9. Alterations of the Non-Canonical WNT/PKC Pathway in Human Osteoarthritis Osteoblasts.

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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 signaling pathway (cWnt), and a reduced mineralization in vitro, alterations linked with an abnormal response to BMP-2. Besides the cWnt pathway, at least two non-canonical signaling pathways, the Wnt/PKC and Wnt/PCP pathway have been described. However, there are no reports of either pathway in OA Ob. The Wnt/PKC pathway is activated after a non-canonical Wnt ligand such as Wnt5a
triggers a leucine-rich G-coupled protein receptor (LGR), typically LGR4 or LGR5 in osteoblasts. This then drives the phosphorylation of PKC (phosphoPKC), and downstream signaling targets. Here, we studied if alterations of the Wnt/PKC pathway could be observed in OA Ob.

Methods: We prepared primary human subchondral Ob 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 involved in Wnt/PKC 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. The Wnt/PKC and JNK pathways were evaluated using Western blot analysis of PKC, phosphoPKC, JNK and phosphoJNK.

Results: OA Ob showed an increased alkaline phosphatase activity and osteocalcin release. The expression of Wnt5a was increased in OA Ob compared to normal. The expression of LGR4 and LGR5 was detected in both normal and OA Ob, however, although the expression of LGR4 was only slightly increased in OA Ob, the expression of LGR5 was significantly increased in these cells. Moreover, Wnt5a directly stimulated the expression of LGR5 both at the mRNA level and protein level. However, Wnt5a did not stimulate the expression of LGR4. Wnt5a increased the expression of phosphoPKC without altering PKC levels, and it also stimulated the phosphorylation of JNK.

Conclusion: These data indicate that Wnt5a, which is increased in OA Ob, can directly increase the expression of LGR5 which in turn stimulates the Wnt/PKC and JNK pathways. The exact role of the non-canonical pathway in OA still remains to be determined