SHORT COMMUNICATION

CHARACTERIZATION OF A DELETION IN TISSUE-NONSPECIFIC ALKALINE PHOSPHATASE (p.F327DEL) AS THE THIRD FREQUENT MUTATION IN THE JAPANESE PATIENTS WITH HYPOPHOSPHATASIA

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Hypophosphatasia is an inherited systemic bone disease caused by mutations of the tissue-nonspecific alkaline phosphatase (TNAP) gene. A total of 254 mutations in the TNAP gene have been reported to date. Among the mutations, a deletion of T at 1559 (c.1559delT; p.L520RfsX86) is the most frequent in Japanese patients and p.F327L is the second frequent. The deletion of Phe at nucleotide 322 (p.F327del) due to c.981_983delCTT in exon 9 appears to the third frequent. p.F327del has been reported in Japanese patients with hypophosphatasia at least 3 cases as compound heterozygotes with c.1559delT and all patients with the deletion were severe form (perinatal or infantile form). The expressed mutant protein showed low enzymatic activity in COS cells. We evaluated in vitro mineralization activity as well as enzymatic activity using U2OS human osteoblast-like cells, which express very low levels of TNAP. We also discuss structural features of the mutation based on the 3D model of human placental alkaline phosphatase. Expression plasmid pcDNA3 containing the mutant TNAP cDNA was constructed by using site-directed mutagenesis. The expression plasmids were transfected into U2OS cells by using Lipofectamine 2000. Enzymatic activity of the cells was assessed at 48 h after the transfection. The remaining cells were cultured for additional 5 days with 10 mM β-glycerophosphate to estimate mineralization. Mineralization was evaluated with 0.5% Alizarin Red S staining and measurement of OD570nm of solubilized materials in 100 mM cetylpyridinium chloride. Enzymatic activity after the 5 days of culture was also assessed. The cells transfected with p.F327del showed approximately 3.4 % of the wild type in enzymatic activity at 48 h after the transfection and 1.3 % after 5 days of culture with β-glycerophosphate. The cells expressed the deletion also exhibited 15.8 % of the wild type in in vitro mineralization. These values were similar to the results of c.1559delT. A 3D model of the mutant protein based on the crystal structure of human placental alkaline phosphatase revealed that p.F327 is located in a core β-sheet (β7 strand) and may interact with β1 and β3 strands. The deletion may cause deformity of the β-sheet, which may affect functions of the active site or stability of the protein. Those results suggest that p.F327del is a severe allele and contributes to the severe phenotype of the patients with the deletion.