome. This domain is composed of 80 to 90 amino residues from the COOH terminus (–2 position). PDZ domains are typically grouped into three classes depending on their target peptides: class I (–S/T-X–Φ), class II (–Φ-X–Φ), and class III (–D/E-X–Φ), where Φ is a hydrophobic residue (2). Melanoma differentiation associated gene-9 (mda-9)/syntenin belongs to PDZ domain family proteins and, through its specific localization, controls a plethora of molecular events (5). One major recently uncovered role of mda-9/syntenin is its involvement in controlling tumor metastasis, which will be elaborated on in the present review.

**mda-9/Syntenin: Cloning and Interacting Partners**

mda-9 was originally cloned as a gene differentially expressed in human melanoma cells reprogrammed to terminally differentiate by combination treatment with IFN-β and the protein kinase C activator mezerein (6, 7). Terminal differentiation of human melanoma cells coincides with an irreversible loss of proliferative capacity, changes in biochemical programs, alterations in surface antigen expression, modifications in cellular morphology, and major changes in gene expression (6, 8). Subtraction hybridization between normal and terminally differentiated human melanoma cells resulted in the identification of melanoma differentiation associated (mda) genes (6, 9). Interestingly, rather than exhibiting a sustained induction, a feature of mda genes regulating growth suppression during terminal differentiation, mda-9 mRNA expression showed a distinct biphasic kinetics peaking 8 to 12 hours after IFN-β + mezerein treatment with a return to basal level by 24 hours, indicating that modulation of mda-9/syntenin expression is disassociated from growth suppression (7, 10). However, the consequence of this biphasic expression pattern remains to be elucidated. IFN treatment alone, and not mezerein treatment, markedly induced mda-9 mRNA expression (7). The mda-9 cDNA is ~2.1 kb with an open reading frame of 894 bp that codes for a protein of 298 amino acid residues with a predicted molecular mass of ~33 kDa (7, 10, 11). mda-9 has two PDZ domains, PDZ-1 (amino acids 110–193) and PDZ-2 (amino acids 194–274; ref. 11). Cloning of mouse and rat mda-9 revealed that mda-9 is highly homologous across species (12). The expression of mda-9 could be detected in all fetal and adult tissues of human origin (10, 11).

The identical gene was subsequently cloned, and named syntenin, by yeast two-hybrid assay as an interacting partner of cell-surface heparan sulfate syndecans, involved in cell-cell and cell-matrix adhesion, signal transduction from the cell surface to the PDZ domain–containing proteins associate with the plasma membrane and they are generally restricted to specific subcellular domains such as synapses, cell-cell contacts, or the apical, basal, or lateral cell surface, thereby supporting a likely role as central organizers of protein complexes at the plasma membrane (4). In general, PDZ domains bind preferentially to peptides that terminate in a hydrophobic amino acid, usually valine or isoleucine, and with serine, threonine, or tyrosine located two residues from the COOH terminus (–2 position). PDZ domains are typically grouped into three classes depending on their target peptides: class I (–S/T-X–Φ), class II (–Φ-X–Φ), and class III (–D/E-X–Φ), where Φ is a hydrophobic residue (2). Melanoma differentiation associated gene-9 (mda-9)/syntenin belongs to PDZ domain family proteins and, through its specific localization, controls a plethora of molecular events (5). One major recently uncovered role of mda-9/syntenin is its involvement in controlling tumor metastasis, which will be elaborated on in the present review.
the interior, and trafficking of lipoproteins and lipases, thus playing prominent roles in cell growth, development, and differentiation (11). There are four known vertebrate syndecans, syndecan-1, syndecan-2, syndecan-3, and syndecan-4, each having a single ectodomain, a membrane-spanning region, and a cytoplasmic domain, with syndecan-4 being the most evolutionary conserved. Syntenin was identified as an interacting molecule with the COOH-terminal domains of all four syndecans (11). Subsequently, a succession of molecules interacting with mda-9/syntenin has been identified, mostly by yeast two-hybrid assays. A list of these molecules and their potential functional significance is given in Table 1.

