Effect of MAPK Pathway Inhibitor on Human Bone and Soft Tissue Tumors

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Introduction: Raf-1, which is an essential serine/threonine kinase, is a downstream effector of the central signal transduction mediator Ras in the MAPK signaling pathway (RAF/MEK/ERK pathway), and the therapeutics targeting Raf-1 are undergoing clinical evaluation on some human malignancies.

The mitogen-activated protein kinase (MAPK) signaling pathway is activated by signals from growth factor receptors, and it plays a crucial role in the cell proliferation. Several authors have reported that dysregulation of the MAPK signaling pathway (RAF/MEK/ERK pathway) exists in many human malignancies, and it is suggested that the MAPK pathway are very important targets as the molecular targeting therapy on some human malignancies. We consider that MAPK signaling inhibition will be demonstrated the antitumor activity on human bone and soft tissue tumors. We examined the inhibitory effect of MEKI1/2 inhibitor on the cell proliferation of human malignant cells.

Materials and Methods: Cell lines and reagent.

2 human osteosarcoma cell lines (KHOS and KTHOS) and 2 human malignant fibrous histiocytosis (MFH) cell lines (GBS-1 and Nara-F) were used in this study. All cell lines were grown in culture medium consisting of Dulbecco’s Modified Eagle Medium (DMEM; Sigma-Aldrich, St. Louis, MO) supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich). The cell lines were routinely maintained at 37°C in a humidified 5% CO2 atmosphere. U0126 (1,4-diamino-2,3-dicyano-1,4-bis[2-aminophenylthio]butadiene; MW= 426.5), a selective MEK1/2 inhibitor, was purchased from Promega.

mRNA expression of Raf-1.

Total RNAs were eluted by selective binding to a silica-gel-based membrane using an RNeasy Mini Kit® (QIAGEN Inc., Valencia, CA). Reverse transcription of RNA into cDNA was performed by using Reverse Transcription System (Promega, Madison, WI). Raf-1 and GAPDH mRNA expression were examined by reverse transcription (RT-) PCR. After PCR amplification, 8-μl aliquots of the PCR products were electrophoresed in a 2% agarose gel, followed by ethidium bromide dye.

The inhibitory effect of S0126.

The cell proliferation was assayed using the MTS assay (CellTiter 96® Aqueous One Solution Cell Proliferation Assay; Promega, Madison, WI). Cells were seeded in 96-well cell culture plates. After 48 hours (h), the medium was refreshed containing U0126 in the indicated concentrations. After 24, 48, 96h, the optical density was measured. The percent viability of each well was calculated. The percent viability of each well was calculated.

Western blotting.

Cells were pretreated for 60 min with 1% FBS containing U0126 in the indicated concentrations before stimulation with or without 10 ng/ml PDGF for 10 min. Whole cell lysates were collected for protein content, and cell lysates were separated by SDS polyacrylamide gel electrophoresis under reducing conditions. Then gels were electrophoretically transferred to PVDF membrane, and immunoblotted with anti-MEK1/2 antibody and anti-phospho-MEK1/2 antibody (Assay Designs, Inc., Ann Arbor, MI). Bound antibodies were detected using the ECL plus western blotting detection system (GE Healthcare Bio-Sciences, Piscataway, NJ).

Results: mRNA expression of Raf-1

The Raf-1 mRNA was expressed in all osteosarcoma and MFH cell lines. The effect of U0126

U0126 inhibited the cell proliferation of all 4 cell lines in a dose- and time-dependent manner. 50μM U0126 inhibited the cell proliferation of KHOS at the percent viability of 50% or less. 50μM U0126 inhibited the cell proliferation of GBS-1, at the percent viability of 50% or less.

Expression of MEK1/2 and phospho-MEK1/2

Western blotting analysis revealed that not only MEK1/2 but phospho-MEK1/2 were expressed in all cell lines under the normal condition. Phosphorylation of MEK1/2 were increased by PDGF stimulation, and S0126 decreased phosphorylation of MEK1/2 in all cell lines.

Discussion: The MAPK pathway is very important as a target of the molecular targeting therapy. In our study, MEKI1/2 inhibitor U0126 showed a dose- and time-dependent inhibitory effect on the cell proliferation and decreased the phosphorylation of ERK1/2. These results suggest that U0126 may be a selective inhibitor of MEK in human osteosarcoma and MFH, and MAPK signaling pathway exists and plays a important role in osteosarcoma and MFH cells. Although further studies are needed to explore the mechanisms for the inhibitory effect on cell proliferation in human osteosarcoma and MFH. MAPK signaling inhibitor will be a potent chemotherapeutic agent for malignant human bone and soft tissue tumor.

References: