HIGH OSTEOGENIC DIFFERENTIATION POTENTIALS OF SKIN AND SPINAL LIGAMENT FIBROBLAST CELLS IN THE PATIENTS WITH OSSIFICATION OF POSTERIOR LONGITUDINAL LIGAMENT OF THE SPINE

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Introduction: Ossification of the posterior longitudinal ligament (OPLL) of the spine is characterized by ectopic bone formation in the spinal ligament. OPLL is often accompanied by ossification of other spinal ligaments and articular ligaments. It has been regarded as one of the manifestations of diffuse idiopathic skeletal hyperostosis (DISH). Therefore, predisposition to systemic ossification may be involved in the pathogenesis of this disease. Ishidou reported that mRNA of decorin, which antagonistically regulated the action of transforming growth factor-beta (TGF-beta), was strongly expressed in the epidermis of the nuchal skin of OPLL patients. This may be interpreted as reflecting abnormalities of the systemic extracellular matrix (ECM) associated with ossification of the ligaments. Recently, Buranasinsup reported that human skin derived precursor cells were isolated and induced into an osteoblastic lineage using osteogenic induction medium and the specific characteristics of osteoblastic cells were detected. We then investigated in vitro osteogenic differentiation potentials of skin and spinal ligament cells in the patients with OPLL compared with non-OPLL.

Materials and Methods: This study was approved by the Ethics Committee of Hirosaki University and all participants gave written informed consent.

Skin tissues and spinal ligament tissues were taken from five OPLL patients and six non-OPLL (cervical spondylotic myelopathy) controls during surgery. Skin cells were harvested from the dermal layer. Spinal ligament cells were harvested from the yellow ligament between C3 and C4 lamina. Collected tissues were minced into about 0.5-mm³ pieces and washed twice with PBS, then plated in 100-mm culture dishes, and maintained in DMEM supplemented with 10% FBS. On confluence, designated hour 0, cells were then exposed to osteogenic medium containing DMEM supplemented with 10% FBS and Dexamethasone 10^-7M for 24 and 48 hours. Samples were processed for 8 weeks and alizarin red assay was then performed to determine mineralization. Extracellular matrix mineral-bound stain was photographed under microscopy. Total RNA was extracted from the cell monolayers with an RNeasy Kit (Qiagen, Chatsworth, CA, USA) according to the manufacturer’s protocol. The mRNA expressions of bone-related markers, alkaline phosphatase (ALP), osteopontin (OPN), Runx2 and Osterix were quantified by real time RT-PCR. All products were corrected for glycerol 3-phosphate dehydrogenase (G3PDH) mRNA levels.

Results: In spinal ligament cells, matrix began to mineralize and crystals appeared on collagen fibers at 4 to 8 weeks after confluence. But none of the ligament cells of non-OPLL patients exhibited mineralization.

In OPLL patients, mRNA expression of ALP and OPN in the spinal ligament cells were significantly increased about 2.03-fold and 2.13-fold respectively after exposing to osteogenic medium for 48 hours as compared with the cells maintained under the resting state (0 h). It was also significantly expressed as for ALP and OPN mRNA of the skin cells (about 2.62-fold and 4.48-fold respectively) in OPLL cases, whereas it was not significantly increased expression of both mRNA in non-OPLL controls (Fig.1). Runx2 and Osterix mRNA expression of both skin and ligament cells was higher in OPLL than non-OPLL, but it was not statistically significant.

Discussion: In this study, the specific characteristics of osteoblastic cells, including the expression of enzyme alkaline phosphatase, the deposition of mineral and highly mRNA expression of ALP and OPN were detected from both skin and spinal ligament cells. Especially, it demonstrated that both cells in OPLL appeared to undergo high osteogenic differentiation potentials with exposing to osteogenic medium in vitro. Recent molecular genetic reports have identified several candidate genes for susceptibility to OPLL cells. Thus, it is suggested that there underlies possible genes related osteogenesis in the extracellular matrix which induces systemic ossification conditions.