Melanoma Proteoglycan Modifies Gene Expression to Stimulate Tumor Cell Motility, Growth, and Epithelial-to-Mesenchymal Transition

Jianbo Yang,1 Matthew A. Price,1 Gui Yuan Li,4 Menashe Bar-Eli,2 Ravi Salgia,3 Ramasamy Jagedeeswaran,3 Jennifer H. Carlson,1 Soldano Ferrone,5 Eva A. Turley, and James B. McCarthy1

1Department of Laboratory Medicine and Pathology and Masonic Cancer Center, University of Minnesota, Minneapolis, Minnesota; 2Department of Cancer Biology, University of Texas M.D. Anderson Cancer Center, Houston, Texas; 3Section of Hematology/Oncology, Department of Medicine and University of Chicago Cancer Research Center, University of Chicago, Chicago, Illinois; 4Cancer Research Institute, Xiangya School of Medicine, Central South University, Changsha, Hunan, P.R. China; and 5London Regional Cancer Center, University of Western Ontario, London, Ontario, Canada

Abstract
Melanoma chondroitin sulfate proteoglycan (MCSP) is a plasma membrane–associated proteoglycan that facilitates the growth, motility, and invasion of tumor cells. MCSP expression in melanoma cells enhances integrin function and constitutive activation of Erk1,2. The current studies were performed to determine the mechanism by which MCSP expression promotes tumor growth and motility. The results show that MCSP expression in radial growth phase, vertical growth phase, or metastatic cell lines causes sustained activation of Erk1,2, enhanced growth, and motility which all require the cytoplasmic domain of the MCSP core protein. MCSP expression in a radial growth phase cell line also promotes an epithelial-to-mesenchymal transition based on changes in cell morphology and the expression of several epithelial-to-mesenchymal transition markers. Finally, MCSP enhances the expression of c-Met and hepatocyte growth factor, and inhibiting c-Met expression or activation limits the increased growth and motility of multiple melanoma cell lines. The studies collectively show the importance of MCSP in promoting progression by an epigenetic mechanism and they indicate that MCSP could be targeted to delay or inhibit tumor progression in patients. [Cancer Res 2009;69(19):7538–47]

Introduction
Human melanoma proteoglycan (MCSP), and the rat homologue NG2, are transmembrane proteoglycans in which the core protein is modified with chondroitin sulfate (1–3). Normal melanocytes express little or no MCSP in situ, whereas increased levels of MCSP are detected in both benign and dysplastic nevi (1) and these levels are largely maintained throughout progression. MCSP expression in certain tumors (such as acute lymphoblastic leukemia and acral lentiginous melanoma) portends a poor prognosis (4–7), implicating MCSP as a mediator of malignant potential in certain tumors.

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/). J. Yang and M.A. Price contributed equally to these studies.

Requests for reprints: James B. McCarthy, Department of Laboratory Medicine and Pathology, University of Minnesota, MMC 609, 420 Delaware Street Southeast, Minneapolis, MN 55455. Phone: 612-625-7454; Fax: 612-625-1121; E-mail: mccar001@umn.edu.

doi:10.1158/0008-5472.CAN-08-4626

Published OnlineFirst September 8, 2009; DOI: 10.1158/0008-5472.CAN-08-4626
like scaffold/adaptor proteins control signaling downstream of growth factor receptors (e.g., Ras-Raf-Mek-Erk pathway; ref. 28).

