How does hypoxia-inducible factor (HIF) regulate osteoclastogenesis and bone erosion?

Pathological bone loss and hypoxia co-exist in rheumatoid arthritis, bone metastatic cancer, primary bone cancer and osteoporosis. The hypoxia-inducible transcription factor, HIF, is stabilised in these conditions, correlating with disease progression and poor prognosis. Pharmacological inhibition of HIF protects from bone loss in murine models of osteoporosis, tumour-induced osteolysis and rheumatoid arthritis.

We are investigating the role(s) HIF plays in osteoclast formation and function. We have previously shown that hypoxic enhancement of osteoclast-mediated bone resorption is HIF-1α-dependent. We now examine whether HIF also regulates osteoclast differentiation and consider specific roles for the HIF-regulating prolyl hydroxylase (PHD) enzymes.

$CD14^+$ human monocytes were differentiated into osteoclasts with M-CSF and RANKL. HIF-1α mRNA and protein, as well as HIF target genes (LDHA, Glut-1 (Western blot), PGK-1 (luciferase assay)), were induced from differentiation day 5. siRNA targeting HIF-1α affected neither the number of osteoclasts formed (TRAP-positive cells containing ≥3 nuclei) nor final resorption activity (lacunar resorption of dentine). Neither did HIF induction with $CoCl_2$ enhance differentiation. Interestingly, 24 hours hypoxic (2% O_2) exposure at any time after differentiation day 5 increased resorption up to 2-fold in the mature cells (lacunar resorption of dentine, TRAP activity assay) without affecting osteoclast number.

Bone marrow-derived osteoclasts were differentiated *ex vivo* from mice with constitutive knock-down of PHD1-3, and so stabilisation of HIF. $PHD2^{+/-}$ marrow formed the same number of osteoclasts as wild-type controls, but the mature osteoclasts were 4-fold more resorptive (CTXI ELISA, TRAP expression). $PHD3^{-/-}$ marrow exhibited an accelerated rate of osteoclast formation. No phenotype was evident in $PHD1^{-/-}$ cells. Increased resorption by $PHD2^{+/-}$ osteoclasts was associated with over-expression of ANGPTL4 and altered mitochondrial metabolism, pathways which drive hypoxia-induced bone resorption in human osteoclasts.

This data suggests that effects of HIF on osteoclast bone resorption are predominantly mediated via PHD2, although small effects of HIF on osteoclast differentiation might be regulated by PHD3. This provides insight into how targeted manipulation of the HIF system could improve disease outcome in osteolytic bone loss conditions.

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