### Localization of mda-9/Syntenin

Analysis of the subcellular distribution of mda-9/syntenin revealed its localization at the areas of cell-cell contact in cells of epithelial origin in colocalization with F-actin, syndecan-1, E-cadherin, β-catenin, and α-catenin (12). In fibroblasts, mda-9/syntenin localizes to focal adhesions and in stress fibers. Overexpression of mda-9/syntenin in different cells induces the formation of plasma membrane structures, including ruffles, lamellipodia, fine extensions, and neurite-like structures, showing its role in regulating the structure and function of the plasma membrane (12). Anchoring of mda-9/syntenin in the plasma membrane influences its functional properties, as seen in the interactions with various partners listed in Table 1.

### Table 1. mda-9/syntenin interacting partners

<table>
<thead>
<tr>
<th>Interacting molecule</th>
<th>Possible function</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-5 receptor α</td>
<td>Sox4 activation; B-cell development and differentiation</td>
</tr>
<tr>
<td>ProGFrα</td>
<td>Intracellular trafficking</td>
</tr>
<tr>
<td>Neurofascin</td>
<td>Synaptic transmission</td>
</tr>
<tr>
<td>Neuroglian</td>
<td>Not identified</td>
</tr>
<tr>
<td>Syndecans</td>
<td>Cell polarization; plasma membrane integrity</td>
</tr>
<tr>
<td>Ephrin-B</td>
<td>Eph receptor signaling</td>
</tr>
<tr>
<td>EphA7</td>
<td>Eph receptor signaling</td>
</tr>
<tr>
<td>r-PTPγ</td>
<td>Plasma membrane localization (?)</td>
</tr>
<tr>
<td>Neurexin-I</td>
<td>Not identified</td>
</tr>
<tr>
<td>Unc51.1</td>
<td>Axon formation</td>
</tr>
<tr>
<td>Rab5 GTPase</td>
<td>Axon formation</td>
</tr>
<tr>
<td>Schwannomin</td>
<td>Plasma membrane localization (?)</td>
</tr>
<tr>
<td>Ionotropic and metabotropic glutamate receptors</td>
<td>Glutamate signaling</td>
</tr>
<tr>
<td>Phosphatidylinositol 4,5-bisphosphate</td>
<td>Plasma membrane anchoring</td>
</tr>
<tr>
<td>Tetraspanin (CD63)</td>
<td>Endocytosis (?)</td>
</tr>
<tr>
<td>Δ1</td>
<td>Epidermal stem cell cohesiveness</td>
</tr>
</tbody>
</table>

Figure 1. Expression of mda-9/syntenin is augmented with progression of melanoma. Nevus, radial growth phase (RGP), and vertical growth phase (VGP) primary melanoma and lymph node metastatic melanoma sections were stained with anti–mda-9/syntenin antibody. Higher magnification shows membrane and cytoplasmic staining in melanoma cells. Magnification: nevus, ×80; RGP, ×300; VGP, ×80; VGP, ×800; lymph node, ×70.
membrane is assisted by its interaction with phosphoinositide phosphatidylinositol 4,5-bisphosphate (PIP2) and phospholipase Cγ (13). mda-9/syntenin also localizes predominantly in the early secretory pathway, such as the endoplasmic reticulum, intermediate compartment, and cis-Golgi, as well as in apical endosomes facilitating trafficking of cell-surface–located molecules (14).