**Materials and Methods**

**Cell lines.** WM164, 1205Lu, WM1552C, and WM1341D human melanoma cells were generously provided by Dr. Meenhard Herlyn (The Wistar Institute, University of Pennsylvania, Philadelphia, PA). A375SM cells were provided by Dr. Isiah Fidler (M.D. Anderson, Houston, TX). MeWo melanoma cells were purchased from American Type Culture Collection. WM and 1205Lu cells were maintained in a 1:1 MCDB 153/Leibovitz’s L-15 medium supplemented with 5 μg/mL of insulin and 2% fetal bovine serum, whereas A375SM cells were cultured in DMEM supplemented with 10% fetal bovine serum, sodium pyruvate, and nonessential amino acids. MeWo cells were cultured in RPMI medium supplemented with 10% fetal bovine serum. The MCSP-negative WM1552C RGP cell line was used to produce WM1552C/MCSP, mock, and cytoplasmic-domain-truncated MCSP (MCSPΔCD) stable transfectant cell lines as described previously (14) and cultured in medium supplemented with 0.25 mg/mL of G418. WM1552C melanoma cells expressing constitutively active Mek-1 were derived clonally. Two clones with enhanced Erk phosphorylation were selected for assay and designated WM1552C/MKK1Δ15 and WM1552C/MKK1Δ17.

**DNA constructs.** The full-length MCSP construct was generated as described previously (14). MCSPΔCD was generated by PCR site-directed mutagenesis of the full-length construct, followed by cloning into the expression vector pCDNA3.1(-), thereby creating the pCDNA3.1-MCSPΔCD plasmid. The DNA fragment encoding the full-length MCSP was amplified by PCR using primers 5’-ATGGCCATATGACGAGCTTGCCTG-3’ (primer 1) and 5’-ATGGAGCTCTAGGCCAATCTC-3’ (primer 2), and the PCR product was cloned into the pMT-Flag vector (Stratagene) using the EcoRI and XhoI restriction enzyme sites. The DNA sequence of the MCSPΔCD construct was confirmed by DNA sequencing.

**Antibodies and reagents.** The anti-MCSP monoclonal antibody 9.2.27 was provided by Dr. Ralph Reisfeld (The Scripps Research Institute, La Jolla, CA). Other antibodies and reagents were purchased from the indicated companies: antitubulin from Oncogene Research Products; anti-phosphorylated p44/42 mitogen-activated protein kinase (pErk1,2), anti-p44/42 mitogen-activated protein kinase (Erk1,2), anti-c-Met and phosphorylated c-Met (p-cMet), Met inhibitor U0126, and control compound U0124 from Cell Signaling Technology, Inc.; anti-glutathione S-transferase (GST) from Abcam, Inc.; anti-E-cadherin from BD Transduction Labs; anti-MIF from Santa Cruz Biotechnology, Inc.; c-Met kinase inhibitor SU11274 from Calbiochem, Inc.; methylcellulose and antimethaglutamin from Sigma; normal mouse monoclonal IgG2a, normal rabbit IgG, and goat anti-mouse FC from ICN Pharmaceuticals; peroxidase-conjugated goat anti-mouse and goat anti-rabbit secondary antibodies from Jackson ImmunoResearch Laboratories; and recombinant human HGF and anti-HGF antibody from R&D Systems.

**Small interfering RNA.** Small interfering RNA (siRNA) specific for MCSP (CUUCCUCCUCCUCCAUGACAU) was designed by our lab and manufactured by Qiagen, Inc. FITC-conjugated negative control siRNA, c-Met-specific siRNA, and HGF-specific siRNA were purchased from Qiagen. Cells were transfected with siRNA using the RNAiFect transfection reagent (Qiagen) suggested by the manufacturer. Transfection efficiency was routinely >90% as determined by flow cytometry analysis of cells transfected with the negative fluorescein-labeled control siRNA.

**In vivo tumor growth.** WM1552C/Mock, WM1552C/MCSP, and WM1552C/MCSPΔCD-transfected tumor cells were harvested from culture, washed twice with serum-free medium, counted and suspended in serum-free medium at 2 × 10⁶ cells/mL. The cell suspension (100 μL; 2 × 10⁶ cells) was injected s.c. into the flank of 7-week-old to 8-week-old female NOD.CB17-Prkdcscid−/− mice (Jackson Laboratory) and monitored over a 6-week period. Forty-two days post-injection, the animals were euthanized, and tumors harvested and weighed to determine tumor mass. Data in Fig. 1 are the combined results from two separate experiments (experiment 1, n = 5; experiment 2, n = 20). Data were analyzed by Student’s two-tailed t test.

