8. Resveratrol Regulates the WNT/ ß-Catenin Pathway in Human Osteoaarthritis Osteoblasts. Elie Abed, Nicolas Duval, Johanne Martel Pelletier, Jean Pierre Pelletier, Daniel Lajeunesse.
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Problem Statement: Clinical and in vitro studies suggest that subchondral bone sclerosis due to abnormal osteoblasts (Ob) is involved in the progression and/or onset of osteoarthritis (OA). Human Ob isolated from sclerotic subchondral OA bone tissue show an altered phenotype, a decreased canonical Wnt/ß-catenin pathway (cWnt), and a reduced mineralization in vitro as in vivo. These alterations were linked with an abnormal response to BMP-2. Recent studies have shown an association between dietary polyphenols and the prevention of osteoarthritis. Resveratrol (3,5,4'-trihydroxy-trans-stilbene) is a polyphenolic phytoestrogen and stimulates osteoblast differentiation and may have a positive effect on cartilage protection. Resveratrol (RSV) regulates the Wnt signaling pathway in different cell systems
and stimulates BMP-2 expression in human Ob, however, the role of RSV and its effect in OA Ob remains unknown. Here we investigated the role of RSV in OA Ob and if it is responsible for the altered response to BMP-2.

Methods: We prepared primary human subchondral osteoblasts using the sclerotic medial portion of the tibial plateaus of OA patients undergoing total knee arthroplasty, or from tibial plateaus of normal individuals at autopsy. The expression of genes was evaluated by qRT-PCR and the protein production by Western blot analysis. Alkaline phosphatase activity (ALPase) and osteocalcin release (OC) were measured by substrate hydrolysis and EIA respectively. Canonical Wnt/bcatenin signaling (cWnt) was evaluated using two approaches: 1) target gene expression was measured using the TOPflash TCF/lef luciferase reporter assay, and 2) intracellular signaling partners b-catenin and phospho b-catenin levels were evaluated by Western blot analysis. Mineralization in response to RSV was evaluated by Alizarin red staining.

Results: BMP-2 dose-dependently (1 to 100 ng/ml) stimulated both ALPase and OC in normal Ob whereas it inhibited them in OA Ob. RSV treatments reversed this response in OA Ob and restored the BMP-2 response. RSV had little effect on cell proliferation and slightly affected the Bax/Bcl2 ratio, an indicator of cell survival. The expression of Runx2/Cbfa1 and PPARg were not affected by increasing doses of RSV. Moreover, Wnt3a increased b-catenin levels and Wnt signaling activity, both parameters are further increased by RSV treatment. The BMP-2-dependent mineralization of OA Ob which is also reduced compared to normal was partially restored by RSV treatment as detected by alizarin red staining.

Conclusion: These data indicate that RSV can promote the Wnt/b-catenin signaling pathway which is altered in these cells. This last situation could explain the role of RSV on alkaline phosphatase activity, osteocalcin release, and in vitro mineralization which are all altered in these cells.