Proteomic Identification of Betaig-h3 as a Lysophosphatidic Acid-Induced Secreted Protein of Human Mesenchymal Stem Cells: Paracrine Activation of A549 Lung Adenocarcinoma Cells by Betaig-h3*$$

Sang Hun Shin‡§, Jaeyoon Kim||, Soon Chul Heo‡§, Yang Woo Kwon‡§, Young Mi Kim‡§, In-San Kim**, Taehoon G. Lee||, and Jae Ho Kim‡§¶‡‡

Lysophosphatidic acid (LPA) is enriched in the serum and malignant effusion of cancer patients and plays a key role in tumorigenesis and metastasis. LPA-activated mesenchymal stem cells promote tumorigenic potentials of cancer cells through a paracrine mechanism. LPA-conditioned medium (LPA CM) from human adipose tissue-derived mesenchymal stem cells (hASCs) elicited adhesion and proliferation of A549 human lung adenocarcinoma cells. To identify proteins involved in the LPA-stimulated paracrine functions of hASCs, we analyzed the LPA CM using liquid-chromatography tandem mass spectrometry-based shotgun proteomics. We identified βig-h3, an extracellular matrix protein that is implicated in tumorigenesis and metastasis, as an LPA-induced secreted protein in hASCs. LPA-induced βig-h3 expression was abrogated by pretreating hASCs with the LPA receptor inhibitor Ki16425 or small interfering RNA-mediated silencing of endogenous LPA1. LPA-induced βig-h3 expression was blocked by treating the cells with the Rho kinase inhibitor Y27632, implying that LPA-induced βig-h3 expression is mediated by the LPA–Rho kinase pathway. Immunodepletion or siRNA-mediated silencing of βig-h3 abrogated LPA CM-stimulated adhesion and proliferation of A549 cells, whereas retroviral overexpression of βig-h3 in hASCs potentiated it. Furthermore, recombinant βig-h3 protein stimulated the proliferation and adhesion of A549 human lung adenocarcinoma cells. These results suggest that hASC-derived βig-h3 plays a key role in tumorigenesis by stimulating the adhesion and proliferation of cancer cells and it can be applicable as a biomarker and therapeutic target for lung cancer. Molecular & Cellular Proteomics 11: 10.1074/mcp.M111.012385, 1–11, 2012.

Tumors are composed of neoplastic cells and non-neoplasticstromal cell components, including fibroblasts, myofibroblasts, endothelial cells, pericytes, and inflammatory cells (1). CarcinoMASS-ASSOCIATED fibroblasts (CAFs1, also called myofi broblasts or cancer stroma) have been shown to play important roles during cancer progression and metastasis (2–5). They stimulate tumorigenesis, angiogenesis, and invasion in a variety of solid tumors, including prostate, breast, and ovarian carcinomas (1, 6–9) by secreting various extracellular matrix proteins, proteases, chemokines, and angiogenic factors (10). CAFs can be identified within tumor stroma by their spindloid appearance and the expression of α-smooth muscle actin (α-SMA). Co-implantation of CAFs with tumor cells has been shown to stimulate the invasiveness of prostate and breast tumors in a xenograft tumor model (8, 9). CAFs have been reported to originate from various cell types, including tissue-resident fibroblasts, cancer cells or epithelial cells undergoing epithelial-to-mesenchymal transition, or mesenchymal stem cells (3, 4).

Mesenchymal stem cells (MSCs) have a self-renewal capacity, long-term viability, and differentiation potential toward diverse cell types such as adipogenic, osteogenic, chondrogenic, and myogenic lineages (11–14); this suggests that MSCs are clinically useful for tissue regeneration. Although

1 The abbreviations used are: CAFs, carcinoma-associated fibroblasts; α-SMA, α-smooth muscle actin; GAPDH, glyceraldehydes-3-phosphate dehydrogenase; hASCs, human adipose tissue-derived mesenchymal stem cells; LC-MS/MS, liquid chromatography tandem mass spectrometry; LPA, lysophosphatidic acid; MSCs, mesenchymal stem cells; LPA CM, LPA-conditioned medium; SDF-1, stromal cell-derived factor-1; siRNA, small interfering RNA; TGF-β1, transforming growth factor-β1.
MSCs exist predominantly in the bone marrow, they are also distributed throughout many other tissues, where they are thought to be the local sources of tissue-resident stem cells (15). Moreover, bone marrow-derived MSCs are recruited into the stroma of developing tumors (16). MSCs constitute a large proportion of non-neoplastic stromal cells within the tumor microenvironment (3). Accumulating evidence suggests that MSCs could also have an adverse effect that favors tumor growth. Tumor cells mixed with MSCs, when transplanted subcutaneously, exhibited elevated capability of proliferation and rich angiogenesis in tumor tissues (17). MSCs stimulated the metastatic potency of breast carcinoma when they were co-injected with human breast carcinoma cells into a subcutaneous site by xenograft transplantation (18). Furthermore, MSCs exposed to tumor-conditioned medium have been reported to exhibit phenotypic and functional characteristics of CAFs, including sustained expression of stromal cell-derived factor-1 (SDF-1) and the ability to promote tumor cell growth in vitro and in vivo co-implantation model (19). These results suggest that tumorigenesis and metastasis of carcinoma cells are acquired by paracrine signals from MSCs within the tumor-associated stroma. However, the paracrine signaling mechanisms by which MSCs stimulate tumorigenesis are largely unknown.

Periostin and βig-h3 are extracellular matrix proteins that are structurally homologous to the axon guidance protein fasciclin I (FAS1) (20). Both periostin and βig-h3 contain four tandem repeats of FAS1 domains and an EMI protein-protein interaction domain, and they play a key role in a variety of cellular responses, including adhesion, migration, proliferation, angiogenesis, wound healing and tumorigenesis (21–23).

We have reported that periostin is secreted from human adipose tissue-derived mesenchymal stem cells (hASCs) in response to LPA treatment, and the recombinant periostin protein stimulates the adhesion and migration of epithelial ovarian cancer cells (24). βig-h3 (also known as transforming growth factor beta-induced protein ig-h3 or TGFBI) was originally identified as transforming growth factor-β1 (TGF-β1)-induced protein in A549 human adenocarcinoma cells (25). βig-h3 is normally expressed in fibroblasts, keratinocytes, and muscle cells (26–28). The expression of βig-h3 was increased or downregulated in various tumor cells, depending on tumor types (21). βig-h3 promoted the adhesion and migration of human hepatoma by interacting with α3β1 integrin (29). Elevated expression of βig-h3 is associated with high-grade human colon cancers and ectopic expression of the βig-h3 enhanced the aggressiveness and altered the metastatic potentials of colon cancer cells (30). Furthermore, βig-h3 has been reported to regulate tumor angiogenesis by regulating endothelial cell adhesion and migration (31). Despite the various reports implicating βig-h3 in tumorigenesis, it is still unclear whether βig-h3 is expressed in cancer stroma and whether βig-h3 is involved in the crosstalk between cancer cells and stromal cells.

