

# Syndecan-2 Functions as a Docking Receptor for Pro-matrix Metalloproteinase-7 in Human Colon Cancer Cells\*

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Heui-Young Ryu<sup>†1</sup>, Jiseon Lee<sup>†1</sup>, Sanghwa Yang<sup>§1</sup>, Haein Park<sup>‡</sup>, Sojoong Choi<sup>‡</sup>, Kyeong-Cheon Jung<sup>¶</sup>,  
Seung-Taek Lee<sup>||</sup>, Je-Kyung Seong<sup>\*\*</sup>, Inn-Oc Han<sup>††</sup>, and Eok-Soo Oh<sup>‡2</sup>

From the <sup>†</sup>Department of Life Sciences, Division of Life and Pharmaceutical Sciences, Ewha Womans University, Seoul 120-750, the <sup>§</sup>Cancer Metastasis Research Center, Yonsei University College of Medicine, Seoul 120-749, the <sup>¶</sup>Department of Pathology, Seoul National University College of Medicine, Seoul 110-799, the <sup>||</sup>Department of Biochemistry, College of Science, Yonsei University, Seoul 120-749, the <sup>\*\*</sup>Department of Anatomy and Cell Biology, College of Veterinary Medicine, Seoul National University, Seoul 151-742, and the <sup>††</sup>Department of Physiology and Biophysics, College of Medicine, Inha University, Incheon 400-712, Korea

Although elevated syndecan-2 expression is known to be crucial for the tumorigenic activity in colon carcinoma cells, how syndecan-2 regulates colon cancer is unclear. In human colon adenocarcinoma tissue samples, we found that both mRNA and protein expression of syndecan-2 were increased, compared with the neighboring normal epithelium, suggesting that syndecan-2 plays functional roles in human colon cancer cells. Consistent with this notion, syndecan-2-overexpressing HT-29 colon adenocarcinoma cells showed enhanced migration/invasion, anchorage-independent growth, and primary tumor formation in nude mice, paralleling their morphological changes into highly tumorigenic cells. In addition, our experiments revealed that syndecan-2 enhanced both expression and secretion of matrix metalloproteinase-7 (MMP-7), directly interacted with pro-MMP-7, and potentiated the enzymatic activity of pro-MMP-7 by activating its processing into the active MMP-7. Collectively, these data strongly suggest that syndecan-2 functions as a docking receptor for pro-MMP-7 in colon cancer cells.

The characteristics and functions of cancer cells are critically influenced by the actions of cell adhesion receptors, which mediate interactions of cancer cells with the extracellular matrix (ECM)<sup>3</sup> and the cytoskeleton (1). At different points during carcinogenesis, the cell adhesion receptors regulate various cancer cell functions, including cell growth, differentiation, cell survival, angiogenesis, and inflammation (2, 3). Thus, the cancer-specific characteristics and functions of

cancer cells are due to the expression and utilization of a distinct set of adhesion receptors that show different expression patterns in normal cells. One group of cancer-related cell adhesion receptors are the syndecans, which are cell surface heparan sulfate proteoglycans known to play diverse roles in cell adhesion and cell communication by serving as co-receptors for both cell signaling and ECM molecules (4). At the plasma membrane, syndecans are capable of transmitting signals from the extracellular environment to the intracellular compartment, thereby regulating adhesion-dependent signal transduction during cell growth (5, 6), cell adhesion and migration (6, 7), cytoskeleton organization (7, 8), and cell differentiation (9). Numerous studies have examined the function of syndecans in various human tumors. Syndecan-1 expression is down-regulated in a great number of squamous cell carcinomas, including uterine cervix, lung, and colorectal cancer (2, 10–12). However, in other studies, syndecan-1 expression is reportedly up-regulated in prostate, lung, and breast cancers (13–15). In the case of syndecan-2, it has been reported that, in normal tissues, syndecan-2 is expressed in mesenchymal cells but not in normal epithelial cells. However, we found that syndecan-2 expression is increased in several epithelial-driven colon carcinoma cells, and this up-regulation is necessary for the tumorigenic activity of colon carcinoma cells (16). Therefore, it is likely that altered expression of syndecan-2 allows normal epithelial cells to become tumorigenic. The exact molecular mechanisms underlying syndecan-2-mediated carcinogenesis have not yet been fully elucidated.

ECM remodeling is orchestrated by distinct proteolytic systems capable of hydrolyzing a wide spectrum of ECM components (17). The involved proteases include the matrix metalloproteinases (MMPs), a large group of enzymes capable of degrading many of the ECM components (18). Because ECM remodeling is an important factor in carcinogenesis, MMPs have been extensively studied in the context of carcinogenesis (19). One of the smallest known members of the MMP family, MMP-7, was first discovered as an enzyme of the involuting rat uterus and was later found to be an important marker in human cancer progression (17). MMP-7 gene expression has been reported in human cancers of the colon, breast, prostate, stomach, pancreas, kidney, and lung (20). Recent studies have shown that MMP-7 interacts with the specific molecular genetic and signaling pathways involved in colorectal cancer development

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<sup>1</sup> These authors contributed equally to this work.

<sup>2</sup> To whom correspondence should be addressed: Dept. of Life Sciences, Ewha Womans University, Daehyun-dong, Seodaemun-Gu, Seoul 120-750, Korea. Tel.: 82-2-3277-3761; Fax: 82-2-3277-3760; E-mail: OhES@ewha.ac.kr.

<sup>3</sup> The abbreviations used are: ECM, extracellular matrix; FBS, fetal bovine serum; GST, glutathione S-transferase; MMP, matrix metalloproteinases; SFM, serum-free medium; TIMP, tissue inhibitor of matrix metalloproteinase; S2E, syndecan-2 ectodomain; SDC2, syndecan-2; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; RT, reverse transcription; PBS, phosphate-buffered saline; GPR, GenePix Result; DQ-gelatin, dye-quenched gelatin.

(21, 22); in particular, MMP-7 is activated at an early stage of colorectal tumorigenesis by the  $\beta$ -catenin signaling pathway (23).

Studies have shown that the cell surface localization of MMPs, which is tightly regulated in normal cells, is very important for carcinogenesis-related processing. MMP-2 may be localized to the cell surface through interactions with integrin  $\alpha\beta 3$  (24) or MT1-MMP (24), while the cell surface heparan sulfate proteoglycan, CD44, may dock MMP-7 (25) and MMP-9 (26) to the cell surface. Therefore, the formation of MMP-adhesion receptor complexes appears to be a common pathway through which soluble MMPs are localized to the cell surface.

Because both syndecan-2 and MMP-7 are important regulators in colon carcinogenesis, we herein examined whether syndecan-2 and MMPs might cooperatively regulate tumorigenic activities in human colon cancer. Our results reveal that syndecan-2 expression is up-regulated in colon adenocarcinomas, where it functions as a docking receptor for pro-MMP-7 to regulate tumorigenic activity.

## MATERIALS AND METHODS

**Reagents and Antibodies**—Antibody against syndecan-2 produced by AdipoGen Inc. (Korea) using purified Fc-fused syndecan-2 ectodomain (S2E-Fc) expressed in HT-29 cells. Monoclonal antibody to MMP-7 was purchased from Abcam (Cambridge, UK), monoclonal antibody to MMP-2 was purchased from Oncogene (San Diego, CA), and monoclonal antibody to MMP-9 was purchased from NeoMarkers (Fremont, CA). Polyclonal MMP-7 antibody was purchased from ABR (Golden, CO). Proenzymes of human recombinant MMP-2, MMP-7, and MMP-9 were purchased from Calbiochem (San Diego, CA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Amresco Inc. (Solon, OH). Recombinant human FGF-19 was purchased from R&D Systems (Minneapolis, MN).

**Cell Culture**—Colon cancer cell lines HT-29 were purchased from the Korean cell line bank. SW480 cells were generous gift from Dr. Jung H. Park of the Hallym University in Korea. HT-29 cells were maintained in McCoy's 5A and SW480 cells in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal bovine serum (FBS) and with penicillin (100 units/ml) and streptomycin (10  $\mu$ g/ml, Invitrogen) at 37 °C in a humidified 5% CO<sub>2</sub> incubator.

**Plasmid Construct**—A series of N-terminal truncation syndecan-2 mutants were generated with pairs of degenerate PCR primers and cloned into pGEX-5X-1 vector at the EcoRI/BamHI cloning sites. Human MMP-7 cDNA was amplified by RT-PCR using HT29 cell line RNA as a template. The DNA fragment precisely encompassing the full-length pro-MMP-7, pro-domain, or the catalytic domain mutants were cloned into the pET22b(+) vector at the NdeI/BamHI sites. FLAG-tagged syndecan-2 cDNA was a generous gift from Dr. Y. Yamaguchi (Burnham Institute for Medical Research, San Diego, CA).