**Growth in soft agar.** A layer of 1% agarose in normal growth medium was pipetted into six-well plates and allowed to solidify. Cells were suspended in 6.75 mL regular growth medium at 5,000 cells/ml and incubated for 15 min at 37°C. For assays involving inhibitors, inhibitor or control was added at the indicated concentration prior to incubation at 37°C. Seven hundred and fifty microliters of 2% agarose was then added to the plates, mixed thoroughly by pipetting, and 2 mL of the cell suspension was pipetted into triplicate wells. Plates were placed at 4°C for 15 min to facilitate rapid polymerization of the agarose, the wells overlaid with 2 mL of growth medium and incubated at 37°C/5% CO₂ for 17 days. The medium was replaced every 3 days, with or without inhibitor as appropriate. Colonies were counted in five random fields per well, and data are shown as the average number of colonies from five fields per well from triplicate wells (±SE).

**Migration assays.** Cells were plated at a high density (3 × 10⁴) in six-well culture plates with growth medium and grown to confluence (−24–36 h). Confluent cell monolayers were scratched using a sterilized 200 μL pipette tip, and the wells were washed twice with medium to remove loose cells. Images of the wound area were collected using a 10× objective at 0, 24, 48, and 72 h and the cell-free area quantified by tracing the open wound area using Adobe Photoshop. Bars represent the percentage of change in the cell-free area between the 0 and indicated assay time points, from triplicate wells (±SE).

**Western blot and immunoprecipitation.** Western blot was performed using standard techniques as described previously (14). For immunoprecipitation, cells were lysed on ice by the addition of an immunoprecipitation buffer [20 mmol/L Tris-HCl (pH 7.5), 150 mmol/L NaCl, 1 mmol/L Na₂EDTA, 1 mmol/L EGTA, 1% Triton, 2.5 mmol/L sodium pyrophosphate, 1 mmol/L glyceroophosphate, 1 mmol/L Na₃O₂, 1 μg/mL leupeptin, and 1 mmol/L phenylmethylsulfonyl fluoride] and the insoluble materials were removed by centrifugation. For coimmunoprecipitation experiments, we replaced the 1% Triton X-100 with 1% Nonidet P40. Lysates were precleared with protein A/G Sepharose beads (Amersham Pharmacia) for 30 min at 4°C. Antibodies were incubated with the lysates overnight at 4°C, and the immunocomplexes collected by incubation with protein A/G Sepharose beads for 1 h at 4°C. Immunocomplexes were washed thrice with lysis buffer at 4°C and the bead-associated proteins resolved by SDS-PAGE.

**Confocal microscopy.** Cells plated on coverslips were serum-starved for 4 h, fixed with 4% paraformaldehyde for 15 min, permeabilized with 0.05% Triton-X-100 for 5 min at room temperature, and blocked with 1% donkey serum for 1 h. Coverslips were incubated with the indicated primary antibodies overnight at 4°C. Appropriate Cy2- or Cy3-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories) were added separately. Propidium iodide was added for nuclei staining. Images were taken as described previously (14).

**Recombinant GST fusion proteins.** cDNA fragments encoding the complete MCSP cytoplasmic tail (amino acids 2247–2322) or the aminoterminal half of the MCSP tail (amino acids 2247–2286) were inserted into the bacterial expression vector pGEX-2T to generate the GST-MCSP tail fusion proteins used in pulldown assays. Recombinant GST-MCSP tail proteins were purified as described previously (10).