Lysophosphatidic acid (LPA) is a small bioactive phospholipid produced by activated platelets, mesothelial cells, fibroblasts, adipocytes, and some cancer cells (32–34). Accumulating evidence suggests that LPA is relevant to the tumorigenesis and metastasis (33). We have previously reported that LPA treatment induced the migration of hASCs and stimulated the expression of α-SMA and SDF-1, which have been known as markers for CAFs in vitro (35, 36). Furthermore, co-transplantation of A549 human lung adenocarcinoma cells and hASCs stimulated in vivo growth of A549 cells and tumor angiogenesis and elicited differentiation of hASCs to CAFs expressing α-SMA and vascular endothelial growth factor, an angiogenic cytokine, through an LPA receptor 1 (LPA1)-mediated mechanism. Conditioned medium from A549 lung adenocarcinoma cells induced expression of α-SMA and vascular endothelial growth factor in hASCs through an LPA1-dependent mechanism in vitro. These results suggest a pivotal role of the LPA-LPA1 signaling axis in the differentiation of hASCs to CAFs and in the paracrine function of hASCs within tumor microenvironment. However, the mechanism by which LPA-activated hASCs can regulate tumorigenic potential of cancer cells is largely elusive.

In order to clarify the paracrine mechanisms involved in the crosstalk between cancer cells and hASCs, we characterized secreted proteins included in LPA-conditioned medium from hASCs, using a shotgun proteomic analysis. The present study demonstrates a pivotal role of βig-h3 as an LPA-induced paracrine factor of hASCs on the adhesive and proliferative properties of A549 cells.

**EXPERIMENTAL PROCEDURES**

**Materials**—α-Minimum essential medium, trypsin, and fetal bovine serum were purchased from Invitrogen (Carlsbad, CA, http://www.invitrogen.com). A549 human lung adenocarcinoma, WI-38 human lung fibroblasts, and HeLa human cervical carcinoma cells were purchased from ATCC (Manassas, VA, http://www.atcc.org). Anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody was purchased from Millipore (Temecula, CA, http://www.millipore.com). 1-Oleoyl-sn-glycero-3-phosphate (LPA), Ki16425, SB431542, fatty acid-free bovine serum albumin (BSA), anti-α-SMA antibody, and Protein A agarose were from Sigma-Aldrich (St. Louis, MO, http://www.sigmaaldrich.com). Recombinant human βig-h3 and TGF-β1 proteins were purchased from R&D Systems, Inc. (Minneapolis, MN, http://www.rndsystems.com). Culture plates were purchased from Nunc (Roskilde, Denmark, http://www.nuncbrand.com). Peroxidase-labeled secondary antibodies and enhanced chemiluminescence Western blotting system were from Amersham Biosciences (Pittsburgh, PA, http://www4.gelifesciences.com).

**Cell Culture**—Subcutaneous adipose tissue was obtained from elective surgeries with patient’s consent, and this protocol was approved by the Institutional Review Board of Pusan National University Hospital. For isolation of hASCs, adipose tissues were washed at least three times with sterile phosphate-buffered saline (PBS) and treated with an equal volume of collagenase type I suspension (1 g/L of Hank’s Balanced Salt Solution with 1% BSA) for 60 min at 37 °C with intermittent shaking. The floating adipocytes were separated from the stromal-vascular fraction by centrifugation at 300 × g for 5 min. The cell pellet was resuspended in α-minimum essential medium with intermittent shaking. The floating adipocytes were separated from the stromal-vascular fraction by centrifugation at 300 × g for 5 min. The cell pellet was resuspended in α-minimum essential medium with intermittent shaking.
supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin, and cells were plated in tissue culture dishes at 3500 cells/cm². The primary hASCs were cultured for 4–5 days until they reached confluence and were defined as passage “0.” The passage number of hASCs used in these experiments was 3–10. The hASCs were positive for CD29, CD44, CD73, CD90, and CD105, whereas CD31, CD34, and CD45 were not expressed in hASCs (supplemental Fig. S1).

Preparation of Conditioned Medium—hASCs were seeded on 150-mm cell culture dishes and cultured in growth medium until reaching confluence. The cells were briefly rinsed twice with PBS and then incubated with 15 ml of α-minimum essential medium in the absence or presence of 10 μM LPA for 48 h before collecting media. The conditioned medium was centrifuged at 1000 × g for 10 min to remove cell debris, filtered using 0.45-μm Millipore syringe filters (Millipore, Bedford, MA), and stored at −70 °C for subsequent use.

Cell Adhesion Assay—Ninety-six-well microculture plates (Falcon, Becton-Dickinson, Mountain View, CA) were incubated with recombinant βig-h3 proteins or conditioned medium from hASCs at 37 °C for 1 h and then blocked with PBS containing 0.2% BSA for 1 h at 37 °C. Cells were trypsinized and suspended in the culture media at a density of 2 × 10⁵ cells/ml, and 0.1 ml of the cell suspension was then added to each well of the plates. Cell attachment was analyzed as follows. After incubation for 1 h at 37 °C, unattached cells were removed by rinsing twice with PBS. The number of attached cells was determined by counting the cells under microscopy at 100× magnification after staining with hematoxylin and eosin.

Cell Proliferation—Proliferation was determined with a colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay: MTT is metabolized by NAD-dependent dehydrogenase to form a colored reaction product (formazan), and the amount of dye formed directly correlates with the number of cells. To determine cell numbers, A549 cells were seeded in a 24-well culture plate at a density of 2 × 10⁵ cells/well, cultured for 48 h in normal growth medium, serum-starved for 24 h, and treated with various reagents for the indicated times. The cells were washed twice with PBS and incubated with 100 μl of MTT (0.5 mg/ml) for 2 h at 37 °C. The formazan granules generated by the cells were dissolved in 100 μl of dimethyl sulfoxide, and the absorbance of the solution at 562 nm was determined using a PowerWavex microplate spectrophotometer (Bio-Tek Instruments, Inc.; Winooski, VT) after dilution to a linear range.

Western Blotting—Serum-starved hASCs were treated with appropriate conditions, washed with ice-cold PBS, and then lyzed in lysis buffer (20 mM Tris-HCl, 1 mM EGTA, 1 mM EDTA, 10 mM NaCl, 0.1 mM phenylmethylsulfonyl fluoride, 1 mM Na₂VO₄, 30 mM sodium pyrophosphate, 25 mM β-glycerol phosphate, 1% Triton X-100, pH 7.4). Lysates were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred onto a nitrocellulose membrane, and then stained with 0.1% Ponceau S solution (Sigma-Aldrich). After blocking with 5% nonfat milk, the membranes were immunoblotted with various antibodies, and the bound antibodies were visualized with horseradish peroxidase-conjugated secondary antibodies, using the enhanced chemiluminescence Western blotting system (ECL, Amersham Biosciences).

