The WNT pathway within leptin-receptor lineage cells plays a pivotal role in the process of peri-implant bone formation

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Disclosures: V.J. Suhardi (N), A. Oktarina (N), M.B. Greenblatt (N), L.B. Ivashkiv (Eli Lilly and Company: Non-paid consultant), M.P.G. Bostrom (Smith & Nephew: Paid consultant), Xu Yang (N)

INTRODUCTION: The success of cementless joint replacements relies on rapid peri-implant bone formation (osseointegration). Fibrotic tissue usually forms in the bone-implant interface when osseointegration fails, which results in implant loosening, requiring revision surgery[1]. We have previously identified leptin receptor-lineage cells (LepR+ cells) as the cellular origin of peri-implant fibrotic tissue while partially contributes to peri-implant bone formation[1]. Prior large-scale genome-wide association studies (GWAS) have identified RSPO3, a WNT-signaling positive modulator, as being strongly associated with bone mineral density and fracture risk[2]. Here we report that the deletion of *Rspos* significantly hampers peri-implant bone formation.
METHODS: All experiments were approved by local IACUC.
Animals: Lepr-cre, Rosa26-CAG-loxp-stop-loxp-tdTomato, and RSPO3$^{fl/fl}$ (N=9) were purchased from Jackson Laboratories. We generated Lepr-cre; tdTomato;Rspo3$^{fl/fl}$mice (N=9), in which Rspos3 is deleted from LepR+ cells marked by tdTomato.
Model of hemi knee arthroplasty: We have developed a mouse model using a 3-D printed titanium (Ti6Al4V) implant to mimic the tibial component of a cementless total knee replacement[3]. The implant is press-fit into the medullary canal of proximal tibia. The porous surface of the implant stem facilitates osseointegration and the smooth tray articulates with the femur.
Micro-CT: Scans (µCT 45, Scanco Medical, Switzerland) were performed at 6 µm voxel size, 90 kVp, 145 mA, and 0.36 rotation step (180 angular range) per view. Volumes of interest was defined as peri-implant (the entire region <200 µm from implant stem). Bone volume fraction (BV/TV), trabecular number (Tb.N.), trabecular thickness (Tb.Th), and trabecular separation (Tb.S) were assessed to determine peri-implant bone mass and architecture.
Statistical Analysis: Student's t-test was used. $p<0.05$ was considered as significant.
RESULTS: Deletion of *Rspos3* (in *LepR-Cre;tdTomato;Rspos$^{fl/fl}$* mice) decreased peri-implant bone formation relevant to the controls (*LepR-Cre;tdTomato;Rspos3$^{+/+}$* mice) (Figure 1), as reflected by lower BV/TV, Tb.N, and higher Tb.N (Figure 1c). Consistent with the microCT analysis, histological analysis also revealed the decreased peri-implant bone and increased peri-implant fibrotics tissue in mice with Rspos3 deletion (Figure 2a,c). Furthermore, a higher percentage of spindle-shaped, fibroblast-like LepR$^+$ cells, were found in the peri-implant area in *LepR-Cre;tdTomato;RSPO3$^{fl/fl}$* mice as compared with controls (Figure 2b,d).
DISCUSSION: With the conditional knockout of RSPO3 from the targeted leptin receptor-expressing cells, we effectively induced the downregulation of the WNT pathway, leading to the suppression of peri-implant bone formation. This suggest a novel perspective of WNT pathway whose role has only been reported in the regulation of bone mineral density (BMD) and fracture healing. This underscores the constructive regulatory function of the WNT pathway in the intricate process of peri-implant bone formation.
SIGNIFICANCE/CLINICAL RELEVANCE: Individuals presenting with RSPO3 mutations might necessitate extra screening and interventions, including the administration of anabolic agents, both before and in the immediate aftermath of total joint arthroplasty to enhance peri-implant bone formation and in turn to improve the outcome of the procedure.

REFERENCES: 1, Suhardi V et al, ORS NIRA Presentation 2023, Paper #250. 2, Nilsson KH et al, *Nat Commun*, 2021, 12:4923. 3, Kuyl EV et al., *Bone Joint J*, 2021, 103-B: 135-144.

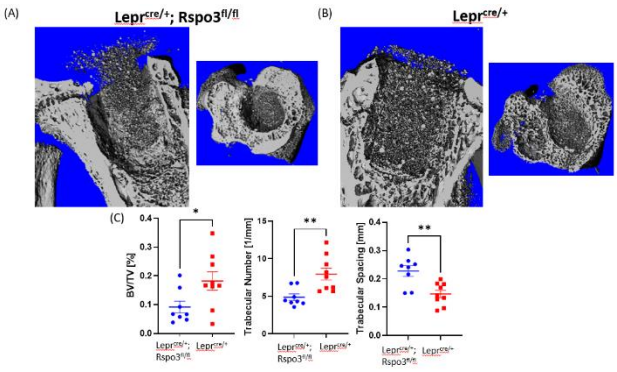

Figure 1. MicroCT of Lepr$^{cre/+}$; Rspo3$^{fl/fl}$ or cre-only control (Lepr$^{cre/+}$) two weeks after undergoing hemi-knee arthroplasty. Representative 3D-reconstructed Micro-CT coronal and axial image of the proximal tibia of Lepr$^{cre/+}$; Rspo3$^{fl/fl}$ (a) or cre-only control (Lepr$^{cre/+}$) (b). (c) BV/TV, trabecular number, and trabecular spacing of the peri-implant bone. Data is represented as mean +/- s.d. *p<0.05, **p<0.01.

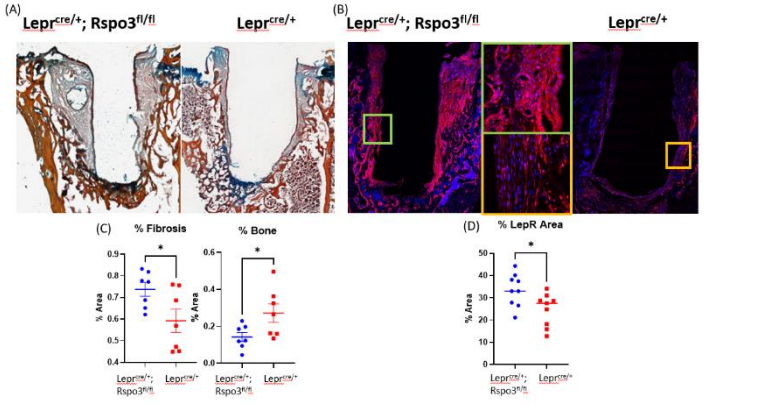

Figure 2. Histology and immunofluorescent imaging of Lepr$^{cre/+}$; Rspo3$^{fl/fl}$ or cre-only control (Lepr$^{cre/+}$) two weeks after undergoing hemi-knee arthroplasty. (a) Representative H&E image of the proximal tibia of Lepr$^{cre/+}$; Rspo3$^{fl/fl}$ or cre-only control (Lepr$^{cre/+}$) two weeks after undergoing hemi-knee arthroplasty. (b) Representative immunofluorescent image of the proximal tibia of Lepr$^{cre/+}$; Rspo3$^{fl/fl}$ or cre-only control (Lepr$^{cre/+}$) two weeks after undergoing hemi-knee arthroplasty. (c) Percent fibrotic area and bone area around the implant measured 200 µm from the implant-host tissue interface. Data is represented as mean +/- s.d. *p<0.05, **p<0.01. (d) Percent Tdtomato positive area around the implant measured 200 µm from the implant-host tissue interface. Data is represented as mean +/- s.d. *p<0.05, **p<0.01.