**Synthesis of Small Interfering RNA Constructs**—Oligonucleotides were designed targeting the human MMP-7 RNA, containing a 9-bp hairpin loop. Oligonucleotides were annealed and cloned into the BglII/EcoRI sites of pSUPERretro vector.

Sequences of the primers are as follows: siMMP-7 sense primer, 5'-GATCCCCCAGGCTCAGGACTATCTCATTCAAGAG-ATGAGATAGTCCTGAGCCTGTTTTGGAAA-3'; anti-sense primer, 5'-AGCTTTTCCAAAAACAGGCTCAGGACTATCTCATCTCTTGAATGAGATAGTCCTGAGCCTGGG-3'. MMP-7 mRNA targeting sequences are underlined. HT-29 cells were transfected with siMMP-7-expression plasmid using Lipofectamine 2000.

**Transfection of Syndecan-2 cDNA**—For stable transfection, HT-29 cells ( $3 \times 10^6$  cells) were trypsinized, harvested, washed twice with cold PBS, and then incubated with 4  $\mu$ g of plasmid DNA on ice for 20 min. Transfection was preceded by electroporation using 0.2 kV, 1 pulse time, and 15 s of length. Clones were selected using G418 (1 mg/ml) for 3 weeks. Syndecan-2 expression was assessed by RT-PCR using a syndecan-2-specific primer. SW480 cells were transfected using Lipofectamine 2000 according to manufacturer's instructions (Invitrogen).

**Immunoblotting**—Immunoblotting was performed as described (16).

**RNA Extraction and RT-PCR**—Total RNA extracted from cultured cells or tissue samples were used as template for reverse transcriptase reaction. Aliquots of cDNA were amplified using the following primers: human syndecan-2, (forward) 5'-ACATCTCCCCTTTGCTAACGGC-3' and (backward) 5'-TAACTCCATCTCCTTCCCCAGG-3'; human MMP-2, (forward) 5'-AATACCTGAACACCTTCTATG-3' and (backward) 5'-ACAAGAAGGGGAAGTTGCAGT-3'; human MMP-7, (forward) 5'-GTTTAGAAGCCAACTCAAGG-3' and (backward) 5'-CTTTGACACTAATCGATCCAC-3'; human MMP-9, (forward) 5'-GGGCTGCTGCTTTGCTGCCCC-3' and (backward) 5'-GGAGGAAAGGCGTGTGCCAGG-3'; and human glyceraldehyde-3-phosphate dehydrogenase, (forward) 5'-CCACCCATGGCAAATTCATGGCA-3' and (backward) 5'-TCTAGACGGCAGGTCAGGTCCACC-3'. After an initial denaturation at 94 °C for 5 min, 30 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s (except, syndecan-2 at 55 °C and MMP-7 at 58 °C), and extension at 72 °C for 60 s were carried out. The reaction products were analyzed in 1.5% agarose gels.

**Invasion and Migration Assay**—For migration assay, gelatin (10  $\mu$ g/ml) was added to each well of a Transwell plate (Coster, Corning, NY, 8- $\mu$ m pore size), and the membranes were allowed to dry at 25 °C for 1 h. The Transwell plates were assembled into a 24-well plate, and the lower chamber was filled with McCoy's 5A media containing 10% FBS, 1% bovine serum albumin, and basis fibroblast growth factor (10  $\mu$ g/ml). Cells ( $5 \times 10^5$ ) were added to each upper chamber, and the plate was incubated at 37 °C in 5% CO<sub>2</sub> incubator. The cells that had migrated on the lower surface of the filters were stained with 0.6% hematoxylin and 0.5% eosin and counted. For *in vitro* invasion assay, Transwell plates were coated with gelatin (10  $\mu$ g/ml) on the lower side of the membrane and with Matrigel (3 mg/ml) on the upper side of the membrane.

**Cell Proliferation Assay**—Cell proliferation was measured by a colorimetric assay using MTT according to the manufacturer's instructions. Briefly, HT-29 cells were harvested with 0.05% trypsin/EDTA and seeded into 48-well plates. After cells were allowed to attach to the plate for 24 h, the medium containing

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0.5 mg/ml MTT was added to each plate, and cells were incubated for 1 h. The medium was then removed, and 200  $\mu$ l of dimethyl sulfoxide was added to each plate for half an hour at room temperature. The mean concentration of absorbance at 570 nm in each set of all samples was measured using a 96-well microtiter plate reader (Dynatech, Chantilly, VA).

**Flow Cytometry**—Cells were washed with PBS and released by the addition of 5% FBS and 0.2% EDTA in PBS through incubation for 30 min at 4 °C. After pelleting, cells were resuspended in PBS and counted. Cells were incubated with anti-syndecan-2 antibody in 10% FBS in PBS for 1 h at 4 °C followed by PBS containing 0.05% Tween 20, washed twice, and incubated with fluorescein isothiocyanate-conjugated anti-mouse in 10% FBS in PBS for 30 min. Syndecan-2 expressions were analyzed by flow cytometry.

**Anchorage-independent Growth in Soft Agarose**—Each well of a 6-well culture plate was coated with 3 ml of bottom agar mixture (McCoy's 5A/10% FBS/0.6% agar). After the bottom layer had solidified, 2 ml of top agar mixture (McCoy's 5A/10% FBS/0.3% agar) containing  $2 \times 10^6$  cells was added to each well, and the cultures were incubated at 37 °C in a 5% CO<sub>2</sub> atmosphere. Every 5 days, normal growth medium was gently layered over the cultures. Colony formation was monitored daily with a light microscope. Colonies in soft agar were photographed with a digital camera after incubation for 3 weeks.

**Gelatinolytic Activity Assay**—Conditioned media was incubated with 20  $\mu$ g of DQ-gelatin as a substrate in a reaction buffer containing 50 mM Tris-HCl (pH 7.6), 150 mM NaCl, 5 mM CaCl<sub>2</sub> (final 200  $\mu$ l). After 2 h at room temperature, increase in fluorescence at 515 nm due to the DQ-gelatin degradation by the overall gelatinase activities was monitored in triplicate in 96-well plates. Collagenase (0.1 unit/ml) was used as a positive control.

**Treatment with NaClO<sub>4</sub>**—To inhibit the maturation of the heparan sulfate chain on the cell surface heparan sulfate proteoglycan, HT-29 cells were treated with NaClO<sub>4</sub> (final concentration 25 mM) for 24 h.

**Detection of Soluble Syndecan-2 in the Conditioned Media**—HT-29 cells were cultured in serum-free media (SFM) for 24 h. Conditioned media (100  $\mu$ l) were collected, briefly centrifuged at 2000  $\times$  g to remove any remaining cells, and used for slot blot analysis. For shedding assays, HT-29 cells cultured in 6-well tissue culture plates ( $6 \times 10^5$  cells per well) were washed two times with SFM and then cells were incubated with fresh SFM supplemented pro-MMP-7 (0.2 unit/ml) for 2 h 37 °C. Conditioned media (100  $\mu$ l) were used for slot blot analysis as described above.

**Microarray Analysis**—Total RNAs from HT-29 control and HT-29 cells stably overexpressing syndecan-2 were isolated using TRIzol reagent (Invitrogen) following manufacturer's protocol. A reference RNA pool was prepared by way of isolation and mixture of total RNAs 11 cancer cells (27). Reference RNA and target RNA were labeled with Cy3 and Cy5, respectively, during reverse transcription reaction. The labeled cDNAs were co-hybridized in microarrays (Genomictree, Daejeon, Korea) made of oligonucleotides representing 22,000 unique human genes (Sigma-Genosys, St. Louis, MO). Three independent biological replicates were prepared for both cells.

The hybridized slides were washed and then scanned using a GenePix 4000B scanner (Axon Instruments, Foster City, CA), and the data were saved in GenePix Result (GPR) format. Six GPRs were imported into GeneSpring software (Agilent Technologies, Foster City, CA) and "print-tip" normalized using the Lowess function. If Cy5 or Cy3 intensities are not greater than 1.5-fold of background intensities, the spots were removed from further analysis. To select for the genes that show significant difference in expression level between HT-29 control and HT-29 cells stably overexpressing syndecan-2, a two-sample *t* test was performed. Genes were considered significant if selected at *p* < 0.001. A total of 47 genes was selected (Table 1). For heat map presentation of the 47 genes and 6 array samples, a correlation coefficient was used as a gene similarity metric, and a hierarchical clustering algorithm based on the average-linkage method was employed. The microarray data have been deposited in the ArrayExpress data base (available on-line).

