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Oncostatin M promotes mesenchymal stem cell-stimulated tumor growth through a paracrine mechanism involving periostin and TGFBI

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ABSTRACT

Oncostatin M, a member of the interleukin-6 family of cytokines, has been implicated in tumorigenesis of human prostate cancer. In the current study, we demonstrate that oncostatin M promotes human adipose tissue-derived mesenchymal stem cell-stimulated tumor growth in an *in vivo* xenograft transplantation model of the human prostate cancer cell line PC-3M-luc-C6, a PC3M cell line expressing the luciferase gene. Conditioned medium derived from oncostatin M-treated mesenchymal stem cells stimulated adhesion of PC-3M-luc-C6 cells. We identified TGFBI and periostin, extracellular matrix proteins implicated in tumorigenesis and metastasis, as oncostatin M-induced secreted proteins in mesenchymal stem cells. Treatment with oncostatin M stimulated secretion of periostin and TGFBI from mesenchymal stem cells in a time-dependent manner. Immunodepletion of TGFBI and periostin from conditioned medium derived from oncostatin M-treated mesenchymal stem cells resulted in abrogation of adhesion of PC-3M-luc-C6 cells stimulated by oncostatin M-conditioned medium. In addition, small interfering RNA-mediated silencing of TGFBI and periostin resulted in abrogation of cell adhesion stimulated by oncostatin M-conditioned medium. These results suggest that mesenchymal stem cell-derived TGFBI and periostin play a key role in tumorigenesis by stimulating adhesion of prostate cancer cells.

1. Introduction

Prostate cancer continues to be the most common lethal malignancy diagnosed in men and the second leading cause of cancer deaths in males (Greenlee et al., 2000). Although prostate cancer cells initially proliferate within a local area, but they may later metastasize, preferentially to bone, where the metastases may cause clinical problems, such as bone pain, suppressed mobility, replacement of hematopoietic tissue, and, in the vertebral column, compression of the spinal cord (Nelson et al., 2003). Prostate cancer tissues are composed of carcinoma cells and non-cancerous stromal cells, including endothelial cells, immune cells, and carcinoma-associated fibroblasts (Cunha et al., 2003). Crosstalk between epithelial prostate cancer cells and stromal cells is important for progression and metastasis of prostate cancer (Karlou et al., 2010). Carcinoma-associated fibroblasts constitute a substantial volume of the tumor stroma and play a pivotal role in maintenance, dissemination, and drug resistance of prostate cancer (Franco and Hayward, 2012). Therefore, anti-cancer therapy targeting the stroma may be a promising therapeutic option for treatment of prostate cancer.

Mesenchymal stem cells (MSCs) have a capacity for self-renewal, long-term viability, and potential for differentiation toward diverse cell types, such as adipogenic, osteogenic, chondrogenic, and myogenic lineages (Barry and Murphy, 2004; Pittenger et al., 1999; Prockop, 1997; Short et al., 2003), suggesting the clinical usefulness of MSCs for tissue regeneration. Although MSCs exist predominantly in bone marrow, they are also distributed

Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; hASCs, human adipose tissue-derived MSCs; MSCs, mesenchymal stem cells; OSM, oncostatin M; OSM CM, conditioned medium from OSM-treated hASCs; PBS, phosphate-buffered saline; SDF-1, stromal cell-derived factor-1; siRNA, small interfering RNA.

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throughout many other tissues, where they are thought to be local sources of tissue-resident stem cells (Crisan et al., 2008). MSCs constitute a large proportion of non-neoplastic stromal cells within the tumor microenvironment (De Wever et al., 2008) and differentiate into carcinoma-associated fibroblasts (Jeon et al., 2008). According to accumulating evidence, MSCs could also have an adverse effect that favors tumor growth. When transplanted subcutaneously, Tumor cells mixed with MSCs exhibited elevated capacity for proliferation and rich angiogenesis in tumor tissues (Zhu et al., 2006). Co-injection of MSCs with human breast carcinoma cells into a subcutaneous site by xenograft transplantation resulted in stimulation of the metastatic potency of breast carcinoma (Karnoub et al., 2007). In addition, MSCs exposed to tumor-conditioned medium have been reported to exhibit phenotypic and functional characteristics of carcinoma-associated fibroblasts, including sustained expression of stromal cell-derived factor-1 (SDF-1) and the ability to promote growth of tumor cells *in vitro* and in an *in vivo* co-implantation model (Mishra et al., 2008). And, co-transplantation of A549 human lung adenocarcinoma cells and human adipose tissue-derived MSCs (hASCs) resulted in stimulation of *in vivo* growth of A549 cells and tumor angiogenesis (Jeon et al., 2010). Conditioned medium of human MSCs have been reported to promote proliferation, migration, and invasion of human prostate cancer PC-3 cells (Ye et al., 2012). These results suggest that tumorigenesis of prostate cancer is acquired by paracrine signals from MSCs within the tumor microenvironment. However, the paracrine signaling mechanisms by which MSCs stimulate tumorigenesis are largely unknown.

