SIGNIFICANCE OF DIFFERENTIATIONAL COMPARTMENTALIZATION OF PTEN GENE IN OSTEOSARCOMA

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Introduction
Phosphatase and tensin homologue deleted on chromosome 10 (PTEN), a candidate tumor suppressor gene located at 10q23.3, was found to be mutated and have abnormal expressions in several tumors. However, expression patterns and pathogenetic roles of PTEN in musculoskeletal tumors have not been examined yet. We conducted this study in order to investigate the mutation of PTEN gene, its alteration of expression pattern in human osteosarcoma and its possible role in the development of osteosarcoma.

Materials and Methods
Tumor specimens comprised 22 osteosarcoma patients and 4 osteosarcoma cell lines (HOS, U2OS, MG-63 and Saos-2). Pure tumor cells were isolated using laser pressure catapulting (LPC) system according to the manufacturer’s instructions. We have studied the mutation of PTEN by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) and if there was aberrant band in PCR-SSCP, direct sequence analysis using autosequencer according to Sanger’s dideoxy chain termination method was done. Also we examined for abnormalities in expression by immunohistochemistry. Monoclonal anti-human PTEN antibody (PTEN/MMAC1 Ab-2, Neomarkers, Fremont, California, USA) was used in immunohistochemical analysis. LNCaP (prostate carcinoma) cell line was used as a positive control and normal iliac bone as a negative control.

Results
Aberrant bands were observed only in LNCaP cell line by PCR-SSCP analysis. DNA sequence analysis of aberrant bands revealed that an AAA nucleotide sequence had been changed into an A in exon 1 region. However, neither deletion nor any other mutation of PTEN was found in all osteosarcoma tissues, osteosarcoma cell lines and normal bone tissue. PTEN protein expression increased in osteosarcoma tissues and osteosarcoma cell lines when compared with normal bone tissue by immunohistochemical analysis. The nuclear stainings were more intense than the cytoplasmic stainings in normal bone tissue and osteosarcoma cell lines. However, PTEN expressions of osteosarcoma tissues were mainly confined to the cytoplasm (Fig.1.2).

Discussion
Although somatic intragenic mutation was not detected in osteosarcoma, we have shown informative abnormal expression patterns. All osteosarcoma cell lines and tissues remained immunopositive. These observations are in contrast to those made in breast cancer, thyroid neoplasia and endometrial cancer, which were strongly associated with decreased PTEN protein level or completely loss of PTEN expression. Also PTEN proteins were localized mainly in nucleus in nonneoplastic osteocyte and osteosarcoma cell lines, but they were localized predominantly in cytoplasm in all osteosarcoma tissues. Differential subcellular localization of PTEN have been observed previously in a large single series ranging from normal thyroid to anaplastic thyroid carcinoma and analysis of endocrine pancreatic tumors. It is possible that PTEN is shuttled between nucleus and cytoplasm by a certain molecule. Similar mechanism has been described for tumor-suppressor P53 that is shuttled via oncoprotein MDM2. Based on current state of knowledge, we would speculate that PTEN could be shuttled and intracellular substrates can show a distinct distribution within different compartments, ie, differential intracellular compartmentalization. Hence, differential compartmentalization of PTEN might play some as yet undefined role in tumorigenetic process. As more studies are performed, it is becoming apparent that inactivation of PTEN relies on multiple diverse mechanisms and not merely structural abnormalities such as somatic mutations. In osteosarcoma, therefore, abnormal expression of PTEN by differential compartmentalization may play a role in the development and progression of osteosarcoma instead of genetic alteration of PTEN.

Fig. 1. Expression of PTEN in normal bone (A) and osteosarcoma tissues (B). Immunoreactivity is mainly seen as nuclear staining in normal bone (A), while it is mainly confined to the cytoplasm in osteosarcoma tissues (B).

Fig. 2. Immunohistochemical staining of osteosarcoma cell line (MG-63) for PTEN. Hematoxylin staining (A) and immunohistochemistry for PTEN (B). Immunoreactivity is mainly confined to the nucleus.

References