**mda-9/Syntenin and Metastasis**

The major characteristic of malignant tumor cells is their ability to invade foreign tissues and form metastatic foci at distant locations in the body. Such a process requires tumor cell attachment to various matrix proteins, degradation of the extracellular matrix (ECM) mainly by matrix metalloproteinases (MMP), followed by migration into the surrounding stroma by tumor cells. Suppression subtractive hybridization between a poorly invasive/nonmetastatic breast cancer cell line, MCF-7, and an invasive/metastatic breast cancer cell line, MDA-MB-435, identified mda-9/syntenin as a potential regulator of metastasis (13). mda-9/syntenin also localizes to tumor cells associated with invasion and migration properties and resulted in morphologic changes with increased pseudopodia formation (15). Deletion analysis of mda-9/syntenin domains identified the PDZ-2 domain to be responsible for its stimulatory effect on cell migration (15).

A model of progression of melanoma suggests that it begins by conversion of a normal melanocyte into a benign nevus, subsequent transformation into a radial and then a vertical growth phase primary melanoma, and finally evolution into a metastatic melanoma. Subtractive suppression hybridization between primary melanomas and melanoma metastases identified increased mda-9/syntenin expression during metastasis (17). Immunohistochemical analysis revealed a statistically significant gradual increase in mda-9/syntenin expression level during progression from acquired melanocytic nevi to primary melanoma without or with conversion to metastatic melanomas (17). Biomarker analysis identified mda-9/syntenin in uveal melanoma cell secretomes of patients with metastatic melanoma (18). In our studies, whereas mda-9/syntenin...
expression was undetected in 9 samples of normal skin and was detected in only 1 of 15 samples of dermal nevi, its expression was significantly augmented as the tumor became more progressed, with 24 of 30 vertical growth phase primary melanomas and 8 of 12 lymph node metastases scoring positive for mda-9/syntenin expression (Fig. 1; ref. 19). Similarly, mda-9/syntenin expression was significantly higher in a series of melanoma cells when compared with SV40 T/t Ag immortalized normal human melanocytes, FM516-SV. In a matched set of low and high metastatic cells, M4Beu. and T1P26, respectively, mda-9/syntenin expression was significantly higher in T1P26 cells than in M4Beu. cells, indicating a possible involvement of mda-9/syntenin in regulating metastasis (19). In vitro studies confirmed that overexpression of mda-9/syntenin by an adenovirus (Ad.mda-9/S) significantly augmented migration, invasion, and anchorage-independent growth of FM516-SV and M4Beu. cells, whereas inhibition of mda-9/syntenin by an adenosine expressing antisense mda-9/syntenin (Ad.mda-9/AS) significantly suppressed these processes in T1P26 cells (19). Treatment with Ad.mda-9/S significantly augmented spontaneous lung metastasis of FM516-SV and M4Beu. cells when implanted s.c. in immunocompromised newborn rats, whereas Ad.mda-9/AS treatment significantly inhibited spontaneous lung metastasis of T1P26 cells in similar experimental conditions, thus confirming the crucial role played by mda-9/syntenin in regulating metastasis (19).

Molecular analysis revealed that mda-9/syntenin displayed preferential association with fibronectin signaling. When plated on fibronectin, mda-9/syntenin–overexpressing FM516-SV and M4Beu. cells displayed more actin stress fibers, whereas mda-9/syntenin antisense–expressing T1P26 cells displayed decreased actin microfilament formation (19). On fibronectin-coated dishes, mda-9/syntenin facilitated phosphorylation of focal adhesion kinase (FAK) with subsequent activation of p38 mitogen-activated protein kinase (MAPK) and c-Jun NH2-terminal kinase (JNK; ref. 19). Activation of FAK and p38 MAPK by mda-9/syntenin leads to activation of nuclear factor κB (NF-κB; refs. 19, 20). Inhibition of FAK, p38 MAPK, JNK, or NF-κB, either by chemical or dominant-negative genetic inhibitors, significantly suppressed the migration- and invasion-augmenting properties of mda-9/syntenin. NF-κB activation by mda-9/syntenin resulted in induction of MT1-MMP that leads to the activation of MMP-2, thus uncovering an end-point molecule mediating mda-9/syntenin–induced phenotypic changes (Fig. 2; ref. 20). However, in breast cancer cells, mda-9/syntenin did not induce either MMP-2 or MMP-9 (15). This discrepancy might be explained by the cell type–specific action of mda-9/syntenin as well as by ECM-induced signaling because the
observations in melanoma cells were obtained by plating the cells on fibronectin.