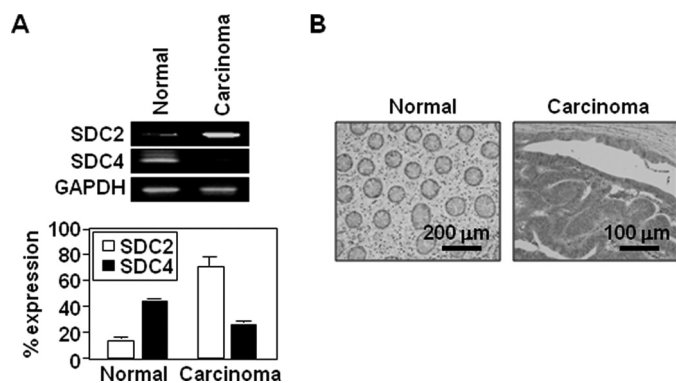
**Primary Tumor Formation**—HT-29 cells were spun down at 1500 rpm for 5 min, resuspended in the SFM, and then injected into nude mice at a density of  $10 \times 10^6$  cells/300  $\mu$ l. After 4 weeks, the mice were sacrificed and the tumor mass was weighed.

**Tissue Sample Preparation**—All experiments using the patient tissues were performed with approval from the Internal Review Board of Yonsei University College of Medicine. Tissues from the patients who underwent surgery at the Yonsei University College of Medicine during 1997–1999 were used with permission from the patients. Tissues that contained at least 70% tumor content were stored in liquid nitrogen immediately after surgery.

**Immunohistochemistry**—Representative core tissue biopsies (2 mm in diameter) were taken from paraffin blocks and arranged in a new recipient block (tissue array block) using a trephine apparatus (Superbiochips Laboratories, Seoul, Korea). 5- $\mu$ m sections from the tissue array block were subjected to immunohistochemical study using the EnVision kit (DAKO, Carpinteria, CA). The deparaffinized sections were boiled for 10 min in citrate buffer (10 mM citric acid, 0.05% Tween 20, pH 6.0) for antigen retrieval, and endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide for 30 min. The sections were preincubated with normal goat serum to prevent nonspecific binding, and then incubated overnight at 4 °C with anti-syndecan-2 antibody. The sections were rinsed with PBS and incubated with horseradish peroxidase-labeled polymer for 30 min at room temperature and then washed with PBS. The enzyme reaction was developed with 0.03% 3',3'-diaminobenzidine tetrahydrochloride containing 0.006% hydrogen peroxide. As a negative control, an irrelevant antibody replaced the primary antibodies.

## RESULTS

**Syndecan-2 Is Up-regulated in Human Colon Adenocarcinoma Tissues**—To investigate the potential role of syndecan-2 in colon cancer, we isolated RNA from tissues obtained from 11 colon cancer patients and used RT-PCR to analyze the mRNA expression of syndecan-2 and -4. Gene expression of syndecan-2 was detected in ~73% of colon cancer tissue samples, but only 18% of neighboring normal epithelium. In contrast, syndecan-

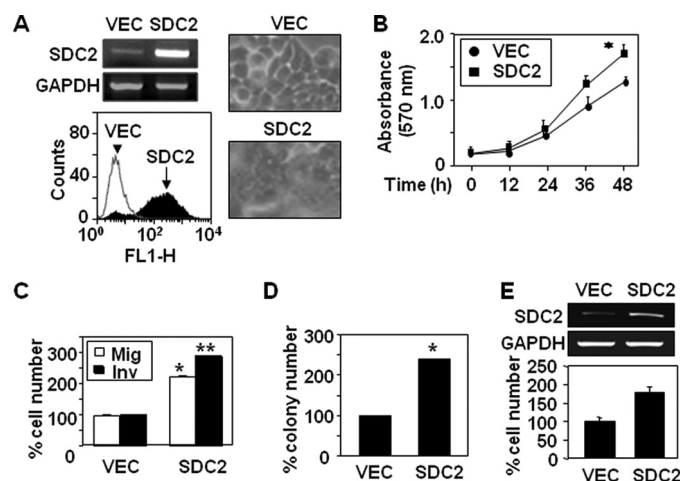


**FIGURE 1. Syndecan-2 expression is increased in human colon cancer tissues.** *A*, total RNA was extracted from human colon carcinoma tissue and neighboring normal tissues, and mRNA expression of syndecan-2 (SDC2) or syndecan-4 (SDC4) was analyzed by RT-PCR (top panel). Data are shown as percentage (%) of SDC2 (open bars)- or SDC4 (closed bars)-positive tissues among the total tissues (bottom panel). *B*, paraffin-embedded tissue sections of 40 cases were immunostained with an anti-SDC2 antibody, and the results were visualized with a peroxidase-conjugated secondary antibody. The presented photographs are representative of 16 syndecan-2-negative and 24 syndecan-2-positive stainings, respectively ( $p < 0.05$ ). Statistical significance was evaluated using Chi-square test.

can-4 mRNA expression was detected in ~40% of normal epithelium but actively repressed in colon cancer tissue (Fig. 1A). Consistently, immunohistochemical studies using paraffin sections revealed that syndecan-2 expression was up-regulated in ~60% of the examined human colon adenocarcinoma tissues, compared with the neighboring normal epithelium (Fig. 1B). These findings confirm that syndecan-2 expression is up-regulated in human colon cancer cells.

**Overexpression of Syndecan-2 Increases the Tumorigenic Potential of Colon Adenocarcinoma Cells**—To further investigate the role of syndecan-2 in colon cancer cells, we transfected an expression vector encoding syndecan-2 into HT-29 human colon adenocarcinoma cells, which express very low basal level of syndecan-2 (Fig. 2). HT-29 cells stably overexpressing syndecan-2 (SDC2) showed increased levels of mRNA and cell surface expression (Fig. 2A). Interestingly, increased syndecan-2 expression was associated with alterations in the epithelial morphology of HT-29 cells, resulting in an aggregated morphology characterized by a lack of clearly discernible cell-cell boundaries (Fig. 2A). Consistent with these morphological changes, syndecan-2 expression was associated with noticeable increases in cell proliferation (Fig. 2B), migration (Fig. 2C; 2.3-fold), invasion (Fig. 2C; 2.9-fold), and anchorage-independent growth (Fig. 2D; 2.4-fold) compared with vector-transfected HT-29 cells. Similarly, SW480 human colon adenocarcinoma cells transfected with syndecan-2 showed increased cell migration, compared with cells transfected with vector control (Fig. 2E; 1.8-fold,  $p < 0.05$ ). Taken together, these data suggest that syndecan-2 expression potentiates the tumorigenic potential in human colon adenocarcinoma cells.

**Syndecan-2 Accelerates Primary Tumor Formation in Vivo**—To evaluate the tumorigenic potential of syndecan-2 *in vivo*, HT-29 or HT-29-SDC2 cells were injected into athymic nude mice and allowed to form tumors over 4 weeks. The mean body weights were not significantly different between the two groups after 4 weeks (data not shown), but the tumor masses were significantly larger in mice injected with HT-29-SDC2 cells *ver-*