**GST pulldown assay.** Melanoma cells (1.0 × 10⁶) were plated in tissue culture plates and incubated overnight at 37°C. Cells were lysed by the addition of 1.0 mL of ice-cold Nonidet P40 lysis buffer, and the cell lysate precleared by incubation with glutathione Sepharose beads (Amersham Pharmacia) for 2 h at 4°C. Precleared cell lysates were mixed with 20 μL of GST-fusion protein-coupled beads and incubated for 2 h at 4°C with rotation. Beads were washed four times by centrifugation with ice-cold lysis buffer. Washed beads were mixed with an equal volume of 2× Laemmli buffer and the bead-associated proteins resolved by SDS-PAGE.

**Statistical analysis.** Statistical analysis was performed using the GraphPad Prism 4 software (GraphPad Software), with the assistance of the University of Minnesota Masonic Cancer Center Statistics Core, Minneapolis, MN.
Results

MCSP-stimulated tumor formation and anchorage-independent cell growth requires intact core protein. WM1552C parental cell line, which is MCSP-negative (Supplementary Fig. S1; ref. 14), was chosen as a model cell line in which to express and evaluate the function of the MCSP core protein. WM1552C MCSP null cells were stably transfected with full-length MCSP core protein or with an empty vector (mock). A portion of the transfected MCSP core protein is modified with chondroitin sulfate, as evidenced by the chondroitinase ABC-induced shift of the higher antibody-reactive band (Supplementary Fig. S1). Mock-transfected WM1552C cells were very poorly tumorigenic in subcutaneous xenograft assays; however, stable expression of the proteoglycan significantly increased both the tumorigenic potential and tumor growth of these RGP cells (Fig. 1A). The difference in tumorigenic potential was also correlated with activation of Erk1,2 and anchorage-independent growth in vitro (Fig. 1B). The colonies formed by WM1552C/MCSP cells were large and contained tightly packed cells, whereas cellular debris were detected in WM1552C/Mock cells with occasional evidence of cells that had undergone a few divisions. Several cell lines expressing endogenous MCSP were treated with siRNA to inhibit the expression of the MCSP core protein. These included WM1341D, WM164 (vertical growth phase), and 1205Lu and A375SM (metastatic) cell lines. Inhibiting MCSP expression in all of these cell lines reduced the activation of Erk1,2 and anchorage-independent growth of these cells, with the exception of MeWo cells, which express low-to-undetectable levels of endogenous MCSP (Fig. 1C and D).

WM1552C cells were also transfected with a construct of MCSP in which the cytoplasmic domain of MCSP was deleted (WM1552C/MCSP ΔCD). Expression of this construct resulted in the formation of both a core protein and a chondroitin sulfate–modified core protein.
In contrast to WM1552C/MCSP cells, WM1552C/MCSPΔCD failed to cause a sustained high level of activated Erk1,2 or form colonies under these conditions (Fig. 2A). WM1552C/MCSPΔCD cells also showed reduced tumor growth compared with the MCSP transfectants (Fig. 2B). MCSP produced an in vitro morphologic phenotype that was distinctly more spread and appeared less differentiated than either MCSPΔCD or mock transfectants when cultured in serum-free medium (Supplementary Fig. S2). MCSP transfectants exhibited sustained (i.e., 72 hours) constitutive activation of Erk1,2, and this high level of activation required the presence of an intact core protein (Fig. 2C). The sustained activation of Erk1,2 resulted in its translocation to the cell nucleus (Fig. 2D), which was not observed in cells expressing MCSPΔCD. To initially probe the role of MCSP in controlling Erk1,2 activity in melanoma cells, we first confirmed an association between Erk1,2 and MCSP in radial phase melanoma cells (Supplementary Fig. S3). MCSP coimmunoprecipitated with active Erk1,2 but this was not detected in immunoprecipitates of β1