Colocalization studies showed that mda-9/syntenin is a component of focal adhesion with overlapping localization with phospho-FAK (19). However, a direct interaction between FAK and mda-9/syntenin was not shown, indicating that mda-9/syntenin might interact with other components of focal adhesion that regulate FAK phosphorylation. Indeed, analysis of FAK sequence also does not reveal any consensus PDZ domain–binding motif in its COOH terminus, thus making FAK-mda-9/syntenin interaction unlikely. Identification of the key interacting partner(s) of mda-9/syntenin in activation of FAK and subsequent signaling remains a priority to understand the molecular mechanism by which this adapter protein controls metastasis.

Deletion mutation analysis revealed that the presence of both PDZ domains of mda-9/syntenin is necessary to facilitate collagen I invasion of HEK 293T cells (21), which is in contrast to the findings in breast cancer cells showing that the PDZ-2 domain plays a major role in conferring mda-9/syntenin function (15). Mutation of K124, R128, and K130, which, based on crystallographic data, form a highly positively charged surface surrounding the peptide-binding interface in PDZ-1 and are involved in lipid binding, inhibits binding of mda-9/syntenin to phosphoinositides and also inhibits invasion (21). Interestingly, this mda-9/syntenin mutation did not interfere with interaction with syndecan-2 but significantly impaired interaction with the tumor suppressor merlin/schwannomin-1 (sch-1), indicating that ligand (lipid/peptide) binding is important for mediating mda-9/syntenin function (21). However, whether interaction with merlin/sch-1 is really important for mediating mda-9/syntenin–induced invasion has not been studied. Augmentation of collagen I invasion by mda-9/syntenin required Rho-family small GTPases, RhoA, cdc42, and Rac1, as well as activated H-ras, as shown by dominant negative mutants. Overexpression of Rac1, as well as activated H-ras, as shown by dominant negative mutants, significantly impaired interaction with the tumor suppressor protein, r-PTP1 diabetes. Expression of r-PTP1 is reduced in several thyroid oncogene-transformed cells and is absent in highly malignant thyroid cells. Overexpression of r-PTP1 in tumorigenic rat thyroid cells suppresses the malignant phenotype. The human homologue of r-PTP1 also shows decreased expression in thyroid carcinomas in comparison with normal thyroid tissues. Although mda-9/syntenin was phosphorylated at tyrosine residues, it was observed that r-PTP1 did not dephosphorylate it, indicating that mda-9/syntenin is not a substrate of r-PTP1 although there is an interaction. The functional significance of this interaction was not investigated. However, an involvement of mda-9/syntenin in the trafficking of r-PTP1 is possible. Considering that mda-9/syntenin plays an active role in mediating tumor progression and metastasis, the functional significance of its interaction with tumor suppressor proteins needs to be scrutinized in detail. It might be possible that by sequestering these proteins to the plasma membrane, mda-9/syntenin might actually inhibit their function. An analogous situation involves sequestration of the Notch ligand Δ1 by mda-9/syntenin, which prevents Notch activation in epidermal stem cells (25).

Additional Important Functions of mda-9/Syntenin

**Interleukin-5 signaling.** mda-9/syntenin interacts with interleukin-5 (IL-5) receptor α and the transcription factor Sox4, thus mediating IL-5–induced Sox4 activation (26). IL-5 and Sox4 regulate B-cell development and differentiation, implicating the involvement of mda-9/syntenin in these processes.