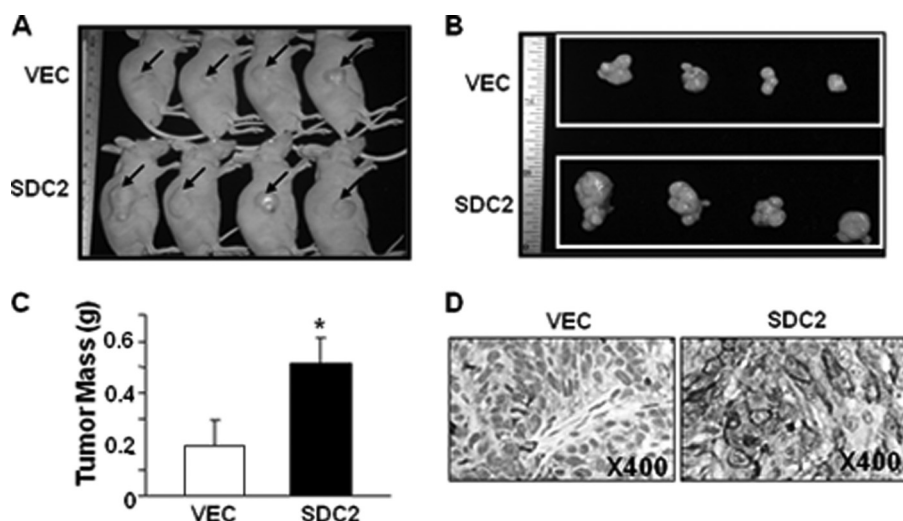


**FIGURE 2. Overexpression of syndecan-2 increases the tumorigenic potential of HT-29 colon cancer cells.** *A*, HT-29 cells were transfected with 4  $\mu$ g of either control (VEC) or syndecan-2 (SDC2) expression vectors. After 4 weeks of clonal selection, the expression level of SDC2 was evaluated with RT-PCR (top panel). Out of six cell clones examined, a representative clone was photographed with a phase-contrast microscope attached to a digital camera (right panel). Cells were incubated with an anti-syndecan-2 antibody, and the cell surface expression levels of syndecan-2 were analyzed by flow cytometry. IgG was used as a control (bottom panel). *B*, cells were split in 48-well plates and incubated for the indicated periods of time. Cell numbers were evaluated with MTT assay, as described under “Materials and Methods.” The given data are the mean absorbance  $\pm$  S.E. from three independent experiments. The asterisk indicates statistically significant increases in proliferation relative to that observed in vector-transfected HT-29 cells ( $p < 0.05$ ). *C*, cells ( $1 \times 10^5$ ) were allowed to migrate on gelatin-coated (10  $\mu$ g/ml, migration) Transwell plates or invade through Matrigel-coated (3  $\mu$ g/ml, invasion) Transwell plates. After 18-h incubation, cells were stained with hematoxylin & eosin, and migrated cells were counted. All data are reported as the percentage change in comparison with the vector control cells, which were arbitrarily assigned 100%. The results shown represent the averages of three independent experiments. One or two asterisks indicate statistically significant increases in migration or invasion relative to that of vector control (\*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ). *D*, cells ( $2.5 \times 10^6$ /plate) were seeded in soft agar and allowed to grow for 21 days, and the number of viable colonies was counted (\*,  $p < 0.05$ ). All data are reported as the percentage change in comparison with the vector control cells, which were arbitrarily assigned 100%. *E*, SW480 colon adenocarcinoma cells were transfected with syndecan-2 cDNA. After 4 weeks of clonal selection, the expression level of SDC2 was evaluated with RT-PCR, using the indicated primers (top panel). Cells ( $1 \times 10^5$ ) were allowed to invade through Matrigel-coated (3  $\mu$ g/ml) Transwell plates as described in *C*. The results shown represent the averages of three independent experiments (\*,  $p < 0.05$ ).

*sus* those injected with the control HT-29 cells (Fig. 3;  $0.49 \pm 0.14$  versus  $0.19 \pm 0.05$ , respectively). As expected, syndecan-2 expression was higher in the tumor tissue derived from HT-29-SDC2 cells versus that from control HT-29 cells (Fig. 3D).

**Syndecan-2 Expression Leads to Increased MMP-7 Expression in HT-29 Cells**—We investigated how syndecan-2 might regulate tumorigenic activity in colon adenocarcinoma cells. To identify downstream targets of syndecan-2, we used microarray analysis to identify genes that were differently expressed in syndecan-2-overexpressing HT-29-SDC2 cells versus HT-29 controls. After screening of a total of 22,000 human genes, 47 genes showed significant differences in expression levels between the two cell types ( $p < 0.001$ , Fig. 4A and Table 1). The differentially expressed 47 genes represent those with diverse molecular functions, such as “binding” with other proteins, nucleic acids, or lipid, “catalytic activities,” including hydrolase, oxidoreductase, or transferases (Fig. 4B). Because MMPs are overexpressed in many cancers, and are

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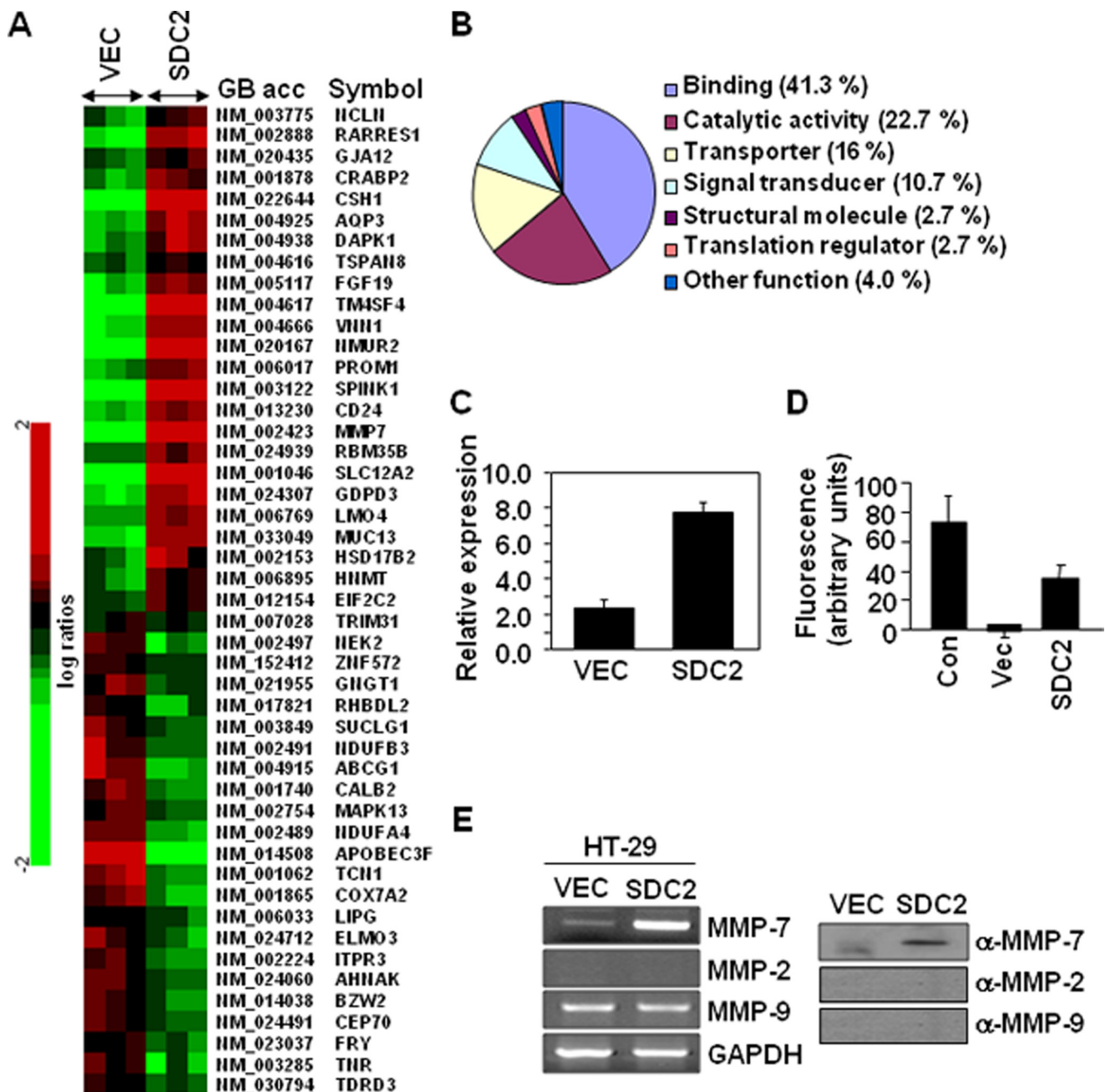
**FIGURE 3. Syndecan-2 accelerates primary tumor formation in vivo.** *A*, vector (VEC)- or syndecan-2 (SDC2)-transfected HT-29 cells ( $10 \times 10^6$ ) were injected into nude mice. After 4 weeks, the mice were sacrificed and photographed. Four representatives were shown for each set. *B*, tumors were extracted and photographed. Two representatives per each set were shown. *C*, data are shown as the mean tumor mass (g)  $\pm$  S.E. from two independent experiments ( $n = 8$  or 13 mice per set; \*,  $p < 0.01$ ). *D*, tumor masses from each set were fixed, sectioned, stained with syndecan-2 antibody, and photographed (400 $\times$  magnification).

capable of mediating invasion and metastasis via ECM degradation (28), we focused on differently expressed members of MMP and tissue inhibitor of matrix metalloproteinase (TIMP) families. We found that one of the highly overexpressed genes in HT-29 following syndecan-2 overexpression was MMP-7 (Table 1). MMP-7 mRNA expression was 3.3-fold higher in HT-29-SDC2 cells (Fig. 4C), and MMP-7 was the only member of the MMPs or TIMP family selected in the current microarray analysis. Consistent with the results from our microarray analysis, gelatinase activities were significantly higher in conditioned media from HT-29-SDC2 cells *versus* controls (Fig. 4D,  $p < 0.01$ ), and syndecan-2 overexpression in HT-29 cells increased MMP-7 expression at the mRNA and protein levels but had no such effect on MMP-2 or MMP-9 (Fig. 4E). These findings collectively suggest that syndecan-2 leads to increased expression of MMP-7, which may be an important regulator in the effects of syndecan-2 in colon cancer cells.

