SHORT COMMUNICATION

IN VITRO CHARACTERIZATION OF TNSALP MUTATIONS FROM TWO NOVEL MOUSE MODELS FOR HYPOPHOSPHATASIA

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The tissue non-specific alkaline phosphatase (TNSALP) is indispensable for correct bone mineralization; it provides inorganic phosphate for hydroxyl apatite crystal formation by cleavage of extracellular inorganic pyrophosphate, phosphoethanolamine and pyridoxal phosphate. Loss or impaired function of TNSALP due to mutations in the ALPL gene causes the clinical syndrome of hypophosphatasia (HPP), a rare inborn error of bone and mineral metabolism. So far only limited therapeutical approaches are available and still in clinical testing, therefore development of additional safe and long-standing therapies for patients is desirable. The large-scale Munich ENU (N-ethyl-N-nitrosourea) mouse mutagenesis project has generated two mouse lines with an impaired function of the TNSALP. One mouse line has a c.755T>C mutation in exon 7, resulting in a TNSALP-p.L252P substitution, the other mouse line has a c.1357A>G mutation in exon 12, leading to a TNSALP-p.T453A exchange. Both lines have reduced serum alkaline phosphatase levels and homozygous animals die three weeks after birth. Sequence alignment of mouse and human display conserved nucleotides in the concerning regions of the ALPL gene, but so far no human mutations are deposited in the TNSALP gene mutation data base (www.sesep.uvsq.fr). Therefore we analyzed the impact of these two mutations on the human TNSALP enzyme. The mutations were introduced into the human ALPL cDNA sequence by site-directed mutagenesis to generate an ALP-L252P and an ALP-T453A construct, respectively. Subsequent transfection of HEK-293 cells alone or in combination with a wild type construct showed no basal enzymatic activity for both mutants. While TNSALP-p.L252P has no effect on the wild type enzyme activity, the TNSALP-p.T453A mutant has a considerable dominant-negative effect. Immunocytochemical staining could not detect any signal of the p.L252P protein, whereas the p.T453A enzyme is localized in the cell membrane. These data confirmed that we generated two novel mouse models to study HPP pathology and new therapeutic strategies. To our knowledge the TNSALP-p.T453A is the first mouse model with a dominant-negative TNSALP mutation. M. Hrabé de Angelis and F. Jakob contributed equally. *corresponding author: f-jakob.klh@uni-wuerzburg.de This study was supported by the German Federal Ministry of Education and Research (BMBFOsteoPath). These data will also be presented on ECTS12-1454 in Stockholm