Increased class 3 semaphorin expression modulates the invasive and adhesive properties of prostate cancer cells

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Abstract. The class 3 semaphorins, sema3A and sema3C, provide important guidance cues in cell development and in cancer; however, the role of these semaphorins in prostate cancer is not known. We report here that sema3A transfected cells exhibit decreased invasion and adhesion in Matrigel-based assays and that sema3C transfected cells exhibit increased invasive and adhesive characteristics. Important adhesion proteins were differentially modulated in sema3A and sema3C cells in a manner consistent with their subsequent invasive and adhesive characteristics. E-cadherin expression as determined by Western blot analysis was strongly upregulated in sema3A transfected cells, but strongly downregulated in sema3C transfected cells compared to untransfected and mock empty vector-transfected PC-3 cells. ß-catenin levels were not changed in sema3A transfected cells; however, sema3C transfected cells had lower expression of this protein. Sema3C transfected cells exhibited greater cellular membrane expression of certain α integrins as compared to untransfected and sema3A transfected cells, a characteristic associated with increased adhesion and invasion. These data indicate that the invasive ability of sema3A and sema3C transfected PC-3 cells is, in part, correlated with adhesion protein expression and adhesive ability to constituents of neighboring cells and the extracellular matrix.

Introduction

Semaphorin 3A (sema3A) and semaphorin 3C (sema3C) are two members of the class 3 semaphorins, a subfamily of highly conserved proteins originally described as guidance cues for developing neurons in the central and peripheral nervous systems (1-5). Expressed in many neuronal and non-neuronal cells, semaphorins regulate numerous pathways involved in proliferation, adhesion, migration, and apoptosis (6-8). It is becoming increasingly evident that sema3A and sema3C, as well as other class 3 semaphorins play an important role in cancer; however, their role is not well-established (9-11).

Sema3A and sema3C often have opposing cellular effects, acting as either attractant or repellant guidance cues respectively during development (8,12,13), and this has also been observed with certain cancers (9,14-17). Sema3A decreases migration, adhesion and disrupts angiogenesis in some cancers (14,18,19), while increased expression of sema3C correlates with cancers that possess higher invasive and metastatic characteristics (7,9,16,17). The role of sema3A and sema3C in androgen-independent prostate cancer has not been studied extensively and their effects in prostate cancer are not known.

Previously we found that the mRNA for sema3A, sema3C, and other class 3 semaphorins was expressed in non-malignant and malignant prostate cells, including the highly invasive androgen independent PC-3 cell lines (Herman *et al*, Proc Am Assoc Cancer Res 45: abs. 5081, 2004). The present study examined the novel role of sema3A and sema3C overexpression on the invasive characteristics of the androgen-independent PC-3 prostate cell line. We found that the regulation of invasion in response to sema3A and sema3C overexpression strongly correlated with the adhesive abilities of these cells. Sema3A overexpression correlated with decreased invasion and adhesion, while sema3C overexpression correlated with increased invasion and adhesion. The expression of several adhesion or adhesion-related proteins, including integrins, E-cadherin and ß-catenin, were differentially modulated in cells that exhibited increased expression of sema3A and sema3C, indicating an underlying mechanism by which these semaphorins can regulate invasion and adhesion.

Materials and methods

Cell culture. PC-3 prostate cancer cells were obtained from the American Type Culture Collection (Manassas, VA). PC-3 cells were cultured in RPMI-1640 (Life Technologies, Grand Island, NY) containing 10% filter sterilized fetal bovine serum [(FBS), Equitech-Bio Inc., Kerrville, TX] in a tissue culture incubator at 37˚C in 5% CO_2 in 75-cm^2 tissue culture flasks (Sarstedt Inc., Newton, NC).

