18. Hypoxia in human osteoarthritic subchondral bone: a risk factor for leptin production by osteoblasts.

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Background and objectives: Venous outflow occlusion, intraosseous hypertension, decreased perfusion, and hypoxia have all been reported to occur in the subchondral bone tissue of the OA-like guinea pig animal model and in human OA. Modeling the relationships among perfusion, intraosseous pressure, and pO2 suggests that venous outflow obstruction, stasis, and intraosseous hypertension are associated with physiologically relevant reductions in perfusion and pO2 in OA bone tissue, and the pathophysiological consequences of venous outflow obstruction may lie in its association with diminished perfusion and hypoxia. Human osteoblasts (Obs) isolated from osteoarthritic (OA) subchondral bone tissue show an altered phenotype and particularly produce higher level of leptin than normal Obs. Therefore, we have investigated whether hypoxia could play a role in the OA-induced leptin production in human Obs.

Methods: Obs from the sclerotic medial portion of the tibial plateaus of OA patients, were cultured either in 20% (normoxia) or 2% (hypoxia) oxygen tension in the presence or not of 1,25[OH]2VitD3. The expression of leptin, osteocalcin (OCN), Hif-1 and -2 was evaluated by qRT-PCR, and leptin production by ELISA in Hif-silencing RNA experiments. In addition to Hif signaling, MAPK signaling was also investigated using several inhibitors.

Results: Although VitD3-induced stimulation of OCN was slightly decreased from 220-fold in normoxic to 175-fold in hypoxic conditions, no statistical significance was reached. Obs leptin expression was stimulated 7-fold under hypoxia. VitD3 stimulated leptin expression 2-fold under normoxia and 28-fold under hypoxia. The hypoxia-induced leptin expression was confirmed at the protein level by ELISA, particularly in presence of vitD3 co-incubation. Compared to Obs incubated in the presence of siScramble RNA, siHif-2 inhibited leptin expression by 70% and leptin production by 60% in presence of vitD3. Immunoblotting showed that vitD3 greatly increased Hif-2 stabilization at the protein level under hypoxic condition. The Hif-2 regulation of VitD3-induced leptin production was confirmed by ELISA. In addition to Hif-2 regulation, the huge increase of leptin expression under hypoxia and vitD3 is mainly controlled by p38 map and PI-3 kinases.

Conclusion: The outflow obstruction and decreased perfusion observed in OA subchondral bone tissue contribute to their hypoxic condition and could trigger Obs to produce leptin, a well-known factor involved in the pathogenesis of osteoarthritis and abnormal OA Obs function.