Sample Preparation and Tryptic Digestion—hASCs were cultured in 150-mm diameter culture dishes until they reached subconfluence and were washed twice with Hank’s balanced salt solution to remove the serum component. The cells were incubated in 20 ml nonsupplemented (no serum, phenol red, or antibiotics) α-minimum essential medium in the absence or presence of 10 μM LPA for 48 h. Conditioned media were collected and centrifuged at 1000 × g for 10 min using a MF 300 centrifuge (Hanil Science Industrial, Inchon, Korea) to remove cell debris, filtered through a 0.2-μm filter, and concentrated using Amicon Ultra-rc-3K (3000 Da) molecular weight cutoff centrifugal filter device (Millipore). For tryptic digestion, each sample was heated at 90 °C for 15 min and 5 μl of 100 mM dithiothreitol was added and incubated in 56 °C for 20 min. Then, 5 μl of 200 mM iodoacetamide was added and incubated at room temperature in the dark for 15 min. To consume any unreacted iodoacetamide, an additional 10 μl of 100 mM dithiothreitol was supplemented. Reduced and alkylated proteins were digested with 500 ng of trypsin (Promega, Madison, WI) for 12 h at 37 °C.

LC-MS/MS—Mass spectrometry analysis was performed using nano-sized liquid chromatography tandem mass spectrometry (LC-MS/MS) system consisting of an Agilent 1100 high-pressure liquid chromatography (HPLC) system (Agilent Technologies, Santa Clara, CA) and a QSTAR quadrupole-time-of-flight mass spectrometer (MDS SCIEX, Concord, Ontario, Canada) equipped with a nano-electrospray ionization source. To achieve high-resolution separation, a nanoscale reversed phase chromatography analytical column (ZORBAX C18, 0.1 mm, 0.075 mm i.d.; Agilent Technologies) was used. Mobile phase A consisted of HPLC-grade water containing 0.1% formic acid and mobile phase B consisted of 64% HPLC grade acetonitrile containing 0.1% formic acid. Separation was performed at a flow rate of 300 nL/min and the applied gradient was 0–40% phase B over 60 min. For MS/MS analysis, each scan cycle consisted of 1 full scan mass spectrum (m/z 400–1500), followed by three MS/MS events. Digested samples were run in duplicate, and representative LC-MS/MS data from three independent experiments are shown (supplemental Table S1).

Database Searching—LC-MS/MS results were transformed to Mascot generic files using Mascot Daemon (version 2.2.2, Matrix Science, Manchester, U.K.). The Mascot generic files were searched against the concatenated database (153,194 sequence entries and their reverse sequences) combining Homo sapiens and Bos taurus databases from UniProt (release 2010_08) using in-house Mascot software (version 2.2.04). The following parameters were used: Trypsin (cuts C-terminal side of KR unless next residue is P); 2 missed cuts; carbamidomethylation (C) as fixed modification; N-acetyl (Protein), oxidation (M), pyroglutamylation (N-term EQ) as variable modification; and charge states +2, +3, and +4. Windows of mass accuracy of 100 ppm and 0.25 Da were used for precursor ions and MS/MS data respectively. Peptide identification and protein assembly were performed in multiple stages. Initial peptide filtering was used to determine an estimated 1% false discovery rate, which was calculated using the target-decoy method (37). Proteins supported by less than 2 spectral counts or no unique peptides were removed. Tandem mass spectra of each protein annotated by a single peptide are shown in the supplemental Fig. S2. Proteins identified with a higher Mascot score in the bovine database than in the human database were considered serum contamination and removed.

Quantitative Analysis of MS Results—To determine the fold-changes in the amounts of identified proteins between experimental groups, we used a normalized spectral index based on fragment ion intensity measurement with modification (38). Briefly, we calculated the spectral index (SI) of each protein, which is the sum of fragment ion intensities for identified peptides (including all its spectra) that are comprised of a protein and is normalized (SIN) by dividing the total SI for all identified proteins (SIN). To avoid taking logarithms on values of zero, we set the values of SI as 0.349 and 0.538 if no peptide was identified in the control and LPA-treated group, respectively, which is half the smallest value among all SI. We compared the protein quantity between two groups using log2 ratios of SIN_i/LPN_i (SIN_i/LPN_i). Bioinformatic Analysis—Identified proteins were assessed to define “putative secretory proteins.” Classical secretory proteins with signal
peptide were predicted by SignalP 3.0 neural network (NN) scoring. Nonclassical secretory proteins without signal peptides were predicted using secretomeP 2.0 mammalian neural network scoring. DAVID 2008 was used for information mining and functional annotation analysis (http://david.abcc.ncifcrf.gov/).

**Immunodepletion of βιg-h3 from Conditioned Medium**—For immunoprecipitation of βιg-h3, aliquots (30 μl) of a suspension (50% slurry) of protein A-agarose beads (Sigma-Aldrich) in PBS were mixed with 1 μg of anti-βιg-h3 and control rabbit antibodies at 4 °C for 1 h with intermittent shaking. After recovery by centrifugation, beads were washed three times and used for immunodepletion of βιg-h3. LPA CM was incubated with protein A-agarose beads immobilized with anti-βιg-h3 and control antibodies for 1 h at 4 °C. Immune complexes absorbed to protein A-agarose beads were precipitated by centrifugation of siRNAs in each well was 100 nM. After incubation of hASCs with serum-free medium and added to each well. The final concentration for 15 min at room temperature, the mixtures were diluted 15 min, and respective siRNAs were then added to the mixtures. Resultant supernatants were collected and immediately used for experiments.

**siRNA-mediated Gene Silencing**—Small interfering RNA (siRNA) duplexes were synthesized, desalted, and purified by Samchully Pharm, Co. Ltd. (Siheung, GyeongGi, Korea) as follows: LPA1,5 duplexes were synthesized, desalted, and purified by Samchully Pharm. Co. Ltd. (Siheung, GyeongGi, Korea) as follows: LPA1,5 and nonspecific control siRNA (D-001206–13-05) were purchased from Dharmacon, Inc. (Chicago, IL). For siRNA experiments, hASCs were seeded on 60-mm dishes at 70% confluence, and they were then transfected with siRNAs using the Lipofectamine plus™ reagent according to the manufacturer’s instructions. Briefly, Lipofectamine plus™ reagent was incubated with serum-free medium for 15 min, and respective siRNAs were then added to the mixtures. After incubation for 15 min at room temperature, the mixtures were diluted with serum-free medium and added to each well. The final concentration of siRNAs in each well was 100 nM. After incubation of hASCs in serum-free medium containing siRNAs for 4 h, the cells were cultured in growth medium for 24 h, and the expression levels of LPA1,5 and GAPDH were then determined by reverse transcription-polymerase chain reaction analysis.

