NRF2 TRANSCRIPTION FACTOR AND HEME OXYGENASE-1 AFFECTS OSTEOCLASTS DIFFERENTIATION

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Objectives. Due to increased activity and number of osteoclasts (OCLs) there is a progressive demineralization/resorption of bone tissue in diseases associated with inflammation (rheumatoid arthritis) or bone metabolic diseases (osteoporosis). Acting as strong cytoprotectants and antioxidants Nrf2 transcription factor and heme oxygenase-1 (HO-1), its downstream target, have been suggested to play a role in osteoclastogenesis. Nevertheless, the studies investigating the effect of genetic manipulation of those mediators are lacking.

Aim. We aimed to investigate the effect of Nrf2 and HO-1 deficiency on OCL differentiation from bone marrow (BM) cells and specifically from bone marrow macrophages (BMMs).

Materials and methods. As a model Nrf2- or HO-1-knockout mice (Nrf2-/- or HO-1-/-,

respectively) and murine monocytic cell line, RAW264.7 were used in combination with pharmacological inducers of HO-1, cobalt protoporphirin IX (CoPPIX) or hemin, as well as siRNA against HO-1.

Results. To test the effect of the absence of Nrf2 and HO-1 in BM cells on osteoclastogenesis, BM cells from Nrf2-/-, HO-1-/- and wild type (WT) mice were counted, seeded in the presence of macrophage colony-stimulating factor (M-CSF) to stimulate macrophage growth (day 0 -> day 6) and with receptor activator of nuclear factor kB ligand (RANKL) to induce osteoclasts differentiation (day 3-> day 6). Macrophage phenotype of cells at day 3 was confirmed irrespective of the genotype. Mature OCLs were identified at day 6 by tartrate-resistant acid phosphatase (TRAP) detection. Nrf2 deficiency resulted in higher number of OCLs. Reversely, after Nrf2 activation by sulforaphane in Nrf2+/+ cells no TRAP-positive signal was observed, confirming inhibitory effect of Nrf2. In addition, osteoclasts-specific genes, such as cathepsin K and integrin β3, were upregulated in Nrf2-/- BM cells (vs. Nrf2+/+). In contrast, the lack of HO-1 diminished the number of TRAP+ cells and expression of OCL markers in the population of M-CSF and RANKL-treated BM cells (vs. HO-1+/+). To confirm that the observed effects are not related to the effect of Nrf2/HO-1 on macrophage abundance, BMMs were harvested and plated in equal numbers for all genotypes. Indeed, BMM Nrf2-/- stimulation with RANKL led to a high increase in TRAP-positive cells. BMMs isolated from HO-1-/- mice formed less TRAP+ cells.

In RAW264.7 cells, CoPPIX and hemin both inhibited NFATc-1 and the transfection with siRNA against HO-1 caused similar effect. Therefore, the combination of siRNA HO-1 with CoPP IX was examined. CoPP IX prestimulation resulted in diminishment of NFATc-1 level, while additional siRNA HO-1 transfection tended to reverse this effect- enhanced the expression of NFATc-1.

Conclusions. In summary, Nrf2 deficiency exerts stimulatory effect on osteoclastogenesis, while the lack of HO-1 in BM cells or its silencing in RAW264.7 cells by siRNA decrease this process. On the other hand, HO-1 enhancement by pharmacological inducers also diminished the formation of OCLs, which could be reversed by HO-1 silencing. Those results suggest that HO-1 has to be present at specific level for proper process of osteoclastogenesis.

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CZYNNIK TRANSKRYPCYJNY NRF2 I OKSYGENAZY HEMOWEJ-1 WPŁYWA NA RÓŻNICOWANIE OSTEOKLASTÓW

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