SHORT COMMUNICATION

ALKALINE PHOSPHATASE BONE ISOFORMS IN SKELETAL MINERALIZATION AND VASCULAR CALCIFICATION

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Tissue-nonspecific alkaline phosphatase (TNALP) is expressed at high levels in human bone and liver tissues and accounts for approximately 95% of the total serum ALP activity, with a ratio of approximately 1:1 between bone and liver isoforms in healthy adults. At least six different TNALP isoforms can be separated and quantified by high-performance liquid chromatography (HPLC) in serum: one bone/intestinal (B/I), two bone (B1 and B2), and three liver ALP isoforms (L1, L2, and L3). In healthy adults, the three bone ALP (BALP) isoforms, B/I, B1 and B2, account on average for 4, 16 and 37% of the total serum ALP activity, respectively. In serum, the minor fraction B/I is not a pure BALP isoform as it co-elutes with intestinal ALP and is composed, on average, of 70% bone and 30% intestinal ALP. The circulating levels of these BALP isoforms can vary independently during the pubertal growth spurt and in several disease states, e.g., growth hormone deficiency, X-linked hypophosphatemia, stress fractures, metastatic bone disease and Paget's disease. These isoforms differ also in their distribution in different skeletal sites with respect to cortical and trabecular bone. Our data also show that mice have identical BALP isoforms as humans, that is, their occurrence and implication intersect osteogenesis across mammalian species. The BALP isoforms have different catalytic properties due to structural differences in their posttranslational N-linked glycosylation. The B2 isoform has significantly higher kcat/KM values in comparison with the other BALP isoforms. Differences in glycosylation result in differences in molecular weight: B/I, 126 kDa; B1, 136 kDa; and B2, 141 kDa. Terminal sialic acid residues affect the immunoreactivity of monoclonal antibodies against the BALP isoforms as demonstrated in an international ALP Workshop. Conclusive evidence of a fourth BALP isoform (B1x) has been demonstrated in serum from patients with chronic kidney disease (CKD) on dialysis treatment (60%), predialysis CKD patients (20%), and occasionally in serum from children with CKD (7%). B1x has not been observed in healthy individuals or in any other group of investigated patients. Associations of B1x to phosphate and the calcium x phosphate product (predictor of cardiac mortality) were also found which led us to hypothesize a possible association with vascular calcification for the B1x isoform. Vascular calcification, which leads to cardiovascular morbidity and mortality, is associated with hyperphosphatemia in patients with CKD. In vitro, phosphate induces the transdifferentiation of vascular smooth muscle cells to cells with osteoblast-like properties that express significant amounts of ALP and other bone-associated proteins/enzymes. We designed an experimental study to investigate the different ALP isoforms in calcifying human aortic smooth muscle cells (hASMCs) and found that these cells express all 4 bone-specific ALP isoforms including the B1x isoform. Phosphate stimulated calcification of hASMCs, which was associated with an increase of the BALP isoforms B/I, B1x and B2. Taken together, our results support the working hypothesis that B1x is associated with vascular calcification, and that the BALP isoforms are pivotal for calcification of hASMCs. Further clinical and experimental studies are necessary to elucidate the biological role and potential differences among the BALP isoforms in skeletal mineralization and vascular calcification; however, these BALP isoforms are potential therapeutic targets for the pathological process of vascular calcification.