**Cell-surface trafficking.** Although mda-9/syntenin is located predominantly in the plasma membrane, it is also identified in the early secretory pathway such as the endoplasmic reticulum, intermediate compartment, and cis-Golgi, thus facilitating cell-surface trafficking of secreted molecules such as proTGF-α, an epidermal growth factor receptor ligand (14). mda-9/syntenin is also located in apical endosomes, thus regulating the correct localization of transmembrane receptors, such as transferrins, via rerouting them through an apical endosomal compartment.

**mda-9/syntenin and ephrin signaling.** Ephrins and their cell-surface tyrosine kinase receptors are implicated in controlling axon guidance and fasciculation, in specifying topographical map guidance and fasciculation, in specifying topographical map

---

Tumor Suppressor Proteins and mda-9/Syntenin

Neurofibromatosis type 2 (NF2) is an autosomal dominant disorder that leads to the development of schwannomas, meningiomas, ependymomas, and other tumors of the central nervous system resulting from inactivating mutations of the NF2 gene (22). The NF2 gene product is known as NF2 protein, schwannomin-1 (sch-1), or merlin. Sch-1 is similar to the ERM (ezrin, radixin, and moesin) proteins, which function as molecular adaptors linking integral membrane proteins to the cytoskeleton. There are two isoforms of the NF2 protein, sch-1 and sch-2, which differ in the COOH-terminal 16 amino acids. Only sch-1 suppresses growth when overexpressed, indicating the importance of the COOH-terminal region in mediating its tumor suppressor function. Yeast-two hybrid assay with sch-1 COOH terminus as bait identified mda-9/syntenin as an interacting molecule (23). mda-9/syntenin and sch-1 colocalized in the cytoplasmic face of the plasma membrane and also in punctate intracellular vesicular structures. Inhibition of mda-9/syntenin by an antisense approach inhibited the plasma membrane localization of sch-1, indicating that mda-9/syntenin–sch-1 interaction plays a role in the targeting of sch-1 to the plasma membrane. However, it is not clear how this plasma membrane sequestration by mda-9/syntenin affects sch-1 function. It has not been tested whether inhibition of mda-9/syntenin interferes with the growth-suppressing function of sch-1. Thus, the functional significance of this interaction in tumor suppression remains to be resolved.

In addition to sch-1, mda-9/syntenin interacts with another tumor suppressor protein, r-PTPα a receptor-type tyrosine phosphatase (24). Expression of r-PTPα is reduced in several thyroid oncogene-transformed cells and is absent in highly malignant thyroid cells. Overexpression of r-PTPα in tumorigenic rat thyroid cells suppresses the malignant phenotype. The human homologue of r-PTPα also shows decreased expression in thyroid carcinomas in comparison with normal thyroid tissues. Although mda-9/syntenin was phosphorylated at tyrosine residues, it was observed that r-PTPα did not dephosphorylate it, indicating that mda-9/syntenin is not a substrate of r-PTPα although there is an interaction. The functional significance of this interaction was not investigated. However, an involvement of mda-9/syntenin in the trafficking of r-PTPα is possible. Considering that mda-9/syntenin plays an active role in mediating tumor progression and metastasis, the functional significance of its interaction with tumor suppressor proteins needs to be scrutinized in detail. It might be possible that by sequestering these proteins to the plasma membrane, mda-9/syntenin might actually inhibit their function. An analogous situation involves sequestration of the Notch ligand Δ1 by mda-9/syntenin, which prevents Notch activation in epidermal stem cells (25).
formation within the central nervous system, in organizing the movements of neural crest cells during development, in directing fusion of epithelial sheets in closure of the palate, and in angiogenesis. EphA7 receptor and ephrin B1 interact with mda-9/syntenin, indicating the potential role of mda-9/syntenin in regulating the diverse ephrin-regulated processes (27).