Figure 2. Full-length MCSP, but not MCSPΔCD, promotes growth, tumor formation, and Erk1,2 activation. A, WM1552C parental and transfected cells were plated in 0.2% agarose for 17 d. Pictures are of representative colonies (bar, 20 μm). Colonies were counted as in Fig. 1B. Columns, average number of colonies per five fields in triplicate wells from one representative experiment; bars, SE (ANOVA P < 0.001; * P < 0.001 versus WM1552C/Mock cells by Bonferroni’s adjusted t test). B, cells (2 × 10⁶) were injected into the flank of NOD.SCID mice. Tumors were excised at 42 d post-injection and weighed to determine the tumor mass (*, P < 0.001, Student’s two-tailed t test; n = 10). C, serum-starved cells were suspended in serum-free 1.6% methylcellulose medium for the indicated time, and harvested for Western blot analysis (1, WM1552C/Mock; 2, WM1552C/MCSP; 3, WM1552C/MCSPΔCD). D, cells were cultured on glass coverslips in serum-free medium overnight, and the cells fixed and stained with antibody against phosphorylated Erk1,2 (pErk1,2, green) and propidium iodide (PI, red) to visualize the nucleus. The merged image (yellow) shows localization of phosphorylated Erk1,2 to the nucleus (bar, 20 μm).
integrin (Supplementary Fig. S3A). MCSP/Erk1,2 coprecipitation required a cytoplasmic domain because Erk1,2 was not detected in immunoprecipitates of MCSPΔCD (Supplementary Fig. S3B). A fusion protein containing the entire cytoplasmic domain (GST-MCSP2247–2322) also pulls down active Erk1,2 (Supplementary Fig. S3C), whereas none were detected when using either GST-MCSP2247–2286 (which lacks the carboxyl terminal half of the cytoplasmic domain) or GST alone (Supplementary Fig. S3D). The results are consistent with recently published data demonstrating that the carboxyl terminal half of the cytoplasmic domain of NG2 contains both an Erk docking site and a phosphoacceptor threonine residue (29).

**Stable expression of MCSP induces EMT changes in RGP cells.** Epithelial-to-mesenchymal transition is an important aspect of primary tumor progression that contributes to the initial invasion of tumor cells. EMT is complex, and involves increased motility, the shedding and loss of E-cadherin, and increased expression of mesenchymal markers such as fibronectin and vimentin. Because expression of MCSP caused a morphologic change in the WM1552C RGP cell line (Supplementary Fig. S2), we decided to compare several phenotypic markers of EMT in the MCSP transfectants. Transfectants were evaluated for the expression of two mesenchymal markers, fibronectin and vimentin (Fig. 3). Cells expressing intact MCSP exhibit easily detectable levels of fibronectin and vimentin by immunofluorescence (Fig. 3A) and Western blot analysis of cell extracts (Fig. 3B). By contrast, vimentin and fibronectin expression in either the mock transfectants or MCSPΔCD expressed levels of these proteins which were lower or not detectable.

WM1552C/MCSP cells also exhibited significantly higher levels of activated c-Met whereas the other two cell lines expressed very low levels of this tyrosine kinase receptor (Fig. 4A). MCSP also

![Figure 3](image-url)
increased the levels of endogenous HGF in a pattern similar to that observed for c-Met in these cell lines (Fig. 4B). Densitometry analysis (normalized to tubulin; data not shown) indicated that the addition of HGF to MCSP-expressing cells causes an ~2-fold increase in c-Met activation within 30 min compared with the Mock or MCSP\(\Delta CD\) cells. B, WM1552C-transfected cells were serum-starved for 24 h followed by transfection with nothing (1), negative control siRNA (2), or MCSP-specific siRNA (3) for 48 h, and cell lysates evaluated for HGF expression. C, WM1552C/MCSP cells were serum-starved for 24 h prior to transfection with nothing (1), negative control siRNA (2), or HGF-specific siRNA (3) for 48 h and cell lysates analyzed by Western blot. D, scratch wound migration assay of the indicated cell lines over 72 h in serum-free medium, 2.5 ng/mL rHGF, as indicated (bar, 100 \(\mu\)m).