**Retroviral Overexpression of βιg-h3 in hASCs**—βιg-h3 was overexpressed in hASCs, using retrovirus-mediated gene transfer as described previously (39).

**Statistical Analysis**—The results of multiple observations are presented as mean ± S.D. Student’s t test were used to analyze differences between two groups. For multivariate data analysis, group differences were assessed with two-way analysis of variance (ANOVA), followed by post hoc comparisons tested with Scheffe’s method.

**RESULTS**

**LPA-conditioned Medium from hASCs Stimulate Adhesion and Proliferation of A549 Human Lung Adenocarcinoma Cells**—To assess whether LPA-activated hASCs can regulate the tumorigenic potential of tumor cells, in the present study, we explored the effects of LPA-conditioned medium (LPA CM) from hASCs on the adhesion and proliferation of A549 human lung adenocarcinoma cells in vitro. To measure the effects of LPA CM on the adhesion of A549 cells, adhesive capacities of A549 cells on LPA CM- or control CM-coated dishes were measured. As shown in Fig. 1A, adhesion of A549 cells onto LPA CM-coated dishes was increased in a dose-dependent manner with LPA CM. The adhesive activity of A549 cells on LPA CM-coated dishes was greater than on control CM-coated dishes. To explore the effect of LPA CM on the proliferation of A549 cells, the cells were exposed to different concentrations of LPA CM for 3 days, and the numbers of A549 cells were determined. LPA CM stimulated proliferation of A549 cells in a dose-dependent manner, with a maximal stimulation at 20% concentration (Fig. 1B). To exclude the possibility that exogenous LPA may be responsible for LPA CM-induced proliferation of A549 cells, we compared the effects of LPA CM, control CM, and LPA on cell proliferation. As shown in Fig. 1C, LPA itself had no significant impact on cell proliferation of A549 cells, in contrast to the potent stimulation of cell proliferation by LPA CM, suggesting that LPA stimulates secretion of mitogenic factors from hASCs. To explore whether protein factors are involved in the LPA CM-induced adhesion and proliferation of A549 cells, LPA CM was heated at 95 °C for 5 min to denature protein factors. As shown in Fig. 1D, A549 cells did not adhere onto the culture dishes coated with heat-denatured LPA CM, in contrast to strong adherence of A549 cells onto LPA CM-coated dishes. Furthermore, LPA CM-induced proliferation of A549 cells was abrogated by heat denaturation of LPA CM (Fig. 1E). These results imply a key role of protein factors in LPA CM-induced adhesion and proliferation.

**Proteomic Identification of βιg-h3 as an LPA-induced Secreted Protein in LPA CM**—To identify the protein factors responsible for LPA CM-stimulated adhesion and proliferation, serum-starved hASCs were incubated with serum-free medium in the absence or presence of 10 μM LPA for 2 days, and the conditioned media were subjected to LC-MS/MS analysis for protein identification. After single spectrum-matched proteins were excluded, 146 proteins were identified from comparative proteomic analysis of hASC conditioned medium (supplemental Table S1). The identified proteins were analyzed for the possibility of secretion using SignalP and SecretomeP. In total, 130 (89%) proteins were considered as “putative secretory proteins”; 116 proteins were considered to be secreted through a classical pathway (endoplasmic reticulum/Golgi apparatus-dependent pathway), because a signal peptide was predicted by SignalP, whereas 14 proteins were secreted through a nonclassical pathway, predicted by SecretomeP.

For the comparative analysis of secretomes in response to LPA treatment, a label-free quantitative approach was adopted, and the relative abundance was based on the sum of total fragment ion intensities of peptides matched in control CM and LPA CM. The identities and relative abundance of the secretome are summarized in supplemental Table S1, and the subcellular localization and molecular function of the whole secretome are summarized in the supplemental Tables S2. Sequences of all peptides assigned are listed in the supplemental Table S3. We classified 16 features as LPA-induced proteins, of which Log2 ratio values are higher than 1. An abbreviated list of the LPA-induced proteins is presented in Table I. These proteins included extracellular matrix proteins (βιg-h3 and periostin), proteases and protease inhibitors (matrix metalloproteinase-14, interstitial collagenase, plas-
minogen activator inhibitor 1, and glia-derived nexin), and cytokine signaling (cytokine insulin-like growth factor-binding protein 3 and interleukin-8). Because periostin is secreted from hASCs in response to LPA treatment (24), these results suggest that βig-h3 and periostin may be responsible for LPA CM-stimulated adhesion and migration of A549 cells. The

![Graphs and figures](image)

**TABLE I**

An abbreviated list of secreted proteins that are up-regulated upon LPA treatment

| ID               | Protein                                      | pep# | Log2 Ratio |
|------------------|----------------------------------------------|------|------------|
| PROF1_HUMAN     | Profilin-1                                   | 2    | 6.74       |
| MMP14_HUMAN     | Matrix metalloproteinase-14                  | 2    | 4.66       |
| MANBA_HUMAN     | Beta-mannosidase                             | 1    | 3.53       |
| IL8_HUMAN       | Interleukin-8                                | 1    | 3.08       |
| MMP1_HUMAN      | Intersitial collagenase                      | 4    | 3.06       |
| BGH3_HUMAN      | Transforming growth factor-beta-induced protein βig-h3 (βig-h3; TGFBI) | 7    | 2.70       |
| PAI1_HUMAN      | Plasminogen activator inhibitor 1            | 5    | 2.66       |
| GNS_HUMAN       | N-acetylglucosamine-6-sulfatase              | 4    | 2.27       |
| CD81_HUMAN      | CD81 antigen                                 | 2    | 1.92       |
| PPGB_HUMAN      | Lysosomal protective protein                 | 5    | 1.60       |
| ISLR_HUMAN      | Immunoglobulin superfamily containing leucine-rich repeat protein | 4    | 1.55       |
| CAD13_HUMAN     | Cadherin-13                                  | 1    | 1.54       |
| POSTN_HUMAN     | Periostin                                    | 12   | 1.30       |
| MFAP4_HUMAN     | Microfibril-associated glycoprotein 4        | 1    | 1.27       |
| GDN_HUMAN       | Glia-derived nexin                          | 4    | 1.27       |
| IBP3_HUMAN      | Insulin-like growth factor-binding protein 3 | 3    | 1.16       |

* Number of peptides identified.

