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2007 Christopher & Maggie Nordin Young Investigator Poster Award Recipient

Winner: Mr Taksum Cheng

Abstract:

V-ATPase subunit d2 promoter is regulated by NFAT in osteoclasts

Cheng, T., Leong, C.L., Feng, H.-T., Pavlos, N., Zheng, M.H. and Xu, J.
Centre for Orthopaedic Research, School of Surgery and Pathology, University of Western Australia, Western Australia, Australia

Vacuolar adenosine triphosphatase (V-ATPase) proton pumps play an essential role in the acidification of the bone matrix during osteoclast-mediated bone resorption. Recently, mice lacking the V-ATPase d2 subunit have been shown to be osteopetrotic due to defective osteoclasts (Lee et al., Nature Med, 2006). Here, to investigate the role of RANKL in the transcriptional regulation of the d2 gene we have cloned and characterized its putative promoter region. Bioinformatic analysis of the cloned 3 kb d2 promoter region revealed several candidate transcription factor binding sites including NFATc1, a key transcription factor for osteoclastogenesis. To explore the influence of RANKL on d2 transcription, we generated a series of d2 promoter constructs using the pGL-3 reporter plasmid. Using luciferase assays, the d2 promoter was found to be induced by RANKL stimulation and/or NFATc1 overexpression.

Furthermore, targeted mutagenesis of the putative NFAT transcription binding sites was found to significantly reduce the luciferase activity as induced by NFATc1 overexpression. By semi-quantitative RT-PCR, expression of d2 and NFATc1 was found to be strongly up-regulated by RANKL but not by other pro-osteoclastic factors including TNF, LPS and M-CSF. Interestingly, the RANKL-induced expression pattern of NFATc1 appeared to precede that of d2 during osteoclastogenesis. Consistently, addition of the NFATc1 inhibitor cyclosporin A was found to blunt the mRNA expression of d2 induced by RANKL in RAW264.7 cells.

Finally, chromatin immunoprecipitation (ChIP) assays demonstrate that NFATc1 forms a complex with the d2 promoter. We propose that NFATc1 is an important regulator of d2 transcription during RANKL-induced osteoclastogenesis.