Figure 4. MCSP stimulates an autocrine loop of HGF/c-Met that activates c-Met. A, mock (1), MCSP (2), and MCSP\(\Delta CD\) (3) transfected WM1552C cells were serum-starved for 24 h, stimulated with 2.5 ng/mL of rHGF for various times and cell lysates analyzed by Western blot. Densitometry analysis normalized to tubulin (data not shown) indicates that the addition of HGF to MCSP-expressing cells causes an ~2-fold increase in c-Met activation within 30 min compared with the Mock or MCSP\(\Delta CD\) cells. B, WM1552C-transfected cells were serum-starved for 24 h followed by transfection with nothing (1), negative control siRNA (2), or MCSP-specific siRNA (3) for 48 h, and cell lysates evaluated for HGF expression. C, WM1552C/MCSP cells were serum-starved for 24 h prior to transfection with nothing (1), negative control siRNA (2), or HGF-specific siRNA (3) for 48 h and cell lysates analyzed by Western blot. D, scratch wound migration assay of the indicated cell lines over 72 h in serum-free medium, 2.5 ng/mL rHGF, as indicated (bar, 100 \(\mu\)m).
Erk1,2 activation of this transcription factor facilitates the expression of c-Met (30–32). We next tested the possibility that MCSP stimulated both motility and growth by an Erk1,2/MITF/c-Met pathway. The addition of Mek-1 inhibitor U0126 decreased anchorage-independent growth of WM1552C/MCSP cells (Supplementary Fig. S5A and B). WM1552C/Mock transfectants were also stably transfected with constitutively active Mek-1 to cause sustained activation of Erk1,2 (Supplementary Fig. S5C) resulting in increased motility and growth (Supplementary Fig. S5D and E, respectively), which were both inhibited by U0126 (data not shown; Supplementary Fig. S5F). Elevated levels of MITF and c-Met expression/activation were almost completely inhibited by U0126 in WM1341D and WM1552C/MCSP cells, consistent with a model in which Erk1,2 stimulates increased levels of MITF (Fig. 6A). Treating WM1552C/MCSP cells with siRNA against MITF inhibited the expression of both MITF and c-Met (Fig. 6B). Furthermore, siRNA against c-Met in these cells had no inhibitory effect on the level of MITF (Fig. 6C). These data collectively support a model in which MCSP expression stimulates motility and growth by an activated Erk1,2/MITF/c-Met pathway.

Discussion

The factors that contribute to melanoma progression in primary tumors include changes in cell adhesion molecules, expression of growth factors and their cognate receptors, and associated signaling pathways. MCSP is a cell surface proteoglycan that is detected in the vast majority of melanomas as well as in benign dysplastic nevi (1) and has been associated with increased growth and motility in tumors (1, 3). It also interacts with a number of signal transduction pathway components important for tumor progression such as activated Erk1,2, activated focal adhesion kinase, and activated small GTPases associated with cytoskeletal reorganization (10, 11). The current studies extend these initial observations to include a function of stimulating changes in gene expression with a corresponding change in expression of HGF and expression/activation of c-Met, both of which are implicated in melanoma progression (33).

Stable transfection and expression of full-length MSCP core protein in the MCSP null WM1552C (RGP) cell enhances the level and duration of Erk1,2 activation. This is especially apparent in cells that are stressed by putting them into suspension in the...
absence of serum. The MCSP-induced increases in motility, growth, and activation of Erk1,2 can be reversed by using RNA interference for the MCSP core protein. The MCSP-induced activation of Erk1,2 is required for subsequent changes in anchorage-independent growth and cell motility \textit{in vitro}. This is supported by the ability of the U0126 Mek-1 inhibitor to reverse MCSP-induced increased growth and motility, and by the ability of constitutively active Mek-1 to bypass the requirement for MCSP expression. Furthermore, inhibiting the expression of endogenous MCSP in vertical growth phase melanoma cells (WM1341D and WM164) and metastatic melanoma cells (A375 and 1205Lu) reduces sustained activation of Erk1,2 and anchorage-independent growth. This indicates that the ability of MCSP to cause sustained activation of Erk1,2 is preserved throughout progression and this may be one explanation for its widespread expression in human melanomas.

