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Role of MEK-ERK pathway in sphingosylphosphorylcholine-induced cell death in human adipose tissue-derived mesenchymal stem cells

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Abstract

Sphingosylphosphorylcholine (SPC) is a bioactive lipid molecule involved in a variety of cellular responses. In the present study, we demonstrated that treatment of human adipose tissue-derived mesenchymal stem cells (hATSCs) with D-*erythro*-SPC resulted in apoptosis-like cell death, as demonstrated by decreased cell viability, DNA strand breaks, the increase of sub-G1 fraction, cytochrome *c* release into cytosol, and activation of caspase-3. In contrast, the exposure of hATSCs to L-*threo*-SPC did not induce the cell death, suggesting that the SPC-induced cell death was selective for the D-*erythro*-stereoisomer of SPC. The D-*erythro*-SPC-induced cell death was prevented by DEVD-CHO, a caspase-3 specific inhibitor, and Z-VAD-FMK, a general caspase inhibitor, suggesting that the SPC-induced cell death of hATSCs occurs through the cytochrome *c*- and caspase-3-dependent pathways. In addition, D-*erythro*-SPC treatment stimulated the activation of mitogen-activated protein kinases, such as ERK and c-*Jun* NH_2-terminal protein kinase (JNK), and the D-*erythro*-SPC-induced cell death was completely prevented by pretreatment with the MEK inhibitor, U0126, but not by pretreatment with the JNK inhibitor, SP600125, and the p38 MAPK inhibitor, SB202190, suggesting a specific involvement of ERK in the D-*erythro*-SPC-induced cell death. Pretreatment with U0126 attenuated the D-*erythro*-SPC-induced release of cytochrome *c*. From these results, we suggest that ERK is involved in the SPC-induced cell death of hATSC through stimulation of the cytochrome *c*/caspase-3-dependent pathway.

Keywords: Mesenchymal stem cell; Adipose tissue; SPC; Cell death; ERK

1. Introduction

Mesenchymal stem cells (MSCs) can be isolated from a variety of tissues, including bone marrow, periosteum, trabecular bone, synovium, skeletal muscle, and adipose tissues [1]. MSCs have self-renewal capacity, long-term viability, and differentiation potential toward the diverse cell types such as adipogenic, osteogenic, chondrogenic, and myogenic lineages [1–6]. It has well been documented that cell fate decision toward the proliferation and multi-lineage differentiation of MSCs is determined by a variety of extracellular hormones and cytokines in each peripheral tissue [1–6]. It has been proposed that the depletion or functional inactivation of stem cell populations in local tissues may be responsible for certain degenerative diseases, which are caused by progressive tissue damage and an inability to repair [1,7]. In spite of the various reports on the proliferation and differentiation, the molecular mechanisms of the functional inactivation of MSCs, which can be caused by apoptosis or loss of self-renal capacity, are still unknown.

Abbreviations: MSCs, mesenchymal stem cells; SPC, sphingosylphosphorylcholine; MAPKs, mitogen-activated protein kinases; ERK, extracellular signal-regulated kinase; JNK, c-*Jun* NH2-terminal protein kinase; GPCRs, G protein-coupled receptors; hATSCs, human adipose tissue-derived mesenchymal stem cells; PBS, phosphate-buffered saline; MTT, 3-(4,5-dimethyl-2-thiozol)-2,5-diphenyl-2*H*-tetrazolium bromide; DMSO, dimethylsulfoxide; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling

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Sphingosylphosphorylcholine (SPC) has been implicated in a number of biological processes, including proliferation, growth inhibition, smooth muscle contraction, wound healing, and angiogenesis [8]. SPC has been reported to stimulate DNA synthesis and proliferation in a variety of cell types, including fibroblasts, endothelial cells, keratinocytes, and vascular smooth muscle cells [9–13]. In contrast, SPC can also inhibit the growth of various cell types, most that of tumor cells such as pancreatic, breast, and ovarian cancer cells, and Jurkat T cells [14,15]. Therefore, it is likely that SPC positively or negatively regulates proliferation, depending on cell types. However, it is still unclear whether the SPC-induced growth inhibition is related to cell death, and whether exogenous SPC affects the proliferation in MSCs.