**MMP-7 Regulates Syndecan-2-mediated Migration of HT-29 Cells**—We next investigated whether MMP-7 is involved in syndecan-2-mediated cell migration. We generated two unique 19-bp siRNA sequences targeted against human MMP-7 mRNA and used these siRNA to knock down MMP-7 expression in HT-29 cells. Co-transfection of HT-29 cells with the syndecan-2 expression vector and MMP-7-targeting siRNA showed decreased expression of MMP-7 protein (Fig. 5A), as well as decreased syndecan-2-induced cell migration activity, compared with cells transfected with the syndecan-2 expression vector alone (Fig. 5B,  $p < 0.01$ ). In contrast, the migration of HT-29 cells was not significantly influenced by the treatment of FGF-19 whose mRNA expression was increased in HT-29-SDC2 cells (Fig. 5C,  $p < 0.01$ ). These results show that MMP-7 is involved in syndecan-2-induced cell migration in HT-29 cells, suggesting that syndecan-2-mediated tumorigenic activity is at least in part dependent on MMP-7.

**Syndecan-2 Functions as a Cell Surface Docking Receptor for Pro-MMP-7**—Because syndecans are known to interact with various ECM components, we investigated whether syndecan-2 functions as a cell surface receptor in regulating MMP-7. Purified GST-fused proteins of syndecan-2 (GST·SDC2), or syndecan-4 (GST·SDC4) were incubated with the conditioned media from wild-type HT-29, HT1080 fibrosarcoma, or HT1080 fibrosarcoma cells pretreated with tumor necrosis factor- $\alpha$ . Western blotting of the precipitated GST·SDC2 complexes showed that the syndecan-2 fusion protein bound to 28-kDa pro-MMP-7 in the condition of equal loading of GST-fused protein (Fig. 6A, bottom panel). Furthermore, purified pro-MMP-7 inter-

acted with GST·S2E and -SDC2, but not -S2TC, which lacks the extracellular domain (Fig. 6B). These findings indicate the direct interaction between the extracellular domain of syndecan-2 and pro-MMP-7. Cell lysates from HT-29 cells were immunoprecipitated with an anti-syndecan-2 antibody and then incubated with conditioned media from either HT-29-vector or HT-29-SDC2 cells. Consistent with the above results, syndecan-2 interacted with pro-MMP-7 in the HT-29-conditioned media (Fig. 6C), suggesting that, like recombinant protein, endogenous syndecan-2 expressed in HT-29 cells, which contain glycosaminoglycan chains, can interact with pro-MMP-7. We also found that FLAG-tagged syndecan-2 expressed in HT-29 cells interacted with pro-MMP-7 protein (Fig. 6D). Pretreatment with NaClO<sub>4</sub> to inhibit the maturation of the heparan sulfate chain on the cell surface heparan sulfate proteoglycan did not affect the interaction of syndecan-2 with MMP-7 (Fig. 6E), suggesting that MMP-7 binds to syndecan-2 through the core protein rather than the glycosaminoglycan chains. To further examine the effect of syndecan-2 on cell surface localization of MMP-7, HT-29-Vector or HT-29-SDC2 were co-immunostained with both syndecan-2- and MMP-7-specific antibodies following staining with fluorescein isothiocyanate- and Texas Red-conjugated secondary antibodies. As expected, the levels of syndecan-2 were significantly elevated in HT-29-SDC2 cells, and syndecan-2 was localized on the cell membrane (Fig. 6F). Taken together these data suggest that syndecan-2 directly interacts with MMP-7. To characterize the binding of MMP-7 to syndecan-2, several recombinant syndecan-2 and MMP-7 constructs were made (Fig. 7A), and purified GST-fused proteins of syndecan-2 were incubated with purified recombinant MMP-7 mutant proteins. Western blotting of the precipitated GST·SDC2 complexes showed that N-terminal extracellular domain of syndecan-2 (amino acid residues 16–50) protein bound to pro-domain of MMP-7 (Fig. 7, B and C). Consistently, when aminophenylmercuric acid was used to convert pro-MMP-7 to the active form, active MMP-7 no lon-



**FIGURE 4. MMP-7 expression is elevated in syndecan-2-overexpressing HT-29 cells.** *A*, gene expression profiling was performed between HT-29 cells transfected with vector only (VEC) and those overexpressing syndecan-2 (SDC2). Significant gene expression differences between VEC and SDC2 was identified with a two-sample *t* test at  $p < 0.001$ , resulting in the selection of 47 genes and are shown in a *heat map* following a two-way hierarchical clustering. *GB acc* refers to a GenBank™ accession number for each gene. A *scale bar* showing the ratio and the corresponding color change is shown at the *left*. The relative gene expression ratios and full names of the 47 genes are listed in the Table 1. *B*, molecular functional categories of the 47 genes are shown in a *pie map*. *C*, the relative expression ratios of MMP7 in HT-29 (VEC) and HT-29 (SDC2), respectively, compared with common reference RNAs are shown as *bar graphs* with standard deviations. Each *bar* represents a mean expression level  $\pm$  S.D. from three independent experiments. *D*, conditioned media were collected from the transfectants, and gelatinase activity was measured using DQ-gelatin as a substrate. The relative activity was normalized by subtracting the background fluorescence from the media. Collagenase was used as a positive control (*con*). Data are shown as the mean fluorescence  $\pm$  S.E. from three independent experiments. *E*, total RNA was extracted from control HT-29 cells or HT-29 cells stably overexpressing syndecan-2 (SDC2), and the expression levels of various MMPs were evaluated with RT-PCR and the indicated primers. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was amplified as a control. The given results are representative from four independent experiments (*left panel*). Conditioned media from control HT-29 cells or HT-29 cells stably overexpressing syndecan-2 (SDC2) was subjected to immunoblotting with the indicated MMP-specific antibodies (*right panel*).

ger interacted with syndecan-2 (Fig. 7D), suggesting that syndecan-2 interacts specifically with pro-MMP-7. Taken together these data suggest that syndecan-2 functions as a docking receptor for pro-MMP-7 on the cell surface to regulate colon cancer activity.

*Syndecan-2 Regulates Activation of Pro-MMP-7*—If syndecan-2 functions as a docking receptor for MMP-7, it might also regulate the activity of MMP-7. To mimic cell surface docking of MMP-7 with syndecan-2, we coated enzyme-linked immunosorbent assay plates with either S2E or S4E (negative con-

## Syndecan-2, a Docking for MMP-7 in Colon Cancer

**TABLE 1**

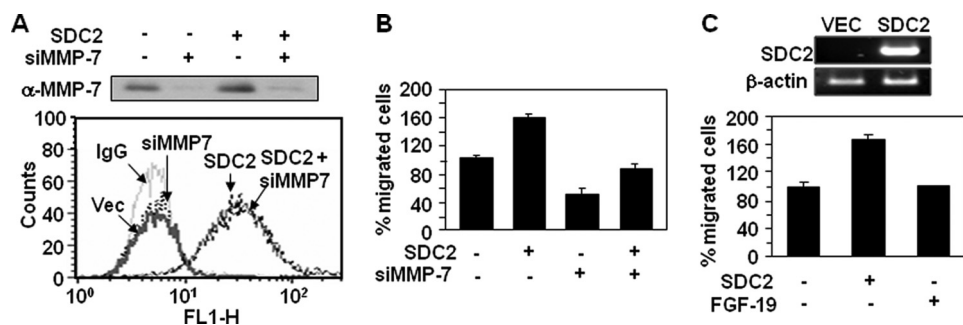
**Genes differentially expressed in HT-29 colon cells overexpressing SDC-2 ( $p < 0.001$ )**

A set of 47 genes shown in the tree view (Fig. 4A) is listed here in the order of relative expression. "GB acc" refers to the GenBank<sup>TM</sup> accession number. "VEC" and "SDC2" columns refer to average expression ratios of genes from HT-29 transfected with vector only and those from overexpressing syndecan-2, respectively. S.D.<sup>VEC</sup> and S.D.<sup>SDC2</sup> are standard deviations for VEC and SDC2, respectively. "Ratio" in the last column is the ratios of SDC2 over VEC and represents -fold changes in expression levels of genes in HT-29 overexpressing syndecan-2 compared with HT-29 transfected with vector only.