RT-PCR and PCR. Total RNA was extracted from cultured prostate cells using TRIzol (Invitrogen, Carlsbad, CA) (20). First-strand DNA synthesis was performed using a DT18 oligo primer custom manufactured by Invitrogen and

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Moloney murine leukemia virus (MMLV)-reverse transcriptase (Promega, Madison, WI) (20). The cDNA was amplified in a polymerase chain reaction (PCR) with gene specific primers that were based on the mRNA coding sequence of sema3A and sema3C with the addition of *Bam*HI and *Not*I restriction sites. The cDNA primer sequence for sema3A is 5'-ATTGCG GCCGCATGGGCTGGT-3'/5'-TAGATGGGATCCGACAC TCCTGGGTGC-3'. The cDNA primer sequence for sema3C is 5'-CTTAGCGGCCGCATGGCATTCCGGACAA-3'/5'-GATGGATCCTGACTCTGGCAACTGATTC-3'. The cDNA sequences for sema3A and sema3C were amplified by PCR. The samples were initially denatured at 95˚C for 5 min, followed by 40 amplification cycles. The first 10 amplification cycles consisted of a denaturation step (95˚C for 30 sec), primer annealing (60˚C and 58˚C for sema3A and sema3C respectively for 30 sec) and extension (72˚C for 2 min). The next 30 amplification cycles consisted of denaturation (95˚C for 30 sec), primer annealing (60˚C or 58˚C) and extension (72˚C for 2 min), and increased by 10 sec with each subsequent cycle. The 40 amplification cycles were followed by a final extension period of 10 min at 72˚C to ensure full extension and proper amplification of the sema3A and sema3C cDNA products. The cDNA products were electrophoresed onto a 2.2% agarose gel containing ethidium bromide using a 150 V current and visualized under ultraviolet light. Hypoxyanthine-guanine phosphoribosyltransferase (HPRT, 5'-GAGAGTTC AAGTTGAGTTTG-3'/5'-TTTTCTAACACACGGTGGTA-3') was used as an internal control.

Ligation and transfection. Ligation and transfection were performed as described previously (21). The amplified sema3A and sema3C PCR products were cleaved using *Not*I and *Bam*HI restriction enzymes obtained from Promega and ligated into the pCMV-Tag1 vector from Stratagene (La Jolla, CA) according to the manufacturer's specifications. The pCMV-Tag1 vector contains a kanamycin and G418 resistance gene for transfection of bacteria and mammalian cells respectively. The vectors containing sema3A and sema3C were transfected into competent XL1-Blue bacteria from Stratagene (22). The bacterial clones transfected with sema3A or sema3C were grown on agar plates and selected with 35 μg/ml of kanamycin (23). PCR was then used to confirm the presence of the specific semaphorin genes. The vector with the gene of interest was purified using the Concert Nucleic Acid Purification system from Marligen (Ijamsville, MD). The gene/vector was then transfected into PC-3 cells using the Transit Prostate Transfection Reagent (Mirus Bio Corporation, Madison, WI). Stably transfected cells were selected with 0.5 μg/ml G418 (Invitrogen) (24). As a control for transfection, a mock empty vector (mEV), pCMV-Tag1 vector from Stratagene without any inserted PCR product was transfected into the cells and similarly selected.

RNA slot blot analysis. Briefly, cultured PC-3 cells were harvested from tissue culture dishes using scrapers after washing the cell monolayers with 0.1% diethylpyrocarbonate (DEPC)-treated phosphate-buffered solution (PBS, 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.47 mM KH_2PO_4) (21). Cells were transferred to a 50-ml tube and centrifuged at 2000 rpm (600 x g). mRNA was extracted and purified using the Invitrogen Micro-FasTrack 2.0 system according to the manufacturer's specifications. mRNA was used in an RT-PCR and subsequent PCR with biotin-labeled CTP to create gene-specific probes to confirm semaphorin expression by slot blot analysis as previously described (25). Briefly the total RNA extracted from the above cells was transferred onto a nylon membrane (Osmonics Inc., Minnetonka, MN) using a slot blot manifold apparatus. The RNA was linked and stabilized to the membrane using a UV-Stratalinker 1800 (Stratagene). The membrane was hybridized with an appropriate cell specific cDNA probe and detected with alkaline-phosphatase streptavidin and CDP-Star from Kirkegaard & Perry Laboratories Inc. (Gaithersburg, MD). The RNA was visualized via X-ray film. Differences in density of sema3A and sema3C RNA were determined in untransfected cells, semaphorin transfectants, and mEV cells as compared to HPRT used as an internal control (Herman *et al*, Proc Am Assoc Cancer Res 44: abs. 2674, 2003).

