ABNORMAL MINERALIZATION OF HUMAN OSTEOARTHRITIC OSTEOBLASTS IS LINKED TO ABNORMAL PRODUCTION OF COLLAGEN TYPE 1.

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Introduction:
Osteoarthritis (OA) is characterized by articular cartilage loss, bone sclerosis and synovial inflammation. Bone sclerosis is the result of an increase in subchondral bone activity which can predict cartilage loss. Moreover, cartilage lesions do not progress in the absence of significant subchondral activity. Alterations of the bonny bed precede cartilage changes in the Macaca fascicularis primate model of OA (1) similar to Dunkin-Hartley guinea pig model (2). Bone sclerosis is characterized by an abundant osteoid matrix that does not mineralize normally in OA bone tissue. The mechanisms responsible for this abnormal mineralization remain unknown. We studied the mineralization profile and the expression of proteins involved in this process in normal and OA osteoblasts (Ob) in primary culture.

Methods:
We prepared primary normal and OA Ob from subchondral bone of tibial plateaus. Phenotypic markers of Ob were determined by alkaline phosphatase activity and osteocalcin release by EIA. The expression of osteoblastic markers Runx2/Cbfa1 and Osx, and of collagen type 1 alpha 1 chains (COLL1A1) and alpha 2 chains (COLL1A2), biglycan, decorin, versican, PHOSPHO1 and S100A4 was determined by real-time PCR. The de novo production of collagen was determined by the release of C-terminal propeptide (CICP) by ELISA and mature collagen levels by Western blot analysis. In vitro mineralization was evaluated by alizarin red staining and the Von Kossa method following the treatment of cells with or without 10 ng/ml bone morphogenetic protein-2 (BMP-2).

In order to better understand the mechanism of mineralization defect in human OA Ob, we used human Ob-like cell model SaOS-2 which shows high rates of mineralization. In this model, we followed both variations of COLL1A1 and COLL1A2 expression and in vitro mineralization. SaOS-2 cells were also transfected with a vector containing the COLL1A1 cDNA, and the results of overexpressing type I collagen α1 chain on mineralization were evaluated.

On the other hand, the defect of mineralization that occurs in OA Ob could be the result of the absence or deficit of a paracrine factor. Briefly, OA Ob were fed with medium previously exposed to SaOS-2 culture and vice versa. Mineralization was evaluated after 7, 14 and 30 days by red alizarin staining.

Results:
Normal and OA Ob showed different levels of alkaline phosphatase activity and osteocalcin release, as previously reported. Among the different proteins analyzed by RT-PCR, only the expression of Osx was downregulated, whereas the expression of S100A4 increased in OA Ob compared to normal. The increase in COLL1A1 expression in OA Ob while COLL1A2 remained normal resulted in a COLL1A1 to COLL1A2 ratio of 7.2 ± 0.2 in OA Ob whereas it was 2.5 ± 0.6 in normal Ob (Figure 1A). The de novo production of collagen type 1 measured by CICP release was also increased in OA Ob possibly due to the increased expression of COLL1A1. Western blot analysis also showed that mature collagen type 1 was increased in OA Ob. Despite this increase in collagen expression and production, in vitro alizarin red and Von Kossa staining were both significantly reduced in OA Ob under basal condition and following BMP-2 treatment indicating a reduced mineralization capacity of these cells (Figure 1B). Additional experiments in SaOS-2 cells showed that the COLL1A1 to COLL1A2 ratio in SaOS-2 cells varied from 7.5 ± 1.5 at day 0 to 1.5 ± 0.2 at day 14 post-mineralization whereas the mineralization progressively increased as assessed by alizarin red staining (Figure 1C).

Conclusion and Pertinence: This study suggests that the abnormal mineralization of OA bone tissue observed in vivo may be linked with the abnormal expression of COLL1A1 by OA Ob. Indeed, the incapacity of these cells to down-regulate the COLL1A1 to COLL1A2 ratio explains, at least in part, their incapacity to mineralize normally in vitro. This assessment is illustrated by the fact that when this ratio is altered in our SaOS-2 model, mineralization is not optimal. This could also be linked with the expression of a soluble factor which may alter the COLL1A1 to COLL1A2 ratio. For example, the abnormal expression of S100A4 may be one of our hypotheses, although its exact role on the mineralization process is still under investigation. Regardless of the exact roles of COLL1A1 and S100A4, their abnormal expression could contribute to the laying down of an abnormal osteoid matrix that does not mineralize normally, which in turn can disturb the overlying articular cartilage.

References:

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