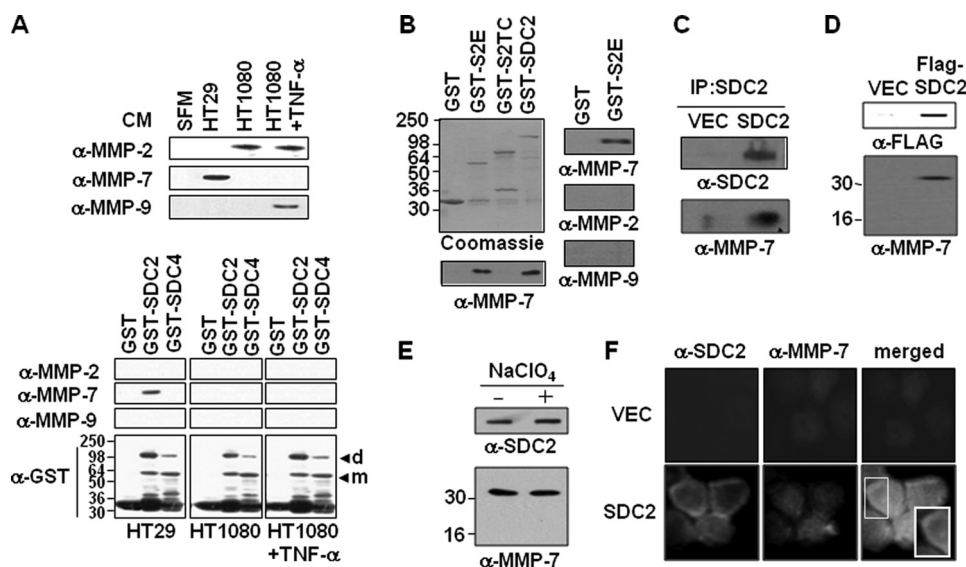
GB acc	Symbol	Name	VEC	S.D. <sup>VEC</sup>	SDC2	S.D. <sup>SDC2</sup>	Ratio
<b>Up-regulated genes</b>							
NM_004617	<i>TMSF4</i>	Transmembrane 4 L six family member 4	0.77	0.08	6.50	0.66	8.47
NM_003122	<i>SPINK1</i>	Serine peptidase inhibitor, Kazal type 1	1.24	0.17	5.12	0.32	4.14
NM_022644	<i>CSH1</i>	Chorionic somatomammotropin hormone 1	0.93	0.30	3.66	0.61	3.93
NM_020167	<i>NMUR2</i>	Neuromedin U receptor 2	0.60	0.08	2.03	0.23	3.38
NM_002423	<i>MMP7</i>	Matrix metalloproteinase 7	2.36	0.46	7.78	0.48	3.30
NM_002888	<i>RARRES1</i>	Retinoic acid receptor responder 1	0.92	0.03	2.56	0.53	2.79
NM_001046	<i>SLC12A2</i>	Solute carrier family 12 member 2	1.27	0.15	3.19	0.40	2.52
NM_033049	<i>MUC13</i>	Mucin 13, cell surface associated	0.78	0.03	1.74	0.05	2.21
NM_002153	<i>HSD17B2</i>	Hydroxysteroid (17- $\beta$ ) dehydrogenase 2	1.29	0.09	2.79	0.62	2.16
NM_024307	<i>GDPD3</i>	Glycerophosphodiester phosphodiesterase domain containing 3	0.91	0.10	1.92	0.19	2.11
NM_004925	<i>AQP3</i>	Aquaporin 3	1.43	0.03	2.89	0.30	2.02
NM_004666	<i>VNN1</i>	Vanin 1	2.39	0.11	4.62	0.19	1.93
NM_006769	<i>LMO4</i>	LIM domain only 4	1.69	0.27	3.22	0.28	1.91
NM_013230	<i>CD24</i>	CD24 molecule	0.92	0.01	1.73	0.05	1.89
NM_004938	<i>DAPK1</i>	Death-associated protein kinase 1	1.75	0.26	3.27	0.41	1.87
NM_001878	<i>CRABP2</i>	Cellular retinoic acid-binding protein 2	3.19	0.43	5.75	0.67	1.80
NM_005117	<i>FGF19</i>	Fibroblast growth factor 19	1.46	0.20	2.43	0.25	1.66
NM_024939	<i>RBM35B</i>	RNA-binding motif protein 35B	2.01	0.26	3.35	0.19	1.66
NM_006017	<i>PROM1</i>	Prominin 1	3.17	0.14	5.15	0.35	1.62
NM_003775	<i>NCLN</i>	Nicalin homolog	1.43	0.08	2.21	0.17	1.55
NM_006895	<i>HNM1</i>	Histamine N-methyltransferase	1.72	0.09	2.56	0.17	1.49
NM_020435	<i>GJA12</i>	Gap junction protein, $\alpha$ 12	2.85	0.10	4.21	0.20	1.48
NM_004616	<i>TSPAN8</i>	Tetraspanin 8	3.15	0.15	4.42	0.20	1.40
NM_012154	<i>EIF2C2</i>	Eukaryotic translation initiation factor 2C 2	2.20	0.13	3.06	0.13	1.39
<b>Down-regulated genes</b>							
NM_007028	<i>TRIM31</i>	Tripartite motif-containing 31	7.23	0.33	5.96	0.37	1.21
NM_152412	<i>ZNF572</i>	Zinc finger protein 572	3.62	0.15	2.90	0.15	1.25
NM_006033	<i>LIPG</i>	Lipase, endothelial	7.57	0.24	5.91	0.40	1.28
NM_023037	<i>FRY</i>	Furry homolog	8.22	0.60	6.25	0.61	1.32
NM_003849	<i>SUCLG1</i>	Succinate-CoA ligase, GDP-forming, $\alpha$	2.61	0.17	1.92	0.12	1.36
NM_002754	<i>MAPK13</i>	Mitogen-activated protein kinase 13	4.58	0.26	3.30	0.04	1.39
NM_014038	<i>BZW2</i>	Basic leucine zipper and W2 domains 2	2.43	0.06	1.75	0.09	1.39
NM_024060	<i>AHNAK</i>	AHNAK nucleoprotein	2.33	0.09	1.65	0.05	1.41
NM_024491	<i>CEP70</i>	Centrosomal protein 70 kDa	1.96	0.08	1.35	0.04	1.45
NM_002491	<i>NDUFB3</i>	NADH dehydrogenase 1 $\beta$ 3	2.06	0.18	1.39	0.03	1.48
NM_002224	<i>ITPR3</i>	Inositol 1,4,5-triphosphate receptor, type 3	2.65	0.11	1.76	0.06	1.51
NM_001740	<i>CALB2</i>	Calbindin 2, 29 kDa	5.63	0.58	3.74	0.18	1.51
NM_017821	<i>RHBDL2</i>	Rhomboid, veinlet-like 2 ( <i>Drosophila</i> )	5.21	0.18	3.46	0.34	1.51
NM_024712	<i>ELMO3</i>	Engulfment and cell motility 3	2.69	0.26	1.77	0.15	1.52
NM_030794	<i>TDRD3</i>	Tudor domain containing 3	1.96	0.21	1.29	0.05	1.53
NM_002489	<i>NDUFA4</i>	NADH dehydrogenase 1 $\alpha$ 4	2.16	0.23	1.34	0.06	1.61
NM_001865	<i>COX7A2</i>	Cytochrome c oxidase subunit VIIa2	1.56	0.13	0.92	0.09	1.68
NM_003285	<i>TNR</i>	Tenascin R (restrictin, janusin)	1.98	0.17	1.17	0.06	1.70
NM_021955	<i>GNGT1</i>	G protein, gamma transducing activity 1	2.12	0.07	1.21	0.10	1.76
NM_004915	<i>ABCG1</i>	ATP-binding cassette, sub-family G, 1	3.90	0.73	2.11	0.27	1.85
NM_001062	<i>TCN1</i>	Transcobalamin 1	4.76	0.12	2.47	0.34	1.93
NM_002497	<i>NEK2</i>	NIMA-related kinase 2	2.87	0.62	1.44	0.27	1.99
NM_014508	<i>APOBEC3F</i>	Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3F	1.66	0.31	0.52	0.07	3.20