$^b$ Log$_2$(TIILPA/TIIcontrol).

![Graphs and figures](image)
Moreover, LPA treatment time-dependently increased the expression levels of **LPA** on the expression levels of **not only LPA** but also **LPA** in hASCs through an LPA receptor 1 (LPA1)-dependent mechanism. To confirm the proteomic data that **LPA** is secreted from hASCs in response to LPA treatment, we determined the expression levels of **LPA** in hASCs by Western blotting. Because **LPA** treatment induced differentiation of hASCs to α-SMA-positive cells (35), we explored the effects of **LPA** on the expression levels of **LPA** and α-SMA. LPA treatment dose-dependently increased the expression levels of not only α-SMA but also βig-h3 in hASCs (Fig. 2A). Moreover, LPA treatment time-dependently increased the expression levels of βig-h3 and α-SMA in hASCs (Fig. 2B). The expression levels of βig-h3 in cell lysates were up-regulated significantly at 12 h after LPA treatment, in contrast to increased expression of α-SMA at 96 h after LPA treatment, suggesting that LPA-induced βig-h3 expression occurred before α-SMA expression. Consistent with the LPA-induced expression of βig-h3 in hASCs, the amounts of βig-h3 protein in conditioned medium from hASCs were increased after a 48-h treatment with LPA (Fig. 2C).

We previously reported that LPA induced α-SMA expression through an LPA receptor 1 (LPA1)-dependent mechanism. To assess the involvement of LPA1 in the LPA-induced expression of βig-h3, we examined the effect of the LPA1-selective antagonist Ki16425 on the expression levels of βig-h3 and α-SMA in hASCs. As shown in Fig. 2D, pretreatment of hASCs with Ki16425 completely abrogated the expression of βig-h3 and α-SMA induced by LPA. Furthermore, depletion of endogenous LPA1 expression using siRNA blocked the LPA-stimulated expression of βig-h3 and α-SMA (Fig. 2E). These results indicate that LPA1 plays a key role in the LPA-induced expression of βig-h3 and α-SMA.

**LPA-induced βig-h3 Expression is Mediated by a Rho Kinase-dependent Pathway**—We have previously reported that the LPA-induced secretion of TGF-β1 from hASCs and the autocrine TGF-β1-dependent pathway mediated LPA-induced α-SMA expression (35). Because βig-h3 was originally identified as a TGF-β1-induced protein (25), we examined the effects of TGF-β1 on βig-h3 expression in hASCs. As shown in Fig. 3A, TGF-β1 dose-dependently increased expression levels of α-SMA and βig-h3 in hASCs. To ascertain whether a TGF-β1-dependent autocrine pathway is involved in the LPA-induced βig-h3 expression, we examined the effects of SB431542, a TGF-β1 receptor I inhibitor, on the expression levels of βig-h3 and α-SMA. As shown in Fig. 3B, the TGF-β1-induced expression of βig-h3 and α-SMA was completely abrogated by pretreatment of hASCs with SB431542. However, LPA-induced βig-h3 expression was not affected by pretreatment of the cells with SB431542, in contrast to significant attenuation of LPA-induced α-SMA expression by SB431542 (Fig. 3C). These results suggest that the TGF-β1-dependent pathway is not involved in LPA-induced βig-h3 expression.

RhoA plays a key role in LPA-induced cellular responses (40). To ascertain whether a Rho kinase is involved in the LPA-stimulated expression of βig-h3, we examined the effect of the Rho kinase inhibitor Y27632 on the expression levels of βig-h3 and α-SMA. As shown in Fig. 3D, pretreatment of hASCs with Y27632 completely abrogated the LPA-induced expression of βig-h3 and α-SMA, implying a pivotal role of the Rho kinase in LPA-induced expression of βig-h3 and α-SMA.
βig-h3 is Responsible for LPA CM-stimulated Adhesion and Proliferation of A549 Cells—To explore the role of βig-h3 on LPA CM-stimulated adhesion and proliferation of A549 cells, βig-h3 was immunodepleted from LPA CM with anti-βig-h3 antibody (Fig. 4A). Adhesion of A549 cells onto culture dishes, which were coated with βig-h3-depleted LPA CM, was markedly attenuated compared with that onto LPA CM-coated dishes (Fig. 4B). Furthermore, LPA CM-stimulated proliferation of A549 cells was attenuated by immunodepletion of βig-h3 from LPA CM (Fig. 4C). These results suggest that βig-h3 is responsible for the LPA CM-stimulated adhesion and proliferation of A549 cells.

To support these findings, βig-h3 expression was depleted by transfection of hASCs with βig-h3-specific siRNA. The LPA-induced secretion of βig-h3 was blocked by transfecting the cells with βig-h3-specific siRNA (Fig. 5A). Adhesion of A549 cells onto LPA CM-coated culture dishes was abrogated by siRNA-mediated knockdown of βig-h3 in LPA CM (Fig. 5B). Furthermore, LPA CM-stimulated proliferation of A549 cells was inhibited by the siRNA-mediated knockdown of endogenous βig-h3 in LPA CM. These results suggest a key role of βig-h3 in LPA CM-induced adhesion and proliferation of A549 cells.

Overexpression of βig-h3 in hASCs Potentiates the Paracrine Functions of hASCs on the Adhesion and Proliferation of A549 Cells—To further confirm the result that βig-h3 secreted from hASCs stimulated the adhesion and proliferation of A549 cells, we overexpressed βig-h3 in hASCs by using a retroviral system. As shown in Fig. 6A, the protein levels of βig-h3 were increased in the cell lysates and conditioned medium from the hASCs infected with the βig-h3 retrovirus. Conditioned medium from the βig-h3-overexpressing hASCs can also stimulate the adhesion and proliferation of A549 cells (Figs. 6B and 6C). These results suggest that βig-h3 secreted from hASCs accelerated the adhesion and proliferation of A549 cells. In order to clarify whether βig-h3 acts as an adhesion molecule, we determined the adhesive capability of A549 cells onto recombinant βig-h3-coated culture dishes. βig-h3 dose-dependently stimulated the adhesion and proliferation of A549 cells, and treatment of A549 cells with purified βig-h3 stimulated the proliferation of A549 cells (supplemental Fig. S4). Moreover, both βig-h3 and LPA CM stimulated the adhesion and proliferation of not only A549 cells but also other cell types, including HeLa human cervical carcinoma cells and WI-38 human lung fibroblasts (supplemental Fig. S5). These results suggest that βig-h3 acts as an extracellular adhesion molecule and stimulates the proliferation of cancer cells.
DISCUSSION

A549 cells induced the differentiation of hASCs to α-SMA-positive CAFs through an LPA-LPA₁-dependent mechanism, and cotransplanted hASCs stimulated in vivo growth of A549 xenograft tumors (41). In the present study, we identified βig-h3 as an LPA-induced secreted protein in hASCs. LPA stimulated the expression of not only α-SMA but also βig-h3 in hASCs by activating the LPA₁ receptor. βig-h3 was originally identified as a TGF-β-induced protein (25). High glucose induced the expression of βig-h3 through the autocrine TGF-β₁-dependent signaling loop in human renal proximal tubule cells and vascular smooth muscle cells (42, 43). LPA-induced α-SMA expression was mediated by an autocrine TGF-β₁-dependent signaling pathway (35), whereas the TGF-β₁-dependent pathway was not associated with the LPA-stimulated βig-h3 expression. We demonstrated that the Rho kinase-dependent pathway plays a pivotal role in the LPA-induced expression of α-SMA and βig-h3. These results suggest that LPA is a unique G protein-coupled receptor agonist stimulating expression of βig-h3 through a TGF-β₁-independent but Rho kinase-dependent mechanism.