The mechanisms by which MCSP facilitates constitutive activation of Erk1,2 remain to be defined. MCSP-mediated sustained activation of Erk1,2 requires the cytoplasmic domain of the core protein. The MCSP core protein cytoplasmic domain is conserved with that of NG2 which contains both Erk docking and phosphoacceptor sites (29). The MCSP core protein or a recombinant fusion protein containing the cytoplasmic domain both coprecipitate/pull down activated Erk1,2. The total amount of Erk1,2 that coprecipitates with the proteoglycan is a very small amount (<5%) of the total activated Erk within the cell, suggesting that only a portion of total Erk1,2 interacts with MCSP. This raises the possibility that MCSP/Erk1,2-related interactions may be controlled by a subpopulation of activated Erk1,2 that could depend on specific scaffolds which help to assemble Erk1,2 pathway components within different subcellular compartments (28). Although the MCSP core protein can interact with Erk1,2, the core protein cannot directly activate Erk1,2 indicating that MCSP must be integrated with other pathways that can directly lead to Erk1,2 activation.

The Ras/Raf/MEK/ERK pathway is a key regulator of melanoma cell proliferation, which is hyperactivated in the vast majority of human melanomas (19). BRAF, which can be constitutively activated by several mutations in melanomas, stimulates constitutive ERK signaling in tumor cells (19, 21). Sequence analysis of BRAF in WM1552C and WM1341D melanoma cell lines indicate that both cells express constitutively activating mutations, V600E and V660R, respectively. Furthermore, kinase assays also showed that both cell lines express constitutively active BRAF (data not shown), indicating that sustained activation of Erk1,2 also requires the expression of the intact MCSP core protein. WM164 and 1205Lu cell lines, both of which also express mutant active BRAF, exhibit the same dependence on MCSP expression for Erk1,2 activation (21, 34). Although mutant active BRAF is associated with constitutive activation of Erk1,2, the action of growth factors has been shown to work in concert with this mutation to cause the strong and sustained activation of the Erk1,2 pathway (21). Constitutively active Mek-1 can phenocopy the effect of the proteoglycan on Erk1,2 activation, growth, and motility, indicating that the core protein per se is not absolutely required for these phenotypic changes. The data favor a model in which the proteoglycan core protein can facilitate the assembly of elements of the Erk1,2 signaling pathway stimulated by upstream activators such as growth factors and/or constitutively active BRAF. Confocal analysis shows that BRAF codistributes at the plasma membrane with MCSP (data not shown), However, neither BRAF nor Mek-1 were detected in immunoprecipitates of the proteoglycan, indicating that the interaction of BRAF and/or Mek-1 with MCSP may be indirect or unable to survive the immunoprecipitation conditions.

Inhibiting c-Met or HGF expression with RNA interference inhibited the ability of MCSP to enhance motility and growth. The results also show that MCSP enhances the total levels of c-Met by 5-fold to 10-fold, as well as increasing MITF, a transcription factor