trol), and then incubated conditioned media from HT-29 cells in the coated plates in the presence of DQ-gelatin as an exogenous substrate. As shown in Fig. 8A, GST-S2E, but not GST-S4E enhanced the gelatinase activity of MMP-7 in the HT-29-conditioned media. Consistently, both S2E and SDC2 enhanced the enzymatic activity of pure pro-MMP-7 by >10-fold *in vitro*, whereas pro-MMP-2 or pro-MMP-9 had no such effect (Fig. 8B). Interestingly, syndecan-2 had no effect on the catalytic activity of active MMP-7 (Fig. 8C) but did enhance the processing of pro-MMP-7 into the active MMP-7 (Fig. 8D). Consistently, in response to tumor necrosis factor- $\alpha$ , syndecan-2 expression enhanced activation of MMP-7 and extracellular cleavage of E-cadherin, a known MMP-7 substrate (29) in HT-29 cells (Fig. 8E). These data strongly suggest that syndecan-2 might directly interact with pro-MMP-7 at the plasma membrane to regulate the processing of pro-MMP-7.

**MMP-7 Causes Extracellular Shedding of Syndecan-2**—Because MMP-7 is known to cleave the extracellular domain of syndecan-1 (31), we investigated whether MMP-7 regulates the shedding of the syndecan-2 extracellular domain. The soluble form of syndecan-2 was detected in the conditioned media from HT-29 cells (Fig. 9A), suggesting constitutive shedding of syndecan-2 in colon cancer cells. Immunoprecipitation with anti-syndecan-2 antibody confirmed that shed syndecan-2 was in the conditioned media (Fig. 9B). We next investigated whether MMP-7 was involved in the shedding of syndecan-2. After being washed with SFM to remove any accumulated shed syndecan-2 and proteases, HT-29 cells were incubated with SFM-containing pro-MMP-7. As expected, treatment with pro-MMP-7 increased shed syndecan-2 in the conditioned media (Fig. 9C). Taken together, these data suggest that MMP-7 causes extracellular domain shedding of syndecan-2.



**FIGURE 5. Syndecan-2-enhanced cell migration requires expression of MMP-7 in HT-29 cells.** *A*, HT-29 cells were co-transfected with syndecan-2 (*SDC2*) and either the pSUPERretro vector or siMMP-7-1 plasmids. After 36-h incubation, cells were washed twice with PBS and then incubated with serum-free media for additional 48 h. Conditioned media were collected and subjected to immunoblotting with an anti-MMP-7 antibody (*top panel*). Cells were incubated with a syndecan-2 antibody, and the cell surface levels of syndecan-2 protein were assessed by flow cytometry. IgG was used as a control (*bottom panel*). *B*, cells ( $1 \times 10^5$ ) were allowed to migrate on gelatin-coated (10  $\mu$ g/ml) Transwell plates. After 18-h incubation, migrated cells were stained with hematoxylin & eosin and counted. The results shown represent the averages of three independent experiments. *C*, HT-29 cells were transfected with syndecan-2 cDNA, and mRNA expression of syndecan-2 (*SDC2*) was analyzed by RT-PCR (*top panel*). Cells ( $1 \times 10^5$ ) were allowed to migrate on gelatin-coated (10  $\mu$ g/ml) Transwell plates, either in the presence or absence of FGF-19 (100 ng/ml). After 18-h incubation, migrated cells were stained with hematoxylin & eosin and counted.



**FIGURE 6. Syndecan-2 directly interacts with MMP-7.** *A*, conditioned media (*CM*) from the indicated cells pretreated with 20 ng/ml tumor necrosis factor- $\alpha$  were collected and subjected to immunoblotting with the specific MMP antibodies (*top panel*). Purified GST, GST-SDC2, or GST-syndecan-4 (GST-SDC4) was incubated with the conditioned media, and the proteins were bound to glutathione-agarose bead and immunoblotted with the specific MMP antibodies (*bottom panel*). The membranes were then stripped and reprobed with anti-GST antibody ( $\alpha$ -GST). Arrowheads point to dimer (*d*) and monomer (*m*) of syndecan-2 and -4. *B*, equivalent amount of purified GST, GST-SDC2, GST-syndecan-2 extracellular domain (GST-S2E), or GST-syndecan-2 mutant lacking the extracellular domain (GST-S2TC) was either separated by 12% SDS-PAGE and stained with Coomassie Blue (*Coomassie*) or incubated with purified MMPs as indicated, bound to glutathione-agarose beads, and immunoblotted with the specific MMP antibody. *C*, cell lysates from HT-29 cells were immunoprecipitated with anti-syndecan-2 antibody, and the precipitates were incubated with the conditioned media from either HT-29 or HT-29-SDC2 cells, and then used for slot blotting with anti-syndecan-2 and anti-MMP-7 antibodies. The presented data are representative of three independent experiments. *D*, HT-29 cells were transfected with 4  $\mu$ g of either control (*VEC*) or FLAG-tagged syndecan-2 (*SDC2*) expression vectors. Cell lysates were subjected to slot blotting with anti-FLAG antibody (*top panel*). Anti-FLAG immunoprecipitates from each cell lysates were incubated with proMMP-7, and the proteins bound were immunoblotted with the specific MMP-7 antibodies (*bottom panel*). *E*, HT-29 cells were preincubated in the absence (-) or presence (+) of 25 mM NaClO<sub>4</sub> for 24 h in SFM. The interaction of syndecan-2 and MMP-7 was assessed as described in *D*. *F*, cells were co-incubated with anti-syndecan-2 and anti-MMP-7-specific antibodies, stained with a Texas Red- and fluorescein isothiocyanate-conjugated secondary, and then photographed under confocal microscopy. The *inset* shows a high power view of the box.

## DISCUSSION

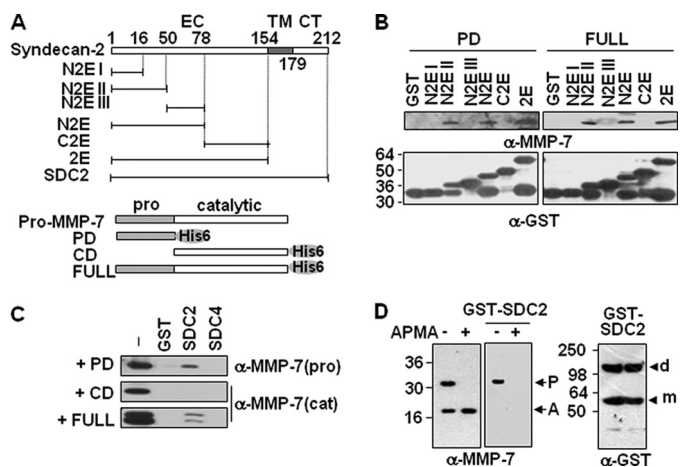
Although our previous study showed that increased syndecan-2 expression led to enhanced cell proliferation and migration/inva-

sion of cancer cell lines (16), the mechanism by which syndecan-2 regulates colon carcinogenesis is not yet known. Consistent with its effects in colon carcinoma cell lines, increased syndecan-2 expression was also observed in the tested human colon adenocarcinoma tissue samples (Fig. 1). We also observed that syndecan-2 overexpression caused the epithelial morphology of HT-29 cells to change to a more aggregated morphology characterized by a lack of clear discernible cell-cell boundaries (Fig. 2B). This is consistent with our previous report that reduction of syndecan-2 expression in the KM12SM colon carcinoma cell line using trichostatin A (a histone deacetylase inhibitor) or antisense cDNA triggered recovery of clear cell boundaries, flattened morphology, and reappearance of actin filaments in the normally aggregated cell morphology characterized by a lack of clear boundaries between cells and cell layering even at low cell density (30). Thus, syndecan-2 expression appears to promote tumorigenic characteristics in colon cancer cell lines. Correlated with these morphological changes, we herein report that overexpression of syndecan-2 increased the tumorigenic activity of HT-29 cells, as shown by enhancement of cell proliferation, migration/invasion, and anchorage-independent growth (Fig. 2). In addition, transplantation of stably syndecan-2-overexpressing HT-29 cells into nude mice led to enhanced primary tumor formation *versus* that by control HT-29 cells (Fig. 3). These data collectively support the hypothesis that syndecan-2 plays a critical role in colon tumorigenesis by altering the cell morphology and tumorigenic activity of colon cancer cells.