To our knowledge, this is the first study showing that βig-h3 secreted from hASCs stimulated the adhesion and proliferation of A549 cells. Immunodepletion or siRNA-mediated silencing of endogenous βig-h3 abrogated LPA CM-induced adhesion and proliferation of A549 cells, whereas conditioned medium from the βig-h3-overexpressing hASCs stimulated the adhesion and proliferation of A549 cells. Accumulating evidence suggests a pivotal role of βig-h3 as an adhesion molecule for various cell types, including dermal fibroblasts, corneal epithelial cells, keratinocytes, astrocytoma cells, and ovarian cancer cells (23, 26, 28, 44–46). βig-h3 secreted from peritoneal cells increased the adhesion of ovarian cancer cells to peritoneal cells and promoted the motility and invasion of ovarian cancer cells (47). Furthermore, βig-h3 stimulated the proliferation of keratinocytes, smooth muscle cells, and renal
proximal epithelial cells (26, 42, 48). βig-h3 expression is elevated in high-grade human colon cancers, and ectopic expression of βig-h3 enhances the aggressiveness and metastatic potential of colon cancer cells in vivo (30). In addition, increased expression of βig-h3 has been reported in various tumor tissues, including lung cancer (23). However, there is conflicting data in the literature reporting the role of βig-h3 in tumor progression. Transfection of βig-h3 into lung cancer cells reduced tumor growth in a xenograft nude mouse model (49). Moreover, the loss of βig-h3 predisposes mice to spontaneous tumor development (50), suggesting a role of βig-h3 as a tumor suppressor. Contrary to the findings of the present study, conditioned medium from Chinese hamster ovary cells transfected with βig-h3 cDNA inhibited the attachment of A549, HeLa, and WI-38 cells to plastic culture plates in vitro (51). The discrepancies in the effects of βig-h3 on cell adhesion are likely because of different experimental conditions. Precoating the culture dishes with βig-h3 stimulated the adhesion of various cell types onto culture dishes (23, 26, 28, 44–46).

Similarly, the adhesion of A549, WI-38, and HeLa cells onto culture plates was augmented by pre-coating the culture plates with recombinant βig-h3 protein or LPA CM. However, co-incubation of cancer cells with βig-h3 protein or LPA CM in the culture medium during the adhesion of the cells onto culture plates inhibited cell adhesion (supplemental Fig. S5). These results suggest that pre-coated βig-h3 stimulates cell adhesion as an extracellular matrix protein, whereas soluble βig-h3 may interfere with the attachment of cells onto culture dishes. Taken together, these results support the notion that βig-h3 secreted from hASCs acts as an adhesion molecule and stimulates the proliferation of cancer cells in vitro.

In addition to βig-h3, using a shotgun proteomic analysis, we have identified a variety of extracellular proteins as LPA-induced secreted proteins, which include periostin, interleukin-8, insulin-like growth factor-binding protein 3/6, and proteases and protease inhibitors (matrix metalloproteinase-14, interstitial collagenase, plasminogen activator inhibitor 1, and glia-derived nexin/Serine protease inhibitor-E2). We have recently reported that LPA stimulated secretion of periostin, an extracellular protein structurally similar to βig-h3, from hASCs through the LPA1-dependent mechanism and recombinant periostin protein augmented the adhesion and migration of epithelial ovarian cancer cells (24), suggesting that both βig-h3 and periostin are involved in the regulation of adhesion, migration, and proliferation of tumor cells. In addition, LPA has been shown to stimulate IL-8 expression in ovarian cancer cells (52) and IL-8 mediated LPA-stimulated invasion of ovarian cancer cells (53). Insulin-like growth factor-binding proteins 3 and 6 bind to insulin-like growth factor, which is implicated in tumorigenesis. Taken together, these results suggest that LPA-induced extracellular protein factors, including extracellular matrix, proteases, and cytokines, play a key role in tumor growth by regulating tumor microenvironment.

CAFs play a key role in tumorigenesis and metastasis of various solid tumors by modulating tumor microenvironment (2–5). Reports have suggested that bone marrow-derived MSCs contributed to 25% of the total myofibroblast population in the tumor stroma in a mouse pancreatic insulinoma model (54) and in a subcutaneous pancreatic xenograft tumor (55). Furthermore, lung cancer stromal fibroblasts have been reported to be derived from blood-borne progenitor cells of patients (56). Incubation of human bone marrow-derived MSCs to conditioned medium from human colorectal cancer cells increased the expression of α-SMA (57). Furthermore, tumor-conditioned medium induced differentiation of human bone marrow-derived MSCs into CAFs, which express α-SMA and SDF-1 (19). We have previously reported that cancer-conditioned medium induced differentiation of hASCs to CAFs, which express α-SMA, SDF-1, and VEGF in vitro (35, 58). Furthermore, co-transplanted hASCs were differentiated into α-SMA-positive CAFs and stimulated tumor angiogenesis through a VEGF-dependent mechanism in an A549 xenograft tumor model (41). Together with the findings that LPA-activated hASCs stimulated the adhesion and proliferation of A549 cells through a βig-h3-dependent mechanism, these results suggest that hASCs promote tumor growth and angiogenesis within tumor microenvironment through secreting paracrine factors, including proteases, extracellular matrix proteins, and angiogenic cytokines.

* This research was supported by programs through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0020274, 2010-0014876) and by the MRC program (2010-0001251).

This article contains supplemental Figs. S1 to S5 and Tables S1 to S3.

†† To whom correspondence should be addressed: Department of Physiology, School of Medicine, Pusan National University, Yangsan 626-870, Gyeongsangnam-do, Republic of Korea. Tel.: 82-51-510-8073. Fax: 82-51-510-8076. E-mail: jhkimst@pusan.ac.kr.