Growth and viability assay. Untransfected PC-3 cells and sema3A-, sema3C-, and mEV-transfected PC-3 cells were plated in 60x15-mm tissue culture dishes at a concentration of $2x10^5$ cells and growth was measured over a span of four days (26). Cells were trypsinized, collected, and counted using the Vi-Cell XR cell counter (Beckman Coulter, Fullerton, CA). Cell viability was determined by the cell counter using trypan blue exclusion (27). Cell counts and cell viability determinants were performed in triplicate for each time-point, and the experiments were repeated twice with similar results.

Chemoinvasion assay. The invasion assay was performed as described previously using a microinvasion chamber (28,29). Briefly, cells were collected at 50-70% confluency. Cells (100,000) were incubated in the upper wells suspended in serum-free media with 0.1% bovine serum albumin (BSA). The lower wells contained complete growth media with 10% FBS, which was used as a chemoattractant to facilitate invasion. An 8-μm porous Nucleopore Track Etch Membrane (Whatman, Alameda, CA) was coated with 50 μg/ml of Matrigel (BD Biosciences, Bedford, MA), separating the upper and lower wells (28,29). PC-3 cells were incubated for 20 h at 37˚C in the tissue culture incubator. Cells that invaded through the Matrigel to the underside of the membrane were counted using a light microscope after being fixed in methanol and stained with Diff-Quik (Dade Behring Inc., Deerfield, IL). Approximately six optical fields were counted per well at magnification x400. Experiments were repeated four to five times and the results were averaged. The results were presented as the percentage of invasion of the semaphorin transfected cells compared to untransfected PC-3 cells ± SEM.

Adhesion assay. Adhesion of PC-3 cells was measured as previously described with some modifications (28,30). Each well of a 24-well plate was coated with 100 μg/ml of Matrigel. In each well, $2x10^4$ cells were added in 0.5 ml serum-free media supplemented with 0.1% BSA. The plates were incubated at 37˚C and adhesion was determined at 20, 30, 45, and 60 min. The plates were fixed with methanol, stained with Diff-Quik and washed with PBS. Adhesion of cells to Matrigel was counted in five random fields per well in duplicate at

Figure 1. Sema3A and sema3C overexpression in PC-3 cells measured by RNA slot blot analysis. PC-3 cells cultured in RPMI-1640 + 10% FBS were harvested at 50-70% confluence. Total RNA was extracted from semaphorin transfected, mEV, and untransfected cells using TRIzol and was transferred to a nitrocellulose membrane using an RNA slot blot manifold. The RNA samples were stabilized by ultraviolet light. The membrane was hybridized with biotin linked with cell specific PCR probe, and HPRT was used as a loading control.

magnification x400, and the results were averaged. The experiments were repeated twice. All transfected cells were compared to untransfected PC-3 cells.

Western blot analysis. E-cadherin and ß-catenin levels were determined by Western blot analysis as previously described (28). Briefly, attached PC-3 cells were washed with PBS and collected by scraping. The cells were transferred to a 50 ml conical tube and centrifuged at 2000 rpm (600 x g) for 10 min. The cell pellet was collected and lysed with an ice cold buffer (100 mM NaCl, 10 mM Tris-HCl, pH 7.4, 1% Triton X-100, 0.1% SDS, 0.5% deoxycholate, 10% glycerol) supplemented with 10 μl of Protease Inhibitor Cocktail, 10 μl Phosphatase Inhibitor Cocktail I and 10 μl Phosphatase Inhibitor Cocktail II all from Sigma (St. Louis, MO). The lysate was centrifuged at 8765 x g for 10 min. The supernatant was collected and the protein concentration was determined using the Bradford assay (Bio-Rad Laboratories, Hercules, CA) as described by the manufacturer. Equal amounts of protein lysate were added to a denaturing loading dye (125 mM Tris-HCl, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol, 0.004% bromphenol blue) and boiled for 5 min. The samples were separated by 4-10% SDS gradient PAGE gel electrophoresis and electro-transferred to a nitrocellulose membrane (Osmonics Inc.). The nitrocellulose membrane was immunoblotted with polyclonal antibodies against E-cadherin and ß-catenin obtained from Cell Signaling Technology (Danvers, MA). Membranes were also immunoblotted with anti-glyceraldehyde 3-phosphate (GAPDH) obtained from Abcam (Cambridge, MA). GAPDH was used as an internal loading control. The relative amount of protein in semaphorin transfectants was expressed as a percentage of untransfected cells.

