GENE EXPRESSION OF ENDOTHELIAL DIFFERENTIATION GENE (EDG) RECEPTORS AND EFFECT OF SHINGOSINE-1-PHOSPHATE AND CERAMIDE ON PROLIFERATION IN THE CELLS OF MUSCULOSKELETAL TUMORS.

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Introduction: Endothelial differentiation gene (EDG) receptors are a novel family of G protein-coupled receptors for lysophosphatitic acid (LPA) and sphingosine-1-phosphate (S1P), which are lipid mediators derived from glycerophospholipids and sphingolipids in the cell membrane.[1] Previous studies have demonstrated that several cultured cell lines including fibroblast, macrophage, lymphocyte, and mesangial cell express EDG receptors and that EDG receptors and its agonists (LPA and S1P) play an important role for cell proliferation, apoptosis, and other physiological responses. The physiological functions of EDG receptors and its agonist, however, remain to be investigated. Moreover, expression and physiological functions of EDG receptors have never been determined in the cells of musculoskeletal tumor. On the other hand, it has been demonstrated that ceramide induces apoptosis in many types of cells and that S1P suppresses apoptosis induced by ceramide. The purposes of this study are to determine whether EDG receptors are expressed in the cells derived from osteosarcoma and malignant fibrous histiocytoma (MFH) and the effect of exogenous SIP on cell proliferation in these cells.

Materials and Methods: We cultured MG 63 (ATCC, Manassas, VA), KHOS (ATCC), and TNMY that we have established as MFH cell line.[2] These cells were maintained in MEM containing 10% FBS, 1% penicillin/streptomycin, and L-glutamine at a humidified incubator with 5% CO2. After confluence, the cells were lysed and total mRNA was isolated using RNeasy kit (Promega, Masison, WI) and then oligo dT primed cDNA was synthesized with use of Reverse Transcription System (Promega). The purified cDNA was used as a template for subsequent polymerase chain reaction (PCR). Primer sequences for EDG 1-7 were chosen according to the sequences described previously.[3] PCR products were separated by 1.2% agarose gel electrophoresis and documented under ultraviolet light. To test the effect of S1P on cell proliferation, MG63, KHOS and TNMY cells were incubated at 10,000 cells/well in MEM containing 0.5% FBS in 96-well plate for 24 hours. Then, S1P (1 µM) and/or C2 ceramide (10 µM) were added for 24 hours. At the end of incubation, we used non-radioactive cell proliferation assay (CellTiter 96® AQuous: Promega) to determine the number of viable cells. The absorbance of the fromazan at 490 nm, that is directly proportional to the number of viable cells, can be measured by this assay. At least three independent cultures were performed for each study. The data were analyzed statistically using analysis of variance (ANOVA) with Fisher's PLSD test. A value of $p<0.05$ was regarded as statistically significant.

Results: In MG63, KHOS, and TNMY cells, mRNA for EDG1, EDG2, and EDG3 receptors was detected. We could not detect mRNA for EDG4, EDG6, and EDG5 in all cell types. In MG63 cells, mRNA for EDG5 receptor was weakly expressed (Fig. 1). The proliferation of TNMY cells was suppressed by 1 µM S1P (Fig. 2). The proliferation of MG63, KHOS, and TNMY cells was significantly suppressed by 10 µM C2 ceramide. We could find that 1µM S1P inhibited suppressive effect of C2 ceramide on the proliferation of MG63 and KHOS cells (Fig. 2).

Discussion: EDG receptors are a novel family of G protein-coupled receptors for lipid mediators of LPA and S1P. Previous reports have demonstrated that mRNA for EDG receptors were detected in many cell types. We could find that mRNA for EDG receptors were expressed in MG63, KHOS, and TNMY cells derived from musculoskeletal tumors. Previous studies have demonstrated that S1P binds to EDG1, EDG3, EDG5, EDG6, and EDG8 receptors more specifically than other EDG receptors and stimulates the cell proliferation. In this study, we could not detect the effect of S1P on the proliferation of MG63 and KHOS cells at the condition we used. Previous reports have demonstrated that ceramide induces apoptosis in many types of cells and that S1P suppresses apoptosis induced by ceramide. We found that the proliferation of MG63, KHOS, and TNMY cells was significantly suppressed by C2 ceramide, presumably through the mechanisms of apoptosis. We also found that 1µM S1P inhibited suppressive effect of C2 ceramide on the proliferation of MG63 and KHOS cells. These results suggest that EDG receptors and S1P play a certain role for the mechanisms of proliferation in MG63 and KHOS cells. Previous reports have shown that mRNA expression of EDG receptor subtypes was different among the cell types. In previous study, suppressive effect of S1P on the proliferation also has been shown in some cell types as well as in TNMY cells used in this study. The role of EDG receptors and S1P in cell proliferation still needs to be investigated.

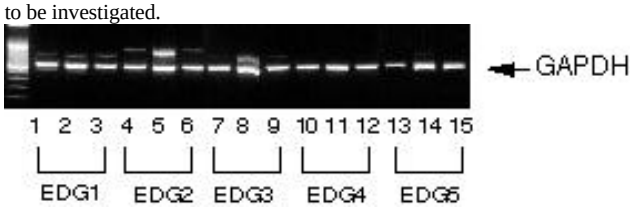
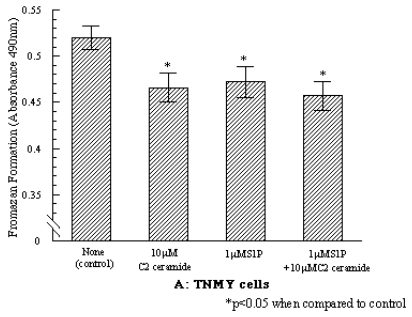
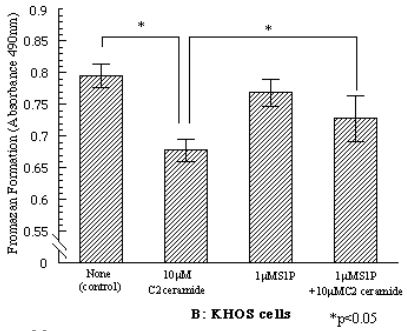


Fig.1 mRNA expression of EDG1-5 receptors.
TNMY: lines 1,4,7,10,13; MG63: lines 2,5,8,11,14; KHOS: lines 3,6,9,12,15





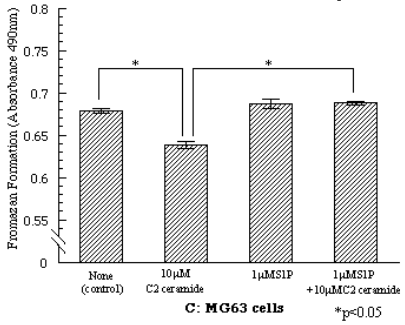


Fig.2 The effect of C2 ceramide and S1P on cell proliferation

References

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2. Nakatani T, et al. Pathol Int. 51:595, 2001
3. Hornuß C, et al. Eur J Pharmacol. 429:303, 2001