Oncostatin M (OSM), a multifunctional cytokine produced by activated monocytes, macrophages, and T lymphocytes, influences inflammation, hematopoiesis, growth, differentiation, and angiogenesis (Tanaka and Miyajima, 2003). OSM belongs to the interleukin-6 (IL-6) family of cytokines, including IL-6, IL-11, leukemia inhibitory factor, ciliary neurotrophic factor, and cardiotrophin 1. OSM was originally identified from conditioned media of phorbol 12-myristate 13-acetate-treated U-937 monocytic cells by its ability to inhibit the growth of melanoma (Zarling et al., 1986). In addition, treatment with OSM inhibited growth of several solid tumor cells, including breast cancer and glioma (Friedrich et al., 2001; Li et al., 2001). However, OSM has been reported to promote proliferation of several tumor cells derived from ovary, cervix, and prostate (Godoy-Tundidor et al., 2005; Li et al., 2011; Mori et al., 1999). OSM and IL-6 have been reported to induce expression of urokinase-type plasminogen activator and vascular endothelial growth factor in metastasizing prostate cancer cells and played a role in the pathogenesis of prostate cancer (Weiss et al., 2011). In addition, treatment with OSM stimulated secretion of SDF-1 from hASCs (Lee et al., 2007). SDF-1 has been implicated in tumor angiogenesis by recruitment of endothelial progenitor cells from bone marrow (Orimo et al., 2005). These results raise the possibility that OSM may play a key role in tumorigenesis through regulation of the paracrine functions of hASCs within the tumor microenvironment.

Periostin and TGFBI (transforming growth factor, beta-induced, 68 kDa also known as βig-h3) are extracellular matrix proteins that are structurally homologous to the axon guidance protein fasciclin I (FAS1) (Kudo et al., 2007). Periostin, originally named osteoblast-specific factor-2, is a disulfide-linked 90-kDa secretory protein that functions as a cell adhesion molecule (Horiuchi et al., 1999). TGFBI was originally identified as a transforming growth factor-β1-induced protein in A549 human adenocarcinoma cells (Skonier et al., 1992). Both periostin and TGFBI contain four tandem repeats of FAS1 domains and an EMI protein-protein interaction domain, and play a key role in a variety of cellular responses, including adhesion, migration, proliferation, angiogenesis, wound healing, and tumorigenesis (Ruan et al., 2009; Thapa et al., 2007). Using a shotgun proteomic analysis, we have reported on secretion of both periostin and TGFBI from hASCs in response to treatment with lysophosphatidic acid (Shin et al., 2012). Periostin and TGFBI stimulate adhesion (Heo et al., 2011; Shin et al., 2012) and proliferation of A549 human lung adenocarcinoma cells, and silencing of periostin expression in hASCs resulted in abrogation of the stimulatory effects of hASCs on tumor growth in a xenograft co-transplantation tumor model (Heo et al., 2011). Despite various reports suggesting the role of periostin and βig-h3 in tumorigenesis, it is still unclear whether OSM-stimulated paracrine function of hASCs is mediated by periostin and TGFBI within the tumor microenvironment.

In order to clarify the role of OSM in the crosstalk between cancer cells and hASCs, we examined the effect of OSM on *in vivo* tumor growth in a xenograft tumor model co-transplanted with hASCs and PC3M prostate cancer cells. In addition, we identified periostin and βig-h3 as OSM-induced secreted proteins of hASCs. Results of the current study demonstrate a pivotal role of periostin and TGFBI as OSM-induced paracrine factors of hASCs in the adhesive and proliferative properties of PC3M cells.

2. Materials and methods

2.1. Materials

α-Minimum essential medium, trypsin, and fetal bovine serum were purchased from Invitrogen (Carlsbad, CA, http://www.invitrogen.com). Recombinant human OSM was purchased from R&D Systems Inc. (Minneapolis, MN). Anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody was purchased from Millipore (Temecula, CA, http://www.milliore.com). Protein A agarose beads were purchased from Sigma–Aldrich (St. Louis, MO). Culture plates were purchased from Nunc (Roskilde, Denmark). Anti-periostin, anti-TGFBI, anti-OSM receptor rabbit antibodies were purchased from Abcam (Cambridge, MA). Rabbit anti-gp130 antibody was purchased from Cell Signaling Technology, Inc. (Danvers, MA). Peroxidase-labeled secondary antibodies and the enhanced chemiluminescence Western blotting system were purchased from Amersham Biosciences (Pittsburgh, PA).

2.2. Cell culture

Subcutaneous adipose tissue was obtained from elective surgeries with patient's consent, and the protocol for this study 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), followed by treatment with an equal volume of collagenase type I suspension (1 g/L of Hank's Balanced Salt Solution with 1% bovine serum albumin) for 60 min at 37 °C with intermittent shaking. 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 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/cm^2. 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 no expression of CD31, CD34, and CD45 was observed in hASCs.

Luciferase-expressing PC-3M-luc-C6 cell line derived from PC-3M metastatic prostate cancer cells was purchased from PerkinElmer (Waltham, MA). PC-3M-luc-C6 cells were cultured