Integrin expression. Cell surface integrin expression was measured by flow cytometry as described previously (31). Sema3A and sema3C transfected PC-3 cells and untransfected cells were collected and suspended in PBS supplemented with 0.1% sodium azide and stained with fluorescein isothiocyanate (FITC)-or phycoerythrin (PE)-labeled monoclonal antibodies against the following human integrin subunits: α1, α2, α3, α4, α5, α6, αv, ß2, and ß3 (BD Pharmingen, San Diego, CA). Ten thousand events were analyzed by flow cytometry on a FACScan (BD Biosciences) for each sample. FITC- and PE-labeled IgG1 were used to identify nonspecific background staining.

Statistical analyses. Statistical analysis of invasion was determined using One-way ANOVA followed by Bonferroni's multicomparison test. Statistical analysis of adhesion was determined using a two-way ANOVA followed by Bonferroni's multicomparison test. Statistics were performed using GraphPad Prism version 4.0 software (San Diego, CA). Values were considered significant at $P \leq 0.001$.

Results

Transfection and growth assay. Fig. 1 shows that the sema3A and sema3C stably transfected PC-3 cells express a 2-fold increase in mRNA compared to untransfected cells. The mock mEV control vector transfected into PC-3 cells did not show any difference in semaphorin expression compared to untransfected cells (Fig. 1). We were unable to determine protein expression by Western blot analysis of the two semaphorins in the transfected cells using available commercial antibodies.

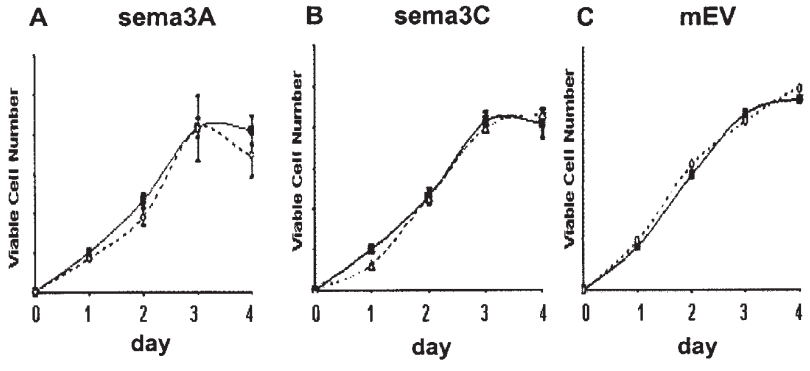

Figure 2. Effect of sema3A, sema3C, and mEV transfection on cell growth. Cells were cultured in RPMI-1640 + 10% FBS. Semaphorin and mEV transfected cells were further cultured in medium containing 0.5 mg/ml of G418. Cells were plated at a seeding density of 2.5×10^5 in 60x15-mm tissue culture dishes. Three separate dishes were utilized for each cell line and time-point. At the indicated times the cell monolayers of each dish were trypsinized, the cells washed with PBS, and then counted with a Vi-Cell XR cell counter. Cell counts were collected daily for 4 days. Fifty counts per sample were analyzed by the cell counter and averaged for each time-point, and this was performed in triplicate for each group. The experiments were performed twice and the graphs represent the mean ± SEM of the two growth assays. A, sema3A; B, sema3C; and C, mEV, all compared to untransfected PC-3 cells. Transfectants are represented by a dashed line and open shapes, and untransfected cells are represented by the black line with solid shapes.

