

## Transcription factor regulation of osteoclastogenesis from Embryonic Stem (ES) cells

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**INTRODUCTION:** Gene inactivation studies in mice have been extremely useful for identifying essential transcription factors and signaling molecules controlling osteoclast lineage commitment, differentiation and function. Knockout of the c-Fos proto-oncogene, a member of the AP-1 transcription factor family, leads to osteopetrosis due to a block in osteoclast differentiation demonstrating that c-Fos is an essential gene for osteoclastogenesis. Moreover, c-Fos null bones contained a greater number of tissue macrophages, implicating c-Fos in macrophage-osteoclast lineage determination. However, histological analysis of mutant long bones during embryonic development revealed a partial bone marrow space, suggesting that there may be some resorptive activity during embryonic development in these mice that might be developmentally regulated. In this study, we have investigated the haematopoietic stem cell and/or osteoclast precursor population which requires c-Fos for differentiation in order to determine whether there is a differential dependence on c-Fos for osteoclastogenesis.

**METHODS:** Two systems were utilised to investigate haematopoietic precursor differentiation to osteoclasts: an *ex vivo* approach analysing the osteoclastogenic potential of c-Fos mutant spleen and bone marrow-derived M-CSF-dependent precursors, and an *in vitro* approach generating osteoclasts using murine Embryonic Stem (ES) cells. Osteoclast differentiation was induced by exposure to soluble recombinant RANKL, and functional assays were performed by measuring resorption pit formation following culture on dentine slices.

**RESULTS:** Comparison of osteoclast precursors cultured with RANKL from wild-type and mutant spleen and bone marrow precursors derived from adult, 2-week old and newborn mice, showed that mutant splenic and bone marrow precursors from adults never gave rise to TRAP-positive osteoclasts. However, 2-week old and newborn mutant precursors retained the ability to differentiate into functional osteoclasts, albeit at a low frequency. This suggests that

there may be an age-dependent pathway for osteoclast differentiation.

This was further addressed by investigating the differentiation potential of wild-type and c-Fos mutant ES cells to differentiate into osteoclasts, using the well-established ability of ES cells to form haematopoietic progenitors within aggregates called Embryoid Bodies (EBs). Dissociated EBs from either wild-type cells or cells containing GFP knocked into the c-Fos locus and cultured with M-CSF and RANKL produced large numbers of TRAP-positive osteoclasts capable of lacunar resorption on dentine slices, with efficient GFP expression in macrophages and osteoclasts. Surprisingly, c-Fos mutant ES cells were also able to differentiate into osteoclasts, at frequencies as high as 30% of wild-type ES cells. Moreover, the precursor pool of cells lacking c-Fos could be expanded following exposure of dissociated EBs to M-CSF, and additionally to TNF  $\alpha$ , but not to TGF  $\beta$ . Molecular analysis showed expression of osteoclast-specific genes, and resorption assays demonstrated pit formation, confirming that c-Fos knockout precursors differentiated into *bona fide* functional osteoclasts,

**DISCUSSION & CONCLUSIONS:** These data suggest that ES cells can provide a manipulatable and expandable population of haematopoietic precursors to study osteoclast lineage determination and differentiation. More importantly, the ES cell system has revealed that there appears to be distinct pathways for osteoclast differentiation which are developmentally-regulated and which display differential dependence on c-Fos. These studies are essential for understanding the molecular controls of osteoclast differentiation and bone remodeling.

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