**Mediation of cohesiveness of epidermal stem cells.** In the basal layer of interfollicular epidermis the stem cells are clustered, a feature known as cohesiveness. These cells express high levels of Notch ligand D1, which is important for maintaining cohesiveness. In the neighboring cells that do not express D1, D1 binds to Notch and induces keratinocyte differentiation. In the D1-expressing stem cells that also express high levels of mda-9/syntenin, D1 interacts with mda-9/syntenin via its COOH-terminal PDZ-binding domain that maintains D1 on the cell surface and facilitates cohesiveness (25). A D1 mutant that cannot bind to mda-9/syntenin or mda-9/ syntenin siRNA inhibits the cohesiveness of epidermal stem cells and facilitates terminal differentiation into keratinocytes by enhancing Notch signaling.

**Regulation of glutamate signaling.** The excitatory neurotransmitter glutamate interacts with its cognate receptors and regulates postsynaptic excitatory currents. Glutamate receptors interact with mda-9/syntenin, indicating the potential role of the latter in regulating synaptic transmission, a hypothesis yet to be proved (28, 29).

**Regulation of axon outgrowth.** Unc51.1 is a serine/threonine kinase that is important for neurite extension/parallel fiber formation in cerebellar granule neurons. mda-9/syntenin interacts with Unc51.1 and Rab5, a member of the Ras-like small GTPases that is important for neurite extension/parallel fiber (28, 29).

**Conclusion and Future Perspectives**

Although the role of mda-9/syntenin in regulating cell migration, invasion, and metastasis has been confirmed by multiple studies, several discrepant findings have been observed depending on the cell type studied and in different environmental contexts. Studies with HEK 293T cells revealed the importance of both PDZ domains in conferring mda-9/syntenin function, whereas with breast cancer cells the PDZ2 domain has been shown to be relevant (15, 21). In melanoma cells, fibronectin engagement was shown to be important for induction of mda-9/syntenin signaling that involves FAK, p38 MAPK, and NF-κB, whereas in HEK 293T cells, Ras, Rho- Rac, PI3K/Akt, and MAPK signaling were shown to mediate mda-9/ syntenin function (19–21). However, it still remains to be determined with which key regulatory molecule mda-9/syntenin interacts to augment invasion and metastasis. It is also not known how mda-9/syntenin expression is augmented with tumor progression. Owing to its interaction with ephrins and their receptors, mda-9/syntenin might also regulate angiogenesis, another important parameter of tumor progression. Indeed, our initial findings indicate that mda-9/syntenin augments tumor angiogenesis by up-regulating vascular endothelial growth factor (4).

Our preliminary studies also show that mda-9/syntenin is overexpressed in different types of brain cancer, such as malignant glioma and meningiomas, indicating a potential involvement of mda-9/ syntenin in regulating tumorigenesis in multiple lineages. Recently, we have also identified that augmentation of c-src signaling plays a critical event in mediating mda-9/syntenin function.6 Unraveling these diverse findings would help comprehend the function of mda-9/syntenin thereby providing a better perspective in developing strategies, such as lentivirus expressing siRNA or small molecule inhibitors, targeting mda-9/syntenin to counteract terminal, metastatic disease.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Acknowledgments**

Received 11/12/2007; revised 1/15/2008; accepted 1/19/2008.

**Grant support:** NIH grant CA056753 (P.B. Fisher) and the Samuel Waxman Cancer Research Foundation (P.B. Fisher). D. Sarkar is the Harrison Endowed Scholar in Cancer Research and P.B. Fisher holds the Thelma Newmeyer Corman Chair in Cancer Research and is a Samuel Waxman Cancer Research Foundation Investigator.

**References**


mda-9/Syntenin: More than Just a Simple Adapter Protein When It Comes to Cancer Metastasis


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/68/9/3087

Cited articles
This article cites 30 articles, 11 of which you can access for free at:
http://cancerres.aacrjournals.org/content/68/9/3087.full.html#ref-list-1

Citing articles
This article has been cited by 16 HighWire-hosted articles. Access the articles at:
content/68/9/3087.full.html#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.