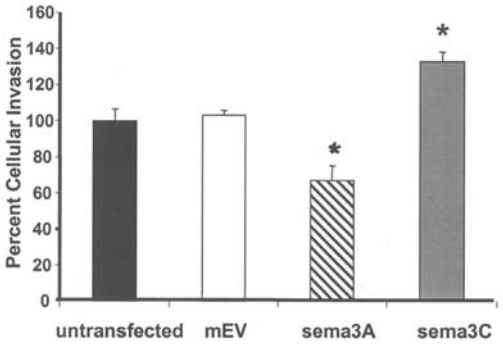

Figure 3. Sema3A and sema3C upregulation differentially modulate invasion of PC-3 cells. The invasion assay was performed as described previously (28). Cells were grown in RPMI-1640 + 10% FBS and harvested at 50-70% confluence. They were then resuspended in 50 μl serum-free cell medium supplemented with 0.1% BSA and seeded into the upper wells of a micro-invasion chamber, separated from the lower wells containing FBS by an 8-μm porous membrane coated with 50 μg/ml Matrigel. Cells invading to the underside of the membrane were stained with Diff-Quik and counted. Six random fields were counted and the results were averaged. The mean ± SEM from three separate experiments are presented as a percentage of cellular invasion of semaphorin transfectants compared to untransfected controls. Invasion of untransfected cells was set at a baseline level of 100%. *Different from untransfected cells at P≤0.001. Black bar, untransfected cells; white bar, mEV; hatched bar, sema3A transfected cells; and gray bar, sema3C transfected cells.

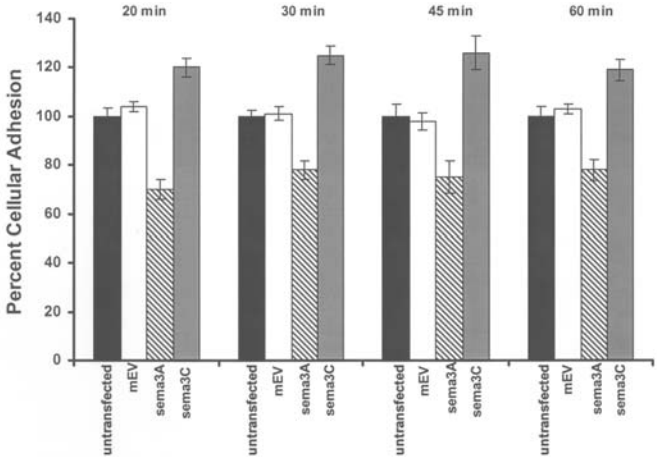

Figure 4. Effect of sema3A and sema3C overexpression on the adhesive characteristics of PC-3 cells. Each well of a 24-well plate was coated with 100 μg of Matrigel. Twenty thousand cultured cells were resuspended in 500 μl of RPMI-1640 + 0.1% BSA per well. Adherent cells were counted at 20, 30, 45 and 60 min. For each time-point, five random fields were counted in two separate wells and the results were averaged. The experiments were repeated twice. The mean ± SEM from the two experiments is presented as a percent of cellular adhesion compared to untransfected cells. Adhesion of untransfected cells was set at a baseline level of 100%. *Different from untransfected cells at P≤0.001. Black bar, untransfected cells; white bar, mEV; hatched bar, sema3A transfected cells; and gray bar, sema3C transfected cells.

The growth rates of stable semaphorin and mEV transfectants were comparable to the growth rate of untransfected cells (Fig. 2). Cellular viability of the transfected cells was ~95% and did not show any significant differences compared to untransfected cells. These data indicate that neither semaphorin transfection nor semaphorin overexpression significantly affect PC-3 cell growth or viability.

Overexpression of sema3A decreases and overexpression of sema3C increases the invasive and adhesive characteristics of PC-3 cells. Transfection of sema3A and sema3C altered the invasive characteristics of PC-3 cells (Fig. 3). Overexpression of sema3A in PC-3 decreased the invasive characteristics of PC-3 cells by 33% compared to the untransfected cells. Sema3C, on the other hand, increased invasion by 33% compared to untransfected cells. Invasion of mEV cells was not significantly different from untransfected cells.