Figure 6. Erk1,2 mediates enhanced c-Met expression through MITF. A, WM1341D and WM1552C/MCSP-transfected cells were incubated with 2.4 μmol/L of U0126 or control compound U0124 overnight in serum-free medium. Lysates were evaluated by Western blot using the indicated antibodies. B, WM1552C/MCSP cells were transfected with MITF-specific or control siRNA for 48 h and assayed for expression of c-Met, MITF, and pErk1,2 by Western blot. C, WM1552C/MCSP cells were transfected with c-Met–specific or control siRNA for 48 h and assayed for expression of c-Met, MITF, and pErk1,2 and MCSP by Western blot.
which acts as a key regulatory factor for transcriptional regulation of c-Met expression in melanoma (30). MCSP-induced activation of Erk1,2 leads to the nuclear translocation of activated Erk1,2 in which it could influence MITF transcription. Furthermore, a MeK-1 inhibitor reverses MCSP-induced elevations in the level of MITF and cells that express the MCSP∆CD mutant (which minimizes the activation of Erk1,2) do not express elevated levels of HGF or activated c-Met. As expected, siRNA interference with MITF expression in WM1552C/MCSP cells also inhibit c-Met expression/activation. Finally, inhibiting MCSP expression in a number of cell lines causes a decreases in the activation of Erk1,2 and the level of activated c-Met indicating that epigenetic changes in these cells produced by MCSP include expression/activation of the c-Met receptor.

An inverse correlation between HGF and the level of E-cadherin suggests that HGF could induce rapid shedding of E-cadherin (35, 36). Down-regulation of E-cadherin in transformed cell lines has been associated with the acquisition of the ability to migrate and invade, which are two features of EMT. MCSP-transfected serum-starved WM1552C RGP cells, which express a relatively high (5-fold to 10-fold increase compared with mock or MCSP∆CD cells) level of c-Met, exhibit an almost complete loss of E-cadherin when a low amount of additional HGF is added to the culture medium. By contrast, this level of HGF suggests that only a partial inhibition of E-cadherin in the mock-transfected and MCSP∆CD mutant cell lines, a result we interpret as indicative of the lower overall level of total and activated c-Met in these two cell lines. MCSP expression in the WM1552C RGP cells also facilitates increased expression of fibronectin and vimentin, which are two phenotypic markers of EMT (37, 38). Thus, MCSP could function in a primary RGP tumor to stimulate the increased motility and invasion associated with EMT.

Previous studies have focused on the function of MCSP as a cell surface adhesion molecule that can signal directly or indirectly to alter the activation of other adhesion receptors (1). The current studies indicate that MCSP can also regulate invasion and growth by an epigenetic mechanism and it may play a dynamic role in melanoma progression by enhancing the expression or function of progression-associated gene products. The current findings show that MCSP stimulates EMT-related changes in RGP cells and additional MCSP-induced epigenetic changes would be anticipated in melanoma cells isolated from different stages of progression. These studies indicate that MCSP is required to cause a robust and sustained activation of Erk1,2 in several melanoma cell lines that express mutant active BRAF by a mechanism which remains to be determined. Although activating BRAF mutations have been the subject of intensive investigation as a therapeutic target, these studies suggest that MCSP is an equally viable target in the treatment of melanoma.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

Received 12/18/08; revised 6/18/09; accepted 7/10/09; published OnlineFirst 9/8/09.

Grant support: National Cancer Institute grants B01 CA82295 and B01 CA92222 (J.B. McCarthy).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked ‘advertisement’ in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

References

[References list is not provided in the image.]


34. Smalley KS, Haass NK, Brafford PA, Lioni M, Flaherty KT, Herlyn M. Multiple signaling pathways must be targeted to overcome drug resistance in cell lines derived from melanoma metastases. Mol Cancer Ther 2006;5:1136–44.


Melanoma Proteoglycan Modifies Gene Expression to Stimulate Tumor Cell Motility, Growth, and Epithelial-to-Mesenchymal Transition

Jianbo Yang, Matthew A. Price, Gui Yuan Li, et al.


Updated version
Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-08-4626

Supplementary Material
Access the most recent supplemental material at:
http://cancerres.aacrjournals.org/content/suppl/2009/09/08/0008-5472.CAN-08-4626.DC1

Cited articles
This article cites 38 articles, 17 of which you can access for free at:
http://cancerres.aacrjournals.org/content/69/19/7538.full.html#ref-list-1

Citing articles
This article has been cited by 8 HighWire-hosted articles. Access the articles at:
/content/69/19/7538.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.