Atg12 maintains skeletal integrity by modulating pro-osteoclastogenic signals and chondrocyte differentiation

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Weightlessness and radiation, two unique elements of space, profoundly decreases bone mass. This bone loss is attributed to increased activity of bone-resorbing osteoclasts and functional changes in bone-forming osteoblasts, cells that give rise to mature osteocytes. Our long-term goal is to identify signaling pathways that may be targeted to mitigate bone loss in scenarios of space exploration, radiotherapy and accidental radiation exposure. We have previously shown that exposure of MLO-Y4 osteocyte-like cells to simulated space radiation (^{56}Fe) increased the expression of the pro-osteoclastogenic gene rankl and decreased protein levels of LC3B-II, a key player in autophagy. In this current study, we aimed to further elucidate the role of autophagy in maintaining structural integrity of the skeleton. We hypothesize that loss of autophagy in bone leads to an imbalance in pro-osteoclastogenic and pro-osteogenic signals, resulting in net bone loss. To test our hypothesis we performed global postnatal deletion of Atg12 using tamoxifen-inducible Cre recombinase under the control of the CAG promoter. Six-week-old CAG-CreERT2/FloxAtg12 animals were treated daily with Tamoxifen or Vehicle (Control, oil only) for five days and euthanasia performed two weeks after the onset of treatment. Percent change in body weights (prior to treatment and at euthanasia) was not significantly different between treatment groups within the same gender. Compared to Vehicle (Control) groups, Tamoxifen (Atg12 iKO) groups showed decreased LC3B-I to II conversion and increased p62 protein levels, consistent with loss of autophagy. Quantitative PCR revealed increased expression of pro-osteoclastogenic cytokines mcp1 and rankl in bone and marrow respectively in male iKOs compared to male controls. Expression levels of these genes were not significantly altered in the Atg12 iKO females compared to females controls. Microcomputed tomography of tibiae revealed decreased cortical bone volume, cortical thickness and periosteal perimeter consistent with bone loss; and a longer primary spongiosa in male Atg12 iKOs display compared to male controls. These decrements were less pronounced in the female Atg12 iKOs. Cancellous bone structure was not significantly different between iKOs and controls in both genders. Histological analysis also revealed that compared to male controls, male iKOs showed a profound increase in chondrocyte column length of the growth plate with hyper-expansion of both proliferating and hypertrophic zones. Taken together, these findings indicate that autophagy plays an important role in the maintenance of bone structural integrity by mediating the production of pro-osteoclastogenic signals and regulating chondrocyte proliferation and differentiation. Supported by NSBRI grant MA02501 (Globus)