Interaction of cancer cells with various key components of the extracellular matrix (ECM) and neighboring cells is a vital regulator of invasion (32-34). The regulation of invasion in response to semaphorin expression strongly correlated with adhesion. Adhesion of PC-3 cells to Matrigel was measured at time-points between 20-60 min. Cellular adhesion did not differ at any of the time-points. Sema3A overexpressed PC-3 cells exhibited decreased adhesion to Matrigel by ~23% at 30 min, while adhesion was increased in sema3C transfected cells by 20% at 30 min (Fig. 4).

E-cadherin and ß-catenin expression are differentially regulated in sema3A and sema3C overexpressing PC-3 cells. To understand the underlying mechanism by which semaphorins regulate invasion and adhesion of PC-3 cells, we measured the expression of E-cadherin and ß-catenin in sema3A and sema3C transfected cells. E-cadherin and ß-catenin

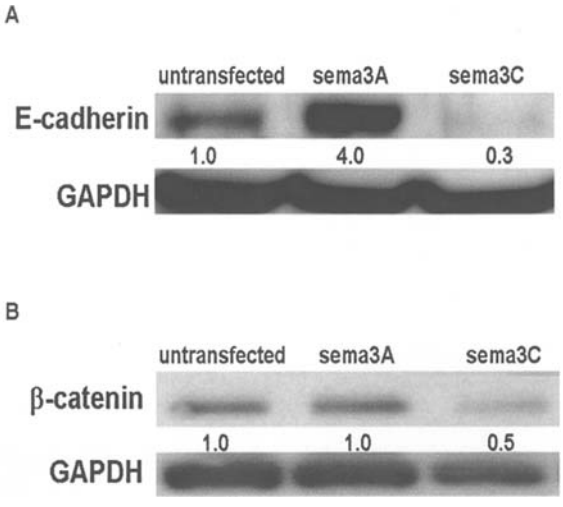

Figure 5. E-cadherin and ß-catenin regulation in sema3A and sema3C transfected PC-3 cells. Cellular protein lysates from untransfected and sema3A and sema3C transfected PC-3 cells were collected, separated by 10% SDS PAGE electrophoresis, transferred to a nitrocellulose membrane, and expression was determined by Western blot analysis. A, total E-cadherin; B, ß-catenin. GAPDH protein expression was used as an internal control. The numbers represent the relative density of protein expression in semaphorin transfected cells normalized to GAPDH compared to normalized protein expression in untransfected cells.

are two important adhesion proteins that function together to form an important transmembrane cell-cell adhesion complex with important anti-invasive properties in prostate cancer (35-39). E-cadherin expression was upregulated 4-fold in PC-3 cells overexpressing sema3A compared to untransfected controls (Fig. 5A); however, ß-catenin levels were not altered (Fig. 5B). Sema3C transfected PC-3 cells exhibited a >3-fold

