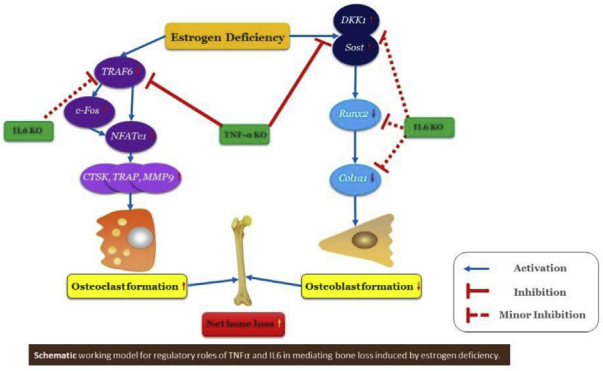
effect in inhibiting bone formation and enhancing TRAF6 mediated osteoclastogenesis than IL6, suggesting the role of different regulatory mechanisms governing TNFα and IL6 action on bone metabolism.



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PROTEOMIC ANALYSIS OF OSTEOBLASTS SECRETOME PROVIDES NEW INSIGHTS IN MECHANISMS UNDERLYING OSTEOARTHRITIS SUBCHONDRAL BONE SCLEROSIS

C. Sanchez, G. Mazzucchelli, C. Lambert, F. Comblain, E. DePauw, Y. Henrotin. *Univ. of Liège, Liège, Belgium*

Purpose: Osteoarthritis (OA) is characterized by cartilage degradation but also by other joint tissues modifications like subchondral bone sclerosis. In this study, we used a proteomic approach to compare secretome of osteoblast isolated from sclerotic (SC) or non sclerotic (NSC) area of OA subchondral bone.
Methods: Secretome was analyzed using differential quantitative and relative label free analysis on nanoUPLC G2 HDMS system. mRNA of the more differentially secreted proteins were then quantified by RT-PCR and the most relevant proteins identified using western-blotting and immunoassays.
Results: 175 proteins were identified in NSC osteoblast secretome. Compared to NSC osteoblast secretome, 13 proteins were significantly less secreted (Osteomodulin, CSF-1, IGFBP5, VCAM-1, IGF2, 78 kDa glucose-regulated protein, versican, calumenin, IGFBP2, thrombospondin-4, periostin, reticulocalbin 1 and osteonectin), and 12 proteins were significantly more secreted by SC osteoblasts (CHI3L1, fibulin-3, SERPINE2, IGFBP6, SH3BGRL3, SERPINE1, reticulocalbin3, alpha-2-HS-glycoprotein, TIMP-2, IGFBP3, TIMP-1, SERPINF1). Similar changes in periostin, osteomodulin, SERPINE1, IGFBP6, fibulin-3 and CHI3L1 mRNA levels were observed. Finally, osteomodulin and fibulin-3 specific sequences were quantified by western blot and immunoassays in serum and culture supernatants.
Conclusions: We highlighted some proteins differentially secreted by the osteoblasts coming from OA subchondral bone sclerosis. These changes contribute to explain some features observed in OA subchondral bone, like the increase of bone remodeling or abnormalities in bone matrix mineralization. Among identified proteins, osteomodulin was found decreased and and fibulin-3 increased in serum of OA patients. These findings suggest that osteomodulin and fibulin-3 fragments could be biomarkers to monitor early changes in subchondral bone metabolism in OA.

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EFFECT OF DENOSUMAB ON BONE FORMATION MARKER P1NP

H. Tanigawa †,‡, T. Maeda †, K. Kumagai †, S. Imai †. † *Shiga Univ. of Med. Sci., Otsu, Japan;* ‡ *Kusatsu Gen. Hosp., Kusatsu-city, Shiga, Japan*

