THE EXPRESSIONS OF EDG RECEPTORS AND SPHINGOSINE KINASES IN MALIGNANT BONE AND SOFT TISSUE TUMORS

Shinichiro Kishimoto, Toshihiro Akisue, Kenta Kishimoto, Hitomi Hara, Masaya Imabori, Yosiyuki Okada, Masahiro Kurosaka
Orthopaedic surgery, Kobe University graduate school of medicine, Kobe, Japan
kishin@med.kobe-u.ac.jp

Introduction: Sphingolipids are ubiquitous components of cell membranes. Their metabolites ceramide, sphingosine, and sphingosine-1-phosphate (S1P) have important physiological functions, including regulation of cell growth and survival. Ceramide and sphingosine are associated with growth arrest and apoptosis, whereas S1P affects diverse biological processes, including cell growth, differentiation, and migration. S1P is proposed to act both as an extracellular mediator and an intracellular second messenger. Extracellular S1P regulates cellular processes by binding to members of the endothelial differentiation gene (EDG) G-protein-coupled receptor family. S1P is generated by phosphorylation of sphingosine catalyzed by two isoforms of sphingosine kinases (SPHK), type1 and type2 [1]. We examined the expression of EDG receptors and SPHK isoforms in malignant bone and soft tissue tumors and their correlation with histologic grade.

Materials and Methods: Tumor samples: Twenty-three tumor samples were obtained by open biopsy at Kobe University Hospital, Japan. Informed consents were obtained from all patients. Tumor tissues were 7 osteosarcomas (OS), 5 MFH, 8 liposarcomas (LS), 3 chondrosarcomas (CS).

RT-PCR: Total RNAs were isolated using an RNeasy Mini Kit® from tumor samples and cell lines. RNA was converted to cDNA by reverse transcription and amplified for 35 cycles by PCR. GAPDH was used as an internal control for RNA integrity. RT-PCR products were run on 2% agarose gel, stained with ethidium bromide, and visualized by UV illumination.

Real time PCR: Real time PCR was carried out for all tumor samples to detect the mRNA expressions of SPHKs. PCR products were measured using ABI PRISM 7700 Sequence Detection System.

Statistical analysis: A Chi square analysis was used to compare for categorical values. Probability values less than 0.05 were considered to be significant.

Results: By RT-PCR analysis, all high-grade malignant tumors (OS and MFH) express EDG1, EDG3, and EDG5 receptors. In low-grade malignant tumors (CS and liposarcoma), the expression of EDG1 receptor is detected in 58%, EDG3 receptor in 75%, and EDG5 receptor in 25% (Fig. 1). The incidence of EDG1, EDG3, and EDG5 receptors expression between high-grade and low-grade malignant tumors is statistically different (p<0.05).

By real-time RT-PCR analysis, in high-grade malignant tumors, the expression level of SPHK1 is higher than that of SPHK2. In low-grade malignant tumors, the expression level of SPHK1 is lower than that of SPHK2 (Fig. 2).

Discussion: S1P may have an important role in a number of disease states. The changes in the phenotypic expression of EDG receptors and SPHKs may have a critical role in governing the concentration of S1P and its efficacy at EDG receptors, and this may impact upon a number of disease processes. S1P binds to EDG1, EDG3, and EDG5 receptors and prevent apoptosis. In this study, we could find that high-grade malignant tumors express EDG receptors more frequently than low-grade tumors. Recently overexpression of SPHK1 has been shown to enhance cell survival and increase cell proliferation. It has been also demonstrated that SPHK2 overexpression induces apoptosis [2]. In this study, the expression level of SPHK2 is lower in high-grade malignant tumors than that in low-grade tumors. These results suggest that the expressions of EDG receptors, SPHK1, and SPHK2 may play an important role for cell proliferation, apoptosis, and may correlate with histologic grade in malignant bone and soft tissue tumors.

Fig. 1. Gene expression of EDG receptors in tumor samples by RT-PCR

Fig.2 SPHK1 and SPHK2 expression levels were quantified using real-time PCR (relative units). High-grade malignant tumors are OS and MFH, low-grade malignant tumors are CS and liposarcoma.


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