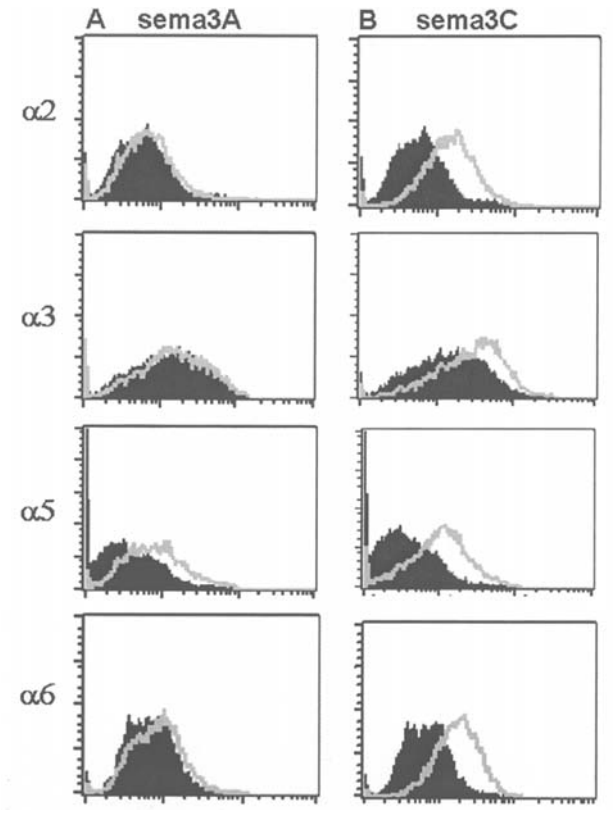

Figure 6. Differential integrin expression in sema3A and sema3C transfected PC-3 cells. Integrin expression was determined in untransfected and in sema3A and sema3C transfected PC-3 cells by flow cytometry using fluorescein isothiocyanate (FITC)-labeled or phycoerythrin (PE)-labeled monoclonal mouse antibodies raised against human integrin proteins, α2, α3, α5, and α6. A, sema3A; and B, sema3C expression compared to untransfected PC-3 cells. The filled black histograms represent untransfected cells and the gray line represents respective sema3A and sema3C transfected cells.

decrease in E-cadherin expression (Fig. 5A) and a 2-fold decrease in ß-catenin levels (Fig. 5B).

Differential integrin expression in sema3A and sema3C over-expressing PC-3 cells. Integrins are essential transmembrane proteins that mediate cellular adhesion to the ECM and are often deregulated in cancer, leading to changes in invasion and metastasis (40,41). Untransfected or transfected PC-3 cells did not express α1, α4, αv, ß2, or ß3 integrins on their surface. Cells overexpressing sema3A exhibited an increased surface expression of the α5 integrin, but did not show any difference in expression of α2, α3, or α6 integrins (Fig. 6A). Membrane integrin levels were expressed differently in sema3C transfected cells as compared to sema3A transfected cells. These cells exhibited increased expression of α2, α3, α5, and α6 integrins (Fig. 6B).

Discussion

Prostate cancer is a severe epidemic and one of the fastest rising neoplasms in the United States. It is a leading cause of cancer-related deaths in men, second only to that of lung cancer (42). Prostate cancer is a very slow growing disease; however, malignant prostate cells often invade and metastasize to various organ systems, including the lung, bone and brain, severely increasing patient mortality (32,43-46).

Previously, we found that several semaphorins, including sema3A and sema3C were expressed in cultured malignant as well as non-malignant prostate cells. During development, sema3A and sema3C, two conserved members of the vertebrate class 3 semaphorin family, often act in an opposing fashion as a chemorepellant or chemoattractant, respectively (6,8,12). This signaling dichotomy of attraction and repulsion in response to sema3A and sema3C in conjunction with other guidance proteins is essential for the proper development of various organ systems (6,8,12). Sema3A and sema3C also have opposing roles in cancer (7,9,16,17).

The role of sema3A and sema3C in invasion has not been studied extensively. Focusing on the highly invasive androgen-independent PC-3 prostate cancer cell line, we show that sema3A and sema3C regulate invasion and adhesion in an opposing fashion. Sema3A transfected PC-3 cells exhibit decreased invasive and adhesive characteristics compared to untransfected and mEV cells, while sema3C transfected cells exhibited increased invasion and decreased adhesion compared to the same control cells. These data are consistent with other reports indicating that sema3A upregulation decreases adhesion of endothelial cells (47) and that increased sema3A expression inhibits breast cancer migration (48). In addition, increased expression of sema3C is correlated with increased lung cancer metastasis (7) and decreased survivability of patients with breast or ovarian cancer (17).

In preliminary studies, we observed that invasion of another androgen-independent prostate cancer cell line, DU145, was similarly modulated by sema3A and sema3c transfection as compared to PC-3 cells (not shown). Transfection of non-invasive, androgen-dependent LNCaP prostate cells with sema3A and sema3C did not alter the invasive characteristics of these cells (not shown). These findings indicate that androgen-dependent and -independent prostate cancer cells respond differently to upregulation of sema3A and sema3C. This could suggest that the functional androgen receptor is involved in the regulation of sema3A and sema3C signaling in androgen-dependent prostate cancer. Although it is known that expression of neuropilin, a receptor for class 3 semaphorins, is upregulated by progesterone (49), a connection between hormones, the androgen receptor, and semaphorins has never been studied among the different prostate cancer cell lines.