Genome-scale microarray analysis showed that syndecan-2 overexpression in HT-29 cells significantly increased the expression level of MMP-7, but not that of MMP-2 or MMP-9, which are centrally involved in tumor invasion and metastasis (Fig. 4). Notably, decreased expression of MMP-7 led to reductions in the syndecan-2-mediated enhancement of cell migration (Fig. 5). These



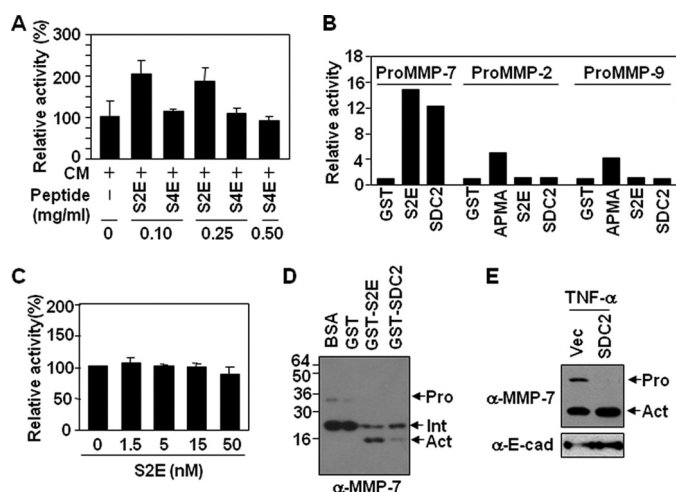
## Syndecan-2, a Docking for MMP-7 in Colon Cancer



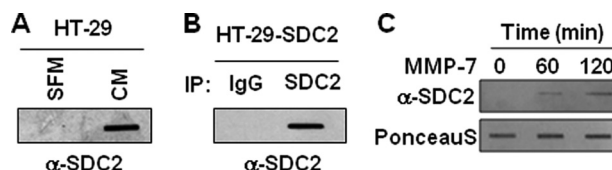
**FIGURE 7. N-terminal syndecan-2 directly interacts with pro-domain of MMP-7.** A, schematic representation of syndecan-2 core proteins and MMP-7. The extracellular domain (EC), the transmembrane domain (TM), and the cytoplasmic domain (CT) of syndecan-2 are noted, together with various deletion mutants. Syndecan-2 is labeled with amino acid number to show the location of deletion (top panel). Pro-domain (PD), catalytic domain (CD), and full-length of pro-MMP (FULL) are noted (bottom panel). B, purified GST or GST-syndecan-2 mutants were incubated with PD or full-length of pro-MMP-7, respectively. Bound materials to glutathione-agarose beads were immunoblotted with the MMP-7-specific antibody (top panel). The membranes were then stripped and reprobed with an anti-GST antibody (bottom panel). C, purified GST, GST-SDC2, or GST-SDC4 were incubated with PD, CD, or FULL. Bound materials to glutathione-agarose beads were immunoblotted with either pro-domain (pro) or catalytic domain-specific (cat) MMP-7 antibody. D, purified GST-SDC2 was incubated with purified pro-MMP-7 (-APMA, P) or active MMP-7 (+APMA, A), bound to glutathione-agarose beads and then immunoblotted with the MMP-7-specific antibody (left panel). The membranes were then stripped and reprobed with an anti-GST antibody (right panel). Arrowheads point to syndecan-2 dimer (d) and monomer (m).

findings are consistent with the notion that syndecan-2 plays a crucial role in colon cancer, because MMP-7 expression is a common feature of colorectal cancer development via multiple pathways (32). Furthermore, MMP-7 is activated at an early stage of colon carcinogenesis, and its expression has been correlated with the depth of invasion, lymph node metastasis, lymphatic invasion, and poor outcome. Therefore, it is likely that syndecan-2 enhances the tumorigenic potential of colon adenocarcinoma cells by inducing key molecules such as MMP-7. Indeed, MMP-7 is involved in syndecan-2-induced cell migration in HT-29 cells (Fig. 5) and the shedding of the syndecan-2 extracellular domain (Fig. 9). However, we cannot exclude the possibility that syndecan-2 regulates cancer cell migration in an MMP-7-independent manner, because syndecan-2-mediated cell migration was observed in MMP-7 knockdown HT29 cells (Fig. 5B, compare lanes 3 and 4). In contrast to our finding, a previous study has shown that syndecan-2 suppresses metastasis of Lewis lung carcinoma cells via inhibition of MMP-2 activation (33). The discrepancy may result from using different cancer cell types.

Previous studies have shown that cell surface heparan sulfate proteoglycans function to dock MMPs at the cell surface (25), and the cell surface localization of MMPs is important for their ability to regulate carcinogenesis (34). Because syndecan-2 is located in the plasma membrane and MMP-7 is known to localize at invasive front in colon cancer cells, it was considered likely that syndecan-2 acts as a cell surface docking receptor for MMP-7. It seemed as though syndecan-2



**FIGURE 8. Syndecan-2 regulates the processing of pro-MMP-7.** A, a 96-well plate was coated with the indicated amount of extracellular domain of either syndecan-2 (S2E) or syndecan-4 (S4E) overnight at 4 °C. Conditioned media from HT-29 cells was collected, and gelatinase activity was measured using DQ-gelatin as a substrate. The relative activity was normalized versus the background fluorescence from the media. Data are shown as the mean fluorescence  $\pm$  S.E. from three independent experiments. B, 96-well plate was coated with GST, S2E, or SDC2 (0.5 mg/ml) overnight at 4 °C. Purified pro-MMP-7, -2, or -9 was added, and gelatinase activity was measured using DQ-gelatin. The relative activity was normalized versus the fluorescence of a GST-coated well, and aminophenylmercuric acid (APMA) was used to activate pro-MMP-2 and pro-MMP-9 as a positive activity control. C, purified recombinant active-MMP-7 was incubated with purified GST-S2E, and the catalytic activity was measured as described in A. The relative activity was normalized versus the background activity from GST. D, purified pro-MMP-7 was incubated with purified S2E or SDC2 as described in B. MMP-7 levels were analyzed by immunoblotting with the MMP-7-specific antibody. E, conditioned media from cells pretreated with 20 ng/ml tumor necrosis factor- $\alpha$  were collected and subjected to immunoblotting with the specific MMP-7 (top panel) or E-cadherin antibody (bottom panel).



**FIGURE 9. MMP-7 causes extracellular shedding of syndecan-2.** A, conditioned media from HT-29 cells were collected and subjected to slot blotting with the specific syndecan-2 antibodies. The presented data are representative of five independent experiments. B, conditioned media from HT-29 cells overexpressing syndecan-2 (HT29-SDC2) were immunoprecipitated with anti-syndecan-2 antibody, and the precipitates were used for slot blotting with anti-syndecan-2 antibody. IgG was used as a control. C, HT-29 cells were treated with 0.2 unit/ml pro-MMP-7 for the indicated time periods. Conditioned medium was collected and subjected to slot blotting with anti-syndecan-2 antibody. The presented data are representative of four independent experiments.

can-2 acts as a pro-MMP-7 docking receptor in at least four possible ways: (i) syndecan-2 could immobilize pro-MMP-7 to restrict its range of action; (ii) syndecan-2 could regulate the enzymatic activity of MMP-7; (iii) syndecan-2 could protect pro-MMP-7 from proteolytic activation; or (iv) syndecan-2 could regulate protease-directed activation of MMP-7. In the present study, we showed that syndecan-2 directly interacts with pro-MMP-7 at the cell surface (Fig. 6), where it regulates the processing of pro-MMP-7 (Fig. 8). These data clearly support the notion that syndecan-2 function as a cell surface docking receptor for pro-MMP-7 in regulating colon adenocarcinoma cells.

In summary, we herein show for the first time that syndecan-2 acts as a docking receptor for pro-MMP-7. At the cell surface, syndecan-2 interacts directly with pro-MMP-7 at the plasma membrane, enhancing its processing into active MMP-7, which in turn regulates tumorigenic activities of colon cancer cells. Although future studies will be required to fully elucidate the mechanism underlying syndecan-2-induced MMP-7-mediated signaling in colon cancer cells, our present findings provide important new insights into colon cancer.

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