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F-SPONDIN (SPONDIN-1) NULL MICE EXHIBIT INCREASED BONE FORMATION, DECREASED OSTEOCLAST FUNCTION AND ACCELERATED OSTEOARTHRITIS

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Purpose: We have previously reported that F-spondin (spondin-1), a neuroregulatory protein, is upregulated by chondrocytes in osteoarthritis. These studies showed that spondin-1, a member of the TSR (thrombospondin) type I class super family, activated latent TGF-β1, which appeared to account for selected *in vitro* effects, including induction of the hypertrophic chondrocyte phenotype. In this study we generated *Spon1* knockout mice to investigate the effect of F-spondin in vivo in i) skeletal development and ii) OA progression following surgical destabilization of the medial meniscus (DMM).

Methods: Knockout mice were generated in collaboration with the Texas Institute of Genomic Medicine by targeted deletion of exon 1 in C57BL/6 mice. Exon deletion was confirmed by Southern blot analysis and PCR using probes specific for wild type (WT) and mutant loci. Total TGFβ-1 was detected using R &D ELISA kit. MicroCt was performed on the Scanco mCT 35 system on proximal tibia and femurs bone evaluation. Values represent the average of 10 WT and 11 KO mice. To assess the role of *Spon1* on OA progression following destabilization of the medial meniscus was performed on 3 month old WT and *Spon1*$^{-/-}$ mice.

Results: *Spon1*$^{-/-}$ null mice were viable and initial macroscopic observations revealed no overt differences in size and body weight compared with WT (mice up to 6 months). Since we previously reported spondin-1 to regulate TGF-β activity, we measured TGF-β1 serum levels in adult mutant mice. Relative to WT, *Spon1* deletion reduced serum levels of total TGF-β1 (82 ± 20 ng/ml vs. 30 ± 25.0 ng/ml; $p<0.002$). Similarly, cultured chondrocytes isolated from the rib cages of 5 day old *Spon1*$^{-/-}$ mice also produced significantly less TGF-β1 (30%) compared to WT controls ($p<0.01$). To determine whether *Spon1* deletion affected bone phenotype, we performed microCT of tibia and femurs in mutant and WT mice aged 1-6 months Relative to WT mice, *Spon1*$^{-/-}$ exhibited increased bone formation at 6 months (Figure 1), evidenced by, a) increased trabecular and cortical bone volume fraction (Bone volume/Total volume: 0.26 ± 0.03 versus 0.16 ± 0.05, $p<0.0002$; Fig 1), b) decreased trabecular spacing (0.14 ± 0.02 versus 0.19± 0.03, $p<0.0003$); and c) increased trabecular number (7.7 ± 1.2, versus 5.5 ± 0.9, $p<0.0005$). Interestingly, no significant changes were observed at 1 or 3 months, suggesting that *Spon1* effects are age-dependent. Histologically, tibia from *Spon1*$^{-/-}$ mice displayed increased bone ingrowth in the bone marrow cavity, particularly within the spongy bone of the ephysiseal regions. This was accompanied by decreased TRAP staining compared to WT mice, suggesting decreased numbers of osteoclasts. Supporting this observation, osteoclast differentiation, performed by RANKL, M-CSF induction of non-adherent bone-marrow cells, revealed impaired differentiation in *Spon1*$^{-/-}$ mice compared to WT.

Preliminary analyses suggest that *Spon1* deletion also accelerated cartilage degradation in the DMM model of osteoarthritis. The increased severity of osteoarthritis-like cartilage destruction 7 weeks post surgery was by accompanied by increased thickening of subchondral bone (Figure 2).

Conclusions: Our studies indicate that spondin-1 (F-spondin), a latent TGF-β1 activating ECM protein, over-expressed in OA bone and cartilage, regulates bone metabolism in aging mice. *Spon1*$^{-/-}$ mice exhibit increased trabecular and cortical bone formation, decreased osteoclast function and increased susceptibility to surgical induced OA. Together these data suggest that a primary function of spondin-1 in skeletal tissue is the regulation of bone mass via latent TGF-β1 activation. Further studies are in progress regarding the potential of spondin-1 as a drugable target in future therapy of osteoporosis or osteoarthritis.

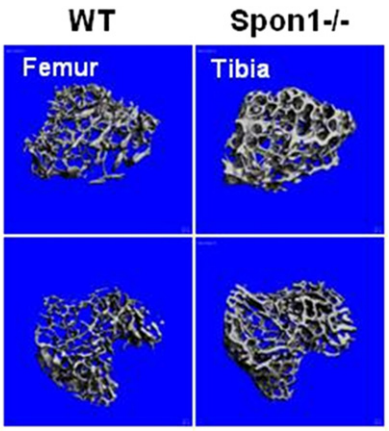

Figure 1

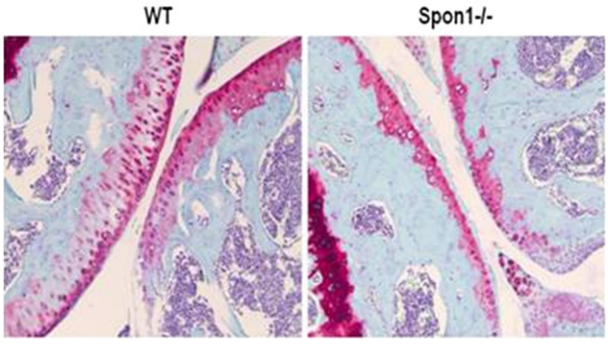

Figure 2

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GENE ARRAY PROFILING OF ARTICULAR CHONDROCYTES IN MICE WITH DIFFERENT SUSCEPTIBILITY TO NATURAL DISEASE REVEALS SPECIFIC GENE SIGNATURES LINKED TO HEALTHY AGEING AND SPONTANEOUS OA.

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Purpose: The mechanisms initiating and driving spontaneous osteoarthritis (OA) progression remain largely unknown. The Str/ort mouse strain has high natural susceptibility to OA with ageing, whereas CBA mice show little, if any, spontaneous OA development. In this study, we used this inter-strain variability to spontaneous OA in mice at various ages to determine articular cartilage (AC) gene expression patterns and biological pathways that contribute to normal, healthy and osteoarthritic AC ageing and that play a role in susceptibility to OA.

Methods: AC from Str/ort and CBA mice at 8 (before overt OA in Str/ort), 18 (early OA) and 40wks of age (late OA) were isolated from tibial and femoral knee joint condyles and RNA extracted (n=4 per group) for gene expression microarray analysis. Ingenuity® pathway analysis was performed for each comparison: 8 vs. 40wks CBA, 8 vs. 40wks Str/ort, 8wks CBA vs. Str/ort, 8 vs. 18wks Str/ort. RT-qPCR was performed on selected genes.

Results: Profiling in 8 and 40wk-old CBA mice was used to pinpoint gene signatures linked to healthy AC ageing. This comparison revealed 190 differentially-regulated genes (50 up, 140 down), which did not include any genes previously linked to OA, except CILP and HDAC5. Pathway analysis revealed that skeletal muscle-specific genes were significantly down-regulated in the AC of CBA mice with ageing from 8-40wks. Changes in this