tured and an ex vivo cartilage graft is produced. Subjecting these chondrocytes to either selenium (SEL), Nivalenol (NIV) or in combination during the growth of the graft has been found to alter the morphology of the cartilage graft. In addition, the quantity of the large aggregating proteoglycan, was significantly reduced in a dose dependent manner in the presence of Nivalenol. This study aimed to examine the composition of aggrecan from cartilage grafts grown in the presence of NIV or SEL alone, or in combination to better understand cellular and molecular mechanisms underlying the pathogenesis of KBD.

**Methods:** Chondrocytes (7 day old bovine cartilage) were seeded at high density in MilliCell filter inserts (12mm diameter; Millipore, MA). Cultures were maintained for 4 weeks in DMEM supplemented with 20% heat-inactivated FBS, ascorbate (100µg/ml) and TGFβ2 (5ng/ml) or additionally supplemented with either SEL, NIV or both at varying concentrations. Media was refreshed thrice weekly and later analysed. At 4 weeks the cartilage grafts were harvested, weighed and extracted in 4M guanidium chloride containing an inhibitor cocktail for biochemical analysis of matrix molecules. Residues were digested with papain. Glycosaminoglycan concentration was determined in all samples and extracts. Aggrecan and GAG composition was determined using Western blotting.

**Results:** The total GAG synthesized in a 4week period was substantially reduced in chondrocytes cultured in the presence of NIV in a dose dependant manner, and to a lesser extent in those cultures exposed to the highest dose of SEL. However, the amount of GAG released into the media remained fairly constant within the treatment groups, but a marked reduction was apparent in the guanidine extracts of the cartilage grafts. Western blot analysis with a series of antibodies on guanidine extracted aggrecan showed no substantial changes in the core protein molecular weights however analysis demonstrated that KS was reduced in NIV treated cultures. Results also indicated that NIV treated cultures appeared to contain less CS substitutions on the aggrecan core protein.

**Discussion:** The data on GAG concentrations indicates that there is an inability of the GAG to remain within the cartilage grafts extracellular matrix when treated with NIV. Western blot analysis indicates minor changes in the composition of the aggrecan in relation to protein core length and CS/NS side chain substitutions or length. Further work will investigate the proportion of aggrecan able to form high molecular weight aggregates, the metabolism of link protein and hyaluronan.

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THE DIFFERENTIAL EXPRESSION OF OPG/RANKL IN HUMAN OSTEOARTHRITIC SUBCHONDRAL BONE OSTEOBLASTS IS AN INDICATOR OF THE METABOLIC STATE OF THESE DISEASE CELLS

J Martel-Pelletier, D Lajeunesse, F Mineau, H Fahmi, M Lavigne, JP Pelletier

1Osteoarthritis Research Unit, University of Montreal Hospital Centre, Notre-Dame Hospital, Montreal, QC, Canada; 2Department of Orthopaedics, Maisonneuve-Rosemont Hospital, Montreal, QC, Canada

**Aim of Study:** Although the OPG/RANK ratio is considered a key element in the regulation of bone metabolism, there is little data on these factor levels in human osteoarthritic (OA) subchondral bone. We previously reported that OA patients can be discriminated into two subgroups identified by the endogenous (low or high) production of PGE2 by osteoblasts that otherwise show no different phenotypic features. We investigated the relationship between the level of expression of OPG and RANKL in both human normal and OA subchondral bone osteoblast subgroups. Furthermore, we analysed the effect of bone remodeling factors on OPG and RANKL expression levels as well as on the mineralization process.

**Methods:** Gene expression was determined using real-time PCR. Gene regulation and the mineralization process were monitored on OA osteoblasts following treatment with vitamin D3 (50 nM), TGF-β1 (5 ng/ml), IL-1β (100 pg/ml), TNF-α (5 ng/ml), PGE2 (500 nM), or PTH (100 nM).

**Results:** Human osteoblasts demonstrated a much higher level of OPG expression than RANKL. OA expressed less OPG than normal; this was more marked in the low OA cells. Compared to normal, RANKL levels were increased in low OA and decreased in high OA. Correlation analysis revealed that in low OA for any given level of OPG expression, the corresponding level of RANKL was about 7.5 times greater than in normal. The OPG/RANKL ratio was diminished in low OA compared to normal or high OA (p<0.02 and p<0.03), and markedly increased in high OA compared to normal. In low OA cells, both vitamin D3 and TGF-β1 significantly reduced the OPG/RANKL ratio, IL-1β had no modification, and the ratio markedly increased in response to TNF-α (336-fold), and to a lesser extent to PTH and PGE2 (30- and 7-fold). Mineralization patterns generally agree with OPG/RANKL levels.

**Conclusion:** Data revealed that OPG and RANKL expression levels, and consequently the OPG/RANKL ratio, differed according to human OA subchondral bone osteoblast classification. The OPG/RANKL balance is disturbed, being decreased in the low OA osteoblasts thus favoring RANKL, and increased in the high OA osteoblasts. This suggests that the low OA osteoblasts are enriched in factors promoting bone resorption, and that the high OA cells have reached a more advanced stage in the progression of the disease with reduced resorptive properties. The latter may be due to a flare in disease activity, in which factors including TNF-α and PGE2 are highly upregulated, thus promoting bone deposition.