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THE SIGNALLING DOMAIN OF THE MULTIADAPTOR PROTEIN P62/SQSTM1 LINKS REACTIVE OXYGEN SPECIES FORMATION AND OBESITY TO INCREASED TNF α -MEDIATED JOINT DAMAGE

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Background The multidomain protein p62 plays a key role in signal transduction through interaction with TRAF6, ERK1 and aPKC. It also contributes to protein degradation by autophagy, the ubiquitin proteasome system or sequestration. Despite an established role as in Paget's disease, it is unclear how p62 acts as a signalling hub between different pathways and how the single domains coordinate the protein functions and link different pathological processes.

Methods Therefore, the authors generated a mouse line carrying a shortened variant of p62, which lacks the binding sites for TRAF6 and aPKC, but still contains all protein degradation modules (p62^{Delta69-251}). The authors analysed the phenotype of these mice and crossed them with hTNFtg arthritic animals to study the effects of the p62 signalling domain on the onset, severity and histological changes of a TNF α dependent arthritis. Isolated cells of these mice were analysed for alterations in their differentiation capacity, ROS production and MAPK signalling.

Results The truncated p62^{Delta69-251} protein was ubiquitously expressed and was still able to oligomerise and to bind LC3, ubiquitinated proteins and ERK1. As expected, p62^{Delta69-251} failed to interact with TRAF6. Of note, cells of homozygote p62^{Delta69-251} mice showed an increased production of ROS, and this effect correlated with elevated levels of autophagy-related acidic membrane vesicles. Stimulation of p62^{Delta69-251} cells with the autophagy-inhibitor Bafilomycin and the proteasome-inhibitor MG132 still resulted in an accumulation of p62 and aggregate formation. Interestingly, the lack of the signaling module was sufficient to produce an obese phenotype accompanied by the enlargement of organs, for example, liver and kidney. PET/CT studies revealed an increased bone metabolism and BMDMs of p62^{Delta69-251} mice showed a significantly increased osteoclastogenesis in vitro, particularly when stimulated with tumour necrosis factor α (TNF α). WB analysis revealed an increased TNF α -induced p38 phosphorylation in cells from p62^{Delta69-251} compared to wt mice. Crossing of p62^{Delta69-251} mice with hTNFtg animals resulted in a dramatic

increase in the severity of joint damage conceivable by an increased number and size of osteoclasts. Interestingly, p62^{Del-ta69-251/+} hTNFtg mice showed a significant increased weight at week 12 compared to hTNFtg mice.

Conclusion The authors show for the first time, that the signalling module of p62 can act as an intracellular antiobesity-factor and that this function does not require the modules for autophagy and ubiquitin-binding. Moreover, p62 is an important regulator of TNF α mediated joint damage indicating that the loss of p62 signalling domains has important consequences both for metabolic activation and for osteoclastogenesis under inflammatory conditions.