Purpose: Type I procollagen N-terminal propeptide (PINP) is considered the most sensitive bone formation marker and it is useful for monitoring osteoporosis treatment such as bone formation or anti-resorptive therapy. In our hospital, we have administered Denosumab, an anti-RANKL antibody as a treatment for osteoporosis. In addition to bone mineral determination (DXA method), P1NP and serum NTx are used as osteogenic markers and bone resorption markers, respectively, for its therapeutic effect determination. In past reports it has been reported that there are many cases in which P1NP falls below the standard lower limit (17.1 μg / L) in patients using bisphosphonate (BP). The purpose of this study was to investigate the state of P1NP value in cases treated with denosumab.
Methods: For January 2015 - December 2016, we evaluated P1NP and NTx for 30 patients with osteoporosis who administered denosumab at our hospital. For each marker, the percentage of cases below the baseline lower limit was compared.
Results: Denosumab administration resulted in P1NP below the reference lower limit value in 15 cases (50%). In NTx, there were no cases below the reference lower limit. There were no cases of apparent atypical femoral fractures during the follow-up period. In 24 patients who were able to measure P1NP from the first dose of denosumab, the transition of the P1NP value was observed. All cases were within the reference values before the initial prescription, but 11 cases (46%) were lower than the reference lower limit value during administration of denosumab for an average of 6 months.
Conclusions: Denosumab strongly suppresses bone turnover and also decreases bone metabolism markers. When both the bone formation marker and the bone resorption marker are below the lower limit value, it is serologically in the state of inhibition of excessive bone turnover (SSBT) and may contribute to atypical fracture (Kitaori et al., 2004).
In this study, P1NP was lower than the lower limit in half cases in patients treated with denosumab, but it is difficult to think of all these cases as SSBT status. There are reports that there are cases in which P1NP is below the lower limit in certain proportions even in BP administered patients (Eastell et al., 2011). It is difficult to use P1NP as a hazard marker of SSBT in patients receiving bone resorption inhibitors including denosumab. It may be necessary to set new reference values for P1NP for cases using bone resorption inhibitors. In patients using denosumab, P1NP fell below the reference lower limit in 50%. In patients with bone resorption inhibitors including denosumab, it may be necessary to examine new reference values for P1NP.

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GENERATION AND PHENOTYPING OF A TARGETED MOUSE MODEL OF ALKAPTONURIA

J.H. Hughes †, K. Liu †, H. Sutherland †, P.J. Wilson †, A.T. Hughes †,‡, A.M. Milan †,‡, L.R. Ranganath †,‡, J.A. Gallagher †, G. Bou-Gharios †. † *Univ. of Liverpool, Liverpool, United Kingdom;* ‡ *Royal Liverpool and Broadgreen Univ. Hosp. NHS Trust, Liverpool, United Kingdom*

Purpose: Alkaptonuria (AKU) is a rare autosomal recessive metabolic disease caused by mutations in the gene homogentisate 1,2-dioxygenase enzyme (HGD). Deficiency of HGD leads to an accumulation of homogentisic acid in the blood and tissues. Overtime, HGA is polymerised to form a pigment which deposits into connective tissues, particularly in cartilage. The cartilage becomes brittle and osteoarthropathy manifests in early adulthood, becoming very debilitating as it progresses. Studying rare diseases with extreme phenotypes like AKU aid our understanding about more common disorders like osteoarthritis. The current mouse model for AKU was generated by chemical mutagenesis. To overcome the uncertainty of this model harbouring other potentially confounding uncharacterised mutations, we generated a specific HGD (Homogentisate 1,2-dioxygenase) null mouse with the advantage of conditional deletion.
Methods: Embryonic stem cells in the C57BL6 background with disrupted HGD gene function were obtained from the Knockout Mouse Project (KOMP). This knockout first plasmid contains the insertion of an IRES:lacZ trapping cassette and a promoter-driven neo cassette into the fifth intron of the HGD gene with the sixth exon flanked by LoxP sequences (HGD tm1a). Flp recombinase reverts the mutation back to wildtype with a floxed critical exon (HGD tm1c). The model then becomes conditional though Cre recombinase which removes the floxed exon resulting in a mutant transcript (HGD tm1d). The Hgd tm1a knock-out first model is characterised here.
Results: Homozygous tm1a mice show black urine stained cage bedding, one of the first AKU symptoms. Homogentisic acid is elevated in the homozygous tm1a in both the urine (99,575 μmol/L) and plasma (100.5 μmol/L) compared to C57BL6 wildtype mice (urine; 0.9 μmol/L, plasma; 1.7 μmol/L). Heterozygous tm1a and tm1c mice have levels of HGA comparable to the C57BL6 wildtype. Ochronosis, pigmentation of chondrocytes found within calcified articular cartilage, was detected at 9 weeks in the tm1a mouse as pericellular pigmentation. At 26 and 40 weeks, numerous fully pigmented chondrocytes can be seen in the homozygous tm1a knee cartilage with no evidence of ochronosis in