REFERENCES

1. De Wever, O., and Mareel, M. (2003) Role of tissue stroma in cancer cell invasion. J. Pathol. 200, 429–447
2. Bhowmick, N. A., Neilson, E. G., and Moses, H. L. (2004) Stromal fibroblasts in cancer initiation and progression. Nature 432, 332–337
3. De Wever, O., Demetter, P., Mareel, M., and Bracke, M. (2008) Stromal myofibroblasts are drivers of invasive cancer growth. Int. J. Cancer 123, 2229–2238
4. Desmoulière, A., Guyot, C., and Gabbiani, G. (2004) The stroma reaction myofibroblast: a key player in the control of tumor cell behavior. Int. J. Dev. Biol. 48, 509–517
5. Orimo, A., and Weinberg, R. A. (2006) Stromal fibroblasts in cancer: a novel tumor-promoting cell type. Cell Cycle 5, 1597–1601
6. Sappino, A. P., Skalli, O., Jackson, B., Schürch, W., and Gabbiani, G. (1988) Smooth-muscle differentiation in stromal cells of malignant and non-malignant breast tissues. Int. J. Cancer 41, 707–712
7. Ganis, R. (2006) Tumor stroma fosters neovascularization by recruitment of progenitor cells into the tumor bed. J. Cell. Mol. Med. 10, 857–865
8. Olimi, A. F., Grosse, G. D., Hayward, S. W., Carroll, P. R., Tlusty, T. D., and Cunha, G. R. (1999) Carcinoma-associated fibroblasts direct tumor progression of initiated human prostatic epithelium. Cancer Res. 59, 5002–5011
9. Orimo, A., Gupta, P. B., Sgriol, D. C., Azenara-Seisdedos, F., Delaunay, T., Naem, R., Carey, V. J., Richardson, A. L., and Weinberg, R. A. (2005)
Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell 121, 335–348

Li, H., Fan, X., and Houghton, J. (2007) Tumor microenvironment: The role of the tumor stroma in cancer. J. Cell. Biochem. 101, 805–815

Barry, F. P., and Murphy, J. M. (2004) Mesenchymal stem cells: clinical applications and biological characterization. Int. J. Biochem. Cell Biol. 36, 568–584

Prockop, D. J. (1997) Marrow stromal cells as stem cells for nonhematopoietic tissues. Science 276, 71–74

Pittenger, M. F., Mackay, A. M., Beck, S. C., Jaiswal, R. K., Douglas, R., Mosca, J. D., Moorman, M. A., Simonetti, D. W., Craig, S., and Marshak, D. R. (1999) Multilineage potential of adult human mesenchymal stem cells. Science 284, 143–147

Shi, S. H., Chang, N., Cao, X.-D., Scott, T., Ramakrishnan, A., and Simon, P. J. (2003) Mesenchymal stem cells. Arch. Med. Res. 34, 565–571

Crisan, M., Yap, S., Castella, L., Chen, C. W., Corselli, M., Park, T. S., Andreiolo, G., Sun, B., Zheng, B., Zhang, L., Norotte, C., Teng, P. N., Traas, J., Schugur, R., Deasy, B. M., Badyuk, S., Buhring, H. J., Giacobino, J. P., Lazzari, L., Huard, J., and Pauthet, B. (2008) A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell 3, 301–313

Hall, B., Andreeff, M., and Marini, F. (2007) The participation of mesenchymal stem cells in the recruitment of immune cells. Exp. Biol. Med. 232, 2219–2230

Zhu, W., Xu, W., Jiang, R., Qian, H., Chen, M., Hu, J., Cao, W., Han, C., and Chen, Y. (2008) Mesenchymal stem cells derived from bone marrow favor tumor growth in vivo. Exp. Mol. Pathol. 80, 267–274

Kamoun, A. E., Dash, A. B., Xu, W., Chen, M., Hu, J., Cao, W., Han, C., and Chen, Y. (2008) Mesenchymal stem cells derived from bone marrow favor tumor growth in vivo. Exp. Mol. Pathol. 80, 267–274

Kudo, Y., Satriawan, B. S., Hatanaka, H., Ogawa, I., and Weinberg, R. A. (2007) Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. Nature 449, 557–563

Mishra, P. J., Mishra, P. J., Humeniuk, R., Medina, D. J., Alexe, G., Mesirov, J. P., Gatt, F., Fourcade, O., Gueguen, G., Gaige, B., Gaussama-Diagne, A., Fauvel, J., Salles, J. P., Maucoc, G., Simon, M. F., and Chop, H. (1997) Lysophosphatidic acid as a phospholipid mediator: pathways of synthesis. FEBS Lett. 410, 54–58

Jen, E. S., Moon, H. J., Lee, M. J., Song, H. Y., Kim, Y. M., Cho, M., Suh, D. S., Yoon, M. S., Chang, C. L., Jung, S. J., and Kim, J. H. (2008) Cancer-derived lysophosphatidic acid stimulates differentiation of human mesenchymal stem cells to myofibroblast-like cells. Stem Cells 26, 789–797

Lee, M. J., Jeon, E. S., Lee, J. S., Cho, M., Suh, D. S., Chang, C. L., and Kim, J. H. (2008) Lysophosphatidic acid in malignant ascites stimulates migration of human mesenchymal stem cells. J. Cell. Biochem. 104, 499–510

Ilh, J. E., and Gygi, S. P. (2007) Target-decoy search strategy for increased confidence in large-scale protein identifications by mass spectrometry. Nat. Methods 4, 207–214

Griffin, N. M., Yu, J., Long, F., Oh, P., Shore, S., Li, Y., Kozlil, J. A., and Schnitzer, J. E. (2010) Label-free, normalized quantification of complex mass spectrometry data for proteomic analysis. Nat. Biotechnol. 28, 83–89

Lee, B. H., Bae, J. S., Park, R. W., Kim, J. E., Park, J. Y., and Kim, I. S. (2006) betaig-h3 triggers signaling pathways mediating adhesion and migration of vascular smooth muscle cells through alphabeta(3) integrin. Exp. Mol. Med. 38, 153–161

Moolenaar, W. H., van Meeteren, L. A., and Giepmans, B. N. (2004) The ins and outs of lysophosphatidic acid signaling. Bioessays 26, 870–881

Jeon, E. S., Lee, I. H., Heo, S. C., Shin, S. H., Choi, Y. J., Park, J. H., Park do, Y., and Kim, J. H. (2010) Mesenchymal stem cells stimulate angiogenesis in a murine xenograft model of A549 human adenocarcinoma through an LPA1 receptor-dependent mechanism. Biochim. Biophys. Acta 1801, 1205–1213

