

monocytes (BMDMs) were isolated from all genotypes and osteoclastogenesis was studied using an established osteoclast formation assay. To investigate the underlying signalling pathways, cells were treated with TNF α at different time points and the activation of mitogen-activated protein kinases was studied by western blot analysis.

Histology and in vivo PET/CT studies revealed increased bone metabolism and bone mass in the hind paws, knees and vertebrae of p62aa Δ 69-251 mice, but only minor changes were seen in the number and size of osteoclasts between p62aa Δ 69-251 and wt animals suggesting that, under physiological conditions, regulatory mechanisms compensate for the lack of the signal transduction domains of p62. Compared with wt cells, however, BMDMs of p62aa Δ 69-251 mice showed a significantly increased osteoclastogenesis, in particular when stimulated with TNF α . Crossing of p62aa Δ 69-251 mice with hTNFtg animals resulted in a dramatic increase in the severity of joint damage in the hTNFtg/p62aa Δ 69-251/wt mice, as determined clinically by histomorphometry and PET/CT analysis. This was accompanied by an increase in the number and size of osteoclasts in vivo. Western blot analysis of osteoclast lysates revealed increased TNF α -induced extracellular signal-regulated kinase phosphorylation in cells from p62aa Δ 69-251 compared with wt mice.

Our data suggest that p62 is an important regulator of TNF α -mediated joint damage. They indicate that the loss of the TRAF6 and aPKCs binding domains has important consequences for osteoclastogenesis under inflammatory conditions.

A48 THE TRAF6 BINDING MOLECULE P62/SQSTM1 IS A CRITICAL REGULATOR OF INFLAMMATORY BONE DESTRUCTION

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The activation of nuclear factor κ B (NF κ B) via RANK is essential in regulating osteoclastogenesis. Under inflammatory conditions this process is enhanced by cytokines such as tumour necrosis factor α (TNF α). P62/SQSTM1 directly modulates these pathways through complex formation with TRAF6, aPKCs and ubiquitin. However, the role of p62/SQSTM1 in regulating bone turnover under inflammatory conditions, and specifically the role of the signal transduction domains of p62/SQSTM1, is unclear.

Mice were generated that carry a shortened but functional mutant of p62 with defective signal transduction domains (p62aa Δ 69-251) for interbreeding studies with arthritic human TNF transgenic (hTNFtg) mice. The resulting genotypes (wild-type (wt), hTNFtg, p62aa Δ 69-251 and hTNFtg/p62aa Δ 69-251/wt) were scored for clinical parameters (paw swelling, grip strength, weight) for 14 weeks. To quantify the extent of inflammation, cartilage degradation and number of osteoclasts, joints of 14-week-old mice were embedded into paraffin and stained with toluidine-blue and tartrate-resistant acid phosphatase. To identify abnormalities in bone metabolism and bone structure, high resolution micro-CT scanning and positron emission tomography using 18-deoxyfluorogluco fluoride (FDG-PET) were performed in 14-week-old mice of all genotypes in vivo. In addition, bone marrow derived