Mitogen-activated protein kinases (MAPKs) are serine/threonine protein kinases, which are involved in the regulation of a large variety of cellular processes: cell growth, differentiation, development, cell cycle, death, and survival [16]. The major groups of MAPKs cascades include the extracellular signal-regulated kinase (ERK), c-*Jun* NH_2-terminal protein kinase (JNK), and p38 MAPK. It is generally accepted that the activation of ERK occurs in response to mitogenic growth factors and physical stress acting through receptor protein tyrosine kinase or G protein-coupled receptors (GPCRs), and that the JNK and p38 MAPK signaling pathways are activated by various and overlapping stimuli such as heat or osmotic shock, radiation, and inflammatory cytokines [16–18]. The ERK1/2 pathway delivers a survival signal which counteracts proapoptotic effects elicited by the JNK and p38 MAPK activation. It has been reported that SPC enhances ERK activity in various cell lines [10,14]. However, it is unclear whether the ERK activation induces apoptosis or enhances cell survival in response to SPC.

In the present study, we demonstrated for the first time that SPC induced cell death through caspase-3-dependent signaling pathway in human adipose tissue-derived mesenchymal stem cells (hATSCs) and the ERK activation plays a key role in the SPC-induced cell death.

2. Materials and methods

2.1. Materials

D-*erythro*- and L-*threo*-SPC were from Matreya (Pleasant Gap, PA). U0126, SP600125, SB202190, Z-VAD-FMK, and DEVE-CHO were from BIOMOL (Plymouth Meeting, PA). Minimum essential medium alpha, phosphate-buffered saline (PBS), and trypsin were from Invitrogen (Carlsbad, CA). Fetal bovine serum was from Hyclone (Logan, UT). The anti-phospho-ERK, anti-phospho-JNK, anti-phospho-p38, anti-phospho-c-*Jun* (Ser-63) and anti-cleaved caspase-3 antibodies were obtained from Cell Signaling Technology Inc. (Beverly, MA, USA). Anti-cytochrome *c* antibody and anti-actin antibody were from BD biosciences (San Diego, CA) and MP Biomedicals (Irvine, CA), respectively. Horseradish peroxidase-conjugated secondary antibodies were from Jackson ImmunoResearch Lab. (West Grove, PA). The enhanced chemiluminescence kit was from Amersham Biosciences (Buckinghamshire, UK). The In Situ Cell Death Detection Kit was purchased from Roche Applied Science (Mannheim, Germany). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and all other reagents were from Sigma-Aldrich Co. (St. Louis, MO).

2.2. Cell culture

Subcutaneous adipose tissue was obtained from elective surgeries with the patient's consent, as approved by the Institution Review Board, and hATSCs were isolated as previously reported [19]. Briefly, liposuction tissues were washed at least three times with sterile phosphate-buffered saline and treated with equal volume of collagenase type I (1 g/l of Hank's buffered saline solution with 1% bovine serum albumin) for 60 min at 37 °C with intermittent shaking. The floating adipocytes were separated from the stromal-vascular fraction by centrifugation (300×*g* for 5 min). The cellular pellet was resuspended in Minimum essential medium alpha supplemented with 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin, and plated in tissue culture dishes at 3500 cells/cm^2. The primary hATSCs were cultured for 4–5 days until they reached confluence and were defined as passage "0". The passage number of the hATSCs used in the experiments was 3–10.

2.3. Cell viability assay

Cell viability was determined by MTT assay previously described [20] and by counting the number of viable cells. For MTT assay, the stock solution (5 mg/ml MTT) was added to each well of the 96-well plate seeded with hATSCs to a final 0.5 mg/ml concentration of MTT. The plate was incubated at 37 °C for 2 h, and the formazan granules generated by the live cells were dissolved in 100% dimethylsulfoxide (DMSO) and absorbance at 570 nm was monitored by using a Power-Wave$_x$ microplate spectrophotometer (Bio-Tek Instruments, Inc. Winooski, VT).

2.4. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis

Total RNAs and subsequent messenger RNAs were purified from hATSCs by using the mRNA isolation system (Novagen, Darmstadt, Germany), as described in the manufacturer's manual. Three microliters of mRNAs was used to make cDNA with oligo dT primers, and then 2 μl of the synthesized cDNA was used as a template for the PCR