Changes in invasion often correlate with differences in regulation of important cell-cell and cell-ECM adhesion proteins (40,41). Cell-cell and cell-matrix proteins are necessary for the proper maintenance of tissue integrity (40,41). These adhesion proteins also have important roles in wound healing; however, if they become deregulated, this can lead to increased malignancy (40,41). In this study we showed that semaphorin transfected cells differentially express important cell-cell (E-cadherin/ß-catenin) and cell-ECM (integrin) proteins. The differences in these cell adhesion proteins between the sema3A and sema3C transfected PC-3 cells strongly correlate with their subsequent invasive and adhesive characteristics. E-cadherin expression is upregulated 4-fold in sema3A-transfected PC-3 cells, although ß-catenin levels are not changed compared to untransfected cells. Upregulation of E-cadherin is correlated with decreased invasion of PC-3

cells (37-39). In contrast, sema3C transfected cells exhibited a strong downregulation of E-cadherin and ß-catenin proteins as compared to untransfected cells. Decreased E-cadherin levels are thought to be a prognostic factor in the progression of malignant cancers to an increased metastatic stage (37,50). E-cadherin expression is also lower in the highly invasive PC-3 cells compared to normal cells (37,51).

While E-cadherin and ß-catenin are important in the regulation of invasion and adhesion of sema3A and sema3C transfected PC-3 cells, integrins, an important class of cell-ECM adhesion proteins, also play a role. Integrin expression and activity is deregulated in PC-3 cells, other forms of prostate cancer, and various other types of neoplastic tissue, leading to aberrant adhesion and invasion (52-55). The level of integrin expression is relatively unchanged in sema3A transfected cells except for an increase in α5 levels compared to untransfected cells. Sema3C also exhibit a similar increase in α5 levels. The exact role that α5 integrin has in semaphorin signaling and prostate cancer invasion is unclear. It is reported that DU145 cells transfected with the androgen receptor exhibit increased surface expression of α5 integrin, and that this correlates with decreased invasion across a Matrigel-coated membrane (56). Although these data might only be pertinent to the androgen receptor-transfected DU145 cells, they could indicate a possible mechanism by which sema3A transfected cells exhibit lower invasive and adhesive properties. However, increased surface expression of α5 integrin alone does not explain the increased invasion and adhesion that is observed in sema3C transfected cells.

In addition to increased cell surface expression of α5 integrin, sema3C transfected cells showed a marked increase in cell surface expression of α2, α3, and α6 integrins, and this pattern is consistent with increased invasion of PC-3 cells. Increased expression of these integrins is correlated with increased adhesion, invasion and metastasis (53,57-60). Increased α2 and α3 expression correlates with increased adhesion of PC-3 cells (61-64). Increased α3 and α6 integrin expression is also indicative of increased invasion and more aggressive prostate tumors (60,65,66). Since sema3C transfected cells exhibit increased expression of α2, α3 and α6, three integrins that correlate with increased malignancy and invasion, this could overshadow the anti-invasive effects of α5 integrin increased expression (53).

The increased surface expression of α2 and α3 integrins in sema3C transfected cells correlates strongly with decreased expression of E-cadherin. It was recently found that down-regulation of E-cadherin leads to a significant increase in α2 and α3 integrin levels in a squamous cell carcinoma (67). In these cells, changes in integrin expression and E-cadherin expression mediate increased invasion (67). These data suggest that sema3C upregulation in PC-3 cells could regulate invasion and adhesion through crosstalk between cell-cell and cell-ECM adhesion pathways.

In this study we showed a novel regulation of sema3A and sema3C on prostate cancer invasion. We found a strong association between cell-cell (E-cadherin/ß-catenin) and cell-ECM (integrin) adhesion proteins and invasion of sema3A and sema3C transfected PC-3 cells. These cells display opposing characteristics with sema3A transfected cells exhibiting decreased invasion and adhesion and sema3C transfected cells exhibiting increased invasion and adhesion. With additional understanding of their ability to regulate invasion, targeting specific semaphorin proteins could lead to the development of new anti-cancer therapies.

Acknowledgements

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