Ha, S. W., Bae, J. S., Yeo, H. J., Lee, S. H., Choi, Y. J., Sohn, Y. K., Kim, J. G., Kim, I. S., and Kim, B. W. (2003) TGFBeta3-induced protein betaig-h3 is upregulated by high glucose in vascular smooth muscle cells. J. Cell. Biochem. 88, 774–782

Lee, S. H., Bae, J. S., Park, S. H., Lee, B. H., Park, R. W., Choi, J. Y., Park, J. Y., Park, H. S., Sohn, Y. S., Lee, D. S., Bae Lee, E., and Kim, I. S. (2003) Expression of TGF-beta induced matrix protein betaig-h3 is up-regulated in myofibroblast-like cells of the diabetic rat kidney and human proximal tubular epithelial cells treated with high glucose. Kidney Int. 64, 1012–1021

Ahmed, A. A., Mills, A. D., Ibrahim, A. E., Temple, J., Blenkiron, C., Vlas, M., Massie, C. E., Iyer, N. G., McGeoch, A., Crawford, R., Nicke, B., Downward, J., Swanton, C., Bell, S. D., Earl, H. M., Laskey, R. A., Caldas, C., and Brenton, J. D. (2007) The extracellular matrix protein TGFBI induces microtubule stabilization and sensitizes ovarian cancers to paclitaxel. Cancer Cell 12, 514–527

Kim, J. E., Kim, S. J., Lee, B. H., Park, R. W., Kim, K. S., and Kim, I. S. (2000) Identification of motifs for cell adhesion within the repeated domains of transforming growth factor-beta-induced gene, betaig-h3. J. Biol. Chem. 275, 30907–30915

Kim, M. O., Yoon, S. J., Kim, I. S., Sohn, S. Lee, and Lee, E. H. (2003) Transforming growth factor-beta-inducible gene product betaig-h3 promotes cell adhesion of human astrocytoma cells in vitro: implication of alphabeta(3) integrin. Neurosci. Lett. 336, 93–96

Ween, M. P., Lokman, N. A., Hoffmann, P., Rodgers, R. J., Ricciardelli, C., and Oehler, M. K. (2011) Transforming growth factor-beta-induced protein secreted by peritoneal cells increases the metastatic potential of...
ovarian cancer cells. Int. J. Cancer 128, 1570–1584

48. Park, S. W., Bae, J. S., Kim, K. S., Park, S. H., Lee, B. H., Choi, J. Y., Park, J. Y., Ha, S. W., Kim, Y. L., Kwon, T. H., Kim, I. S., and Park, R. W. (2004) Beta ig-h3 promotes renal proximal tubular epithelial cell adhesion, migration and proliferation through the interaction with alpha3beta1 integrin. Exp. Mol. Med. 36, 211–219

49. Zhao, Y. L., Piao, C. Q., and Hei, T. K. (2002) Overexpression of Betaig-h3 gene downregulates integrin alpha3beta1 and suppresses tumorigenicity in radiation-induced tumorigenic human bronchial epithelial cells. Br. J. Cancer 86, 1923–1928

50. Zhang, Y., Wen, G., Shao, G., Wang, C., Lin, C., Fang, H., Balajee, A. S., Bhagat, G., Hei, T. K., and Zhao, Y. (2009) TGFBI deficiency predisposes mice to spontaneous tumor development. Cancer Res. 69, 37–44

51. Skonier, J., Bennett, K., Rothwell, V., Kosowski, S., Plowman, G., Wallace, P., Edelhoff, S., Distefano, S., Neubauer, M., Marquardt, H., Rodgers, J., and Purchio, A. F. (1994) beta ig-h3: a transforming growth factor-beta-responsive gene encoding a secreted protein that inhibits cell attachment in vitro and suppresses the growth of CHO cells in nude mice. DNA Cell Biol. 13, 571–584

52. Schwartz, B. M., Hong, G., Morrison, B. H., Wu, W., Baudhuin, L. M., Xiao, Y. J., Mek, S. C., and Xu, Y. (2001) Lysosphospholipids increase interleukin-8 expression in ovarian cancer cells. Gynecol. Oncol. 81, 291–300

53. So, J., Navari, J., Wang, F. Q., and Fishman, D. A. (2004) Lysosphosphatic acid enhances epithelial ovarian carcinoma invasion through the increased expression of interleukin-8. Gynecol. Oncol. 95, 314–322

54. Direkze, N. C., Hodivala-Dilke, K., Jeffery, R., Hunt, T., Poulson, R., Oukrif, D., Alison, M. R., and Wright, N. A. (2004) Bone marrow contribution to tumor-associated myofibroblasts and fibroblasts. Cancer Res. 64, 8492–8495

55. Ishii, G., Sangai, T., Oda, T., Aoyagi, Y., Hasebe, T., Kanomata, N., Endoh, Y., Okumura, C., Okuhara, Y., Magae, J., Emura, M., Ochiya, T., and Ochiai, A. (2003) Bone-marrow-derived myofibroblasts contribute to the cancer-induced stromal reaction. Biochem. Biophys. Res. Commun. 309, 232–240

56. Ishii, G., Ito, T. K., Aoyagi, K., Fujimoto, H., Chiba, H., Hasebe, T., Fujii, S., Nagai, K., Sasaki, H., and Ochiai, A. (2007) Presence of human circulating progenitor cells for cancer stromal fibroblasts in the blood of lung cancer patients. Stem Cells 25, 1469–1477

57. Emura, M., Ochiai, A., Horino, M., Arndt, W., Kamiya, K., and Hirohashi, S. (2000) Development of myofibroblasts from human bone marrow mesenchymal stem cells cocultured with human colon carcinoma cells and TGF beta 1. In Vitro Cell Dev. Biol. Anim 36, 77–80

58. Jeon, E. S., Heo, S. C., Kim, J., Kim, Y. M., Kim, I. S., Lee, T. G., and Kim, J. H. (2012) Proteomic Identification of Betaig-h3 as a Lysosphosphatic Acid-Induced Secreted Protein of Human Mesenchymal Stem Cells: Paracrine Activation of A549 Lung Adenocarcinoma Cells by Betaig-h3. Mol. Cell. Proteomics 11(2): M111.012385. DOI: 10.1074/mcp.M111.012385.

In order to cite this article properly, please include all of the following information: Shin, S. H., Kim, J., Heo, S. C., Kwon, Y. W., Kim, Y. M., Kim, I. S., Lee, T. G., and Kim, J. H. (2012) Proteomic Identification of Betaig-h3 as a Lysosphosphatic Acid-Induced Secreted Protein of Human Mesenchymal Stem Cells: Paracrine Activation of A549 Lung Adenocarcinoma Cells by Betaig-h3. Mol. Cell. Proteomics 11(2): M111.012385. DOI: 10.1074/mcp.M111.012385–11