Knockout of Adhesion G Protein-Coupled Receptor F5 in Leptin Receptor Lineage Cells Attenuates Peri-implant Fibrotic Tissue and Augments Peri-implant Bone Formation

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INTRODUCTION: Peri-implant fibrosis is among the most common reasons for total joint replacement failure[1]. Currently, the only available treatment is a revision surgery with the aim to reset the peri-implant biological environment with so that the host will foster bone regeneration over fibrotic tissue. We have previously established that leptin receptor-expressing (LEPR+) mesenchymal lineage cells are both necessary and sufficient for the formation of peri-implant fibrotic tissue[2]. In this study, we identified adhesion G protein-coupled receptor F5 (ADGRF5) as a key pro-fibrotic mediator in LEPR+ cells. Conditional deletion of ADGRF5 in these cells not only mitigated peri-implant fibrosis but also enhanced peri-implant bone growth offering a promising avenue for therapeutic interventions.

METHODS: All experiments were approved by the local IACUC.

Mice: All surgeries were performed on 10-16 weeks-old LepR-Cre; tdTomato; Adgfr5$^{fl/fl}$ (n=14) and LepR-Cre; tdTomato; Adgrf5$^{+/+}$ mice (cre-only control)

Model of knee arthroplasty aseptic loosening (Fibrous-Integration surgery): 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 medullary canal receiving the implant is over-drilled resulting in micromotion and thus preventing osseointegration and leading to peri-implant fibrosis.

Bulk RNA Sequencing: LEPR+ cells from mouse peri-implant area were subjected to bulk RNA sequencing on postoperative day 14. The cells were isolated, and RNA extracted using an RNAeasy Micro Kit (Qiagen, MD). Reads were mapped to the mouse genome and counted against Gencode v27 using the STAR aligner.

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. Image acquisition was performed on samples obtained at two weeks post-surgery. Volumes of interest was defined as the entire peri-implant region <200 µm from implant stem. Trabecular bone parameters were measured with the software provided by the micro-CT manufacture.

Immunofluorescence: Cryosectioned sections were stained with primary and secondary antibodies. Immunofluorescence imaging was conducted using Zeiss LSM 880 confocal microscope with an Airyscan high-resolution detector.

Biomechanical testing: Pullout testing of the tibial implant was performed to assess the strength of bone-implant interface. Distal tibia and diaphysis were potted in polymethylmethacrylate (PMMA) and tested with an EnduraTEC ELF 3200 system (Bose, USA). Implants were pulled out at 0.03 mm/sec under displacement of failure. Maximum pull-out load in Newtons (N) was calculated from the obtained load-displacement curves.

Statistical Analysis: Data are reported as mean ± standard deviation. Statistical analysis was performed using a Student's t-test. Significance was assigned to $p<0.05$

RESULTS:

The majority of LEPR^{+} cells in peri-implant fibrotic tissue of both mice (Figure 1a) and humans (Figure 1c) expressed ADGRF5. RNA-seq analysis of both mouse and human peri-implant tissue showed upregulation of Adgrf5 in the peri-implant fibrotic tissue as compared to bone (Figure 1b,d). Conditional knockout of Adgrf5 in LEPR-lineage cells (LepR-Cre;tdTomato;Adgfr5$^{fl/fl}$) resulted in decreased peri-implant fibrous tissue formation, increased peri-implant bone formation (Figure 2a-c), and an overall increase in the implant-host tissue interface strength (Figure 2d) as compared to cre only control. Immunofluorescence imaging of the peri-implant area revealed a decrease in LEPR-lineage cells within the peri-implant tissue (Figure 2e-f). Additionally, there was a noticeable decrease in the expression of ACTA2, a commonly used myofibroblast marker, by LEPR-lineage cells (Figure 2g).

DISCUSSION: Through the targeted implementation of a conditional knockout strategy for ADGRF5 in leptin receptor-expressing cells, we effectively induced the downregulation of ADGRF5 expression. This downregulation, in turn, led to the effective suppression of peri-implant fibrosis. This study not only sheds light on a previously unrecognized role of ADGRF5 in promoting peri-implant fibrosis but also underscores its detrimental impact on peri-implant bone formation. Furthermore, these findings provide a new avenue of potential therapeutic development to inhibit peri-implant fibrotic tissue formation and simultaneously boost peri-implant bone formation through the selective inhibition of ADGRF5.

SIGNIFICANCE/CLINICAL RELEVANCE: Selective inhibition of ADGRF5 emerges as a promising and targeted approach for combatting peri-implant fibrosis while simultaneously promoting peri-implant bone formation. This therapeutic intervention could hold significant potential for improving the outcomes of joint replacement surgeries and reducing the risk of implant failure. Further research and development in this direction may lead to novel pharmaceutical interventions that enhance the success and longevity of joint replacements.

REFERENCES: 1, Feng X et al, Am J Transl Res, 2022, 14(10): 7080-7089. 2, Suhardi V et al, ORS NIRA Presentation 2023, Paper #250. 3, Kuyl EV et al., *Bone Joint J*, 2021, 103-B: 135-144.

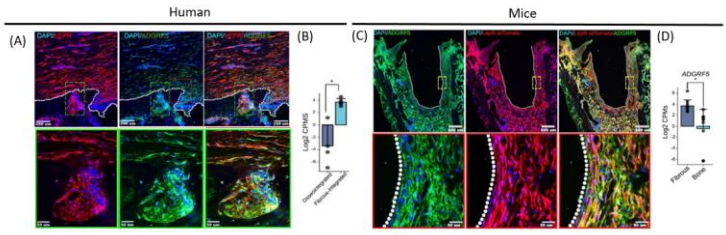

Figure 1. Expression of LEPR and ADGRF5 in peri-implant fibrotic tissue. (A) LEPR (red) and ADGRF5 (green) expressing cells in human peri-implant fibrotic tissue. (B) ADGRF5 is more highly expressed by Lin-LEPR+ in human fibrous membrane than in bone. *p<0.05. (C) LepR-Tdtomato (red) and ADGRF5 (green) expressing cells in LepRcre;tdtomato mice that underwent fibrous-integrated surgery at postoperative day 14. (D) RNA-seq analysis of mouse peri-implant tissue demonstrated Adgrf5 is more highly expressed Lin-LepR-Tdtomato+ from fibrous-integrated mice than from osseo-integrated mice. *p<0.05

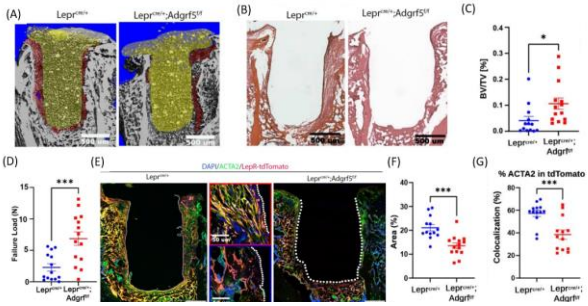

Figure 2. Ablation of Adgrf5 in LEPR lineage cells inhibits peri-implant fibrosis and enhances peri-implant bone formation in vivo. (A-B) Micro-computed tomography (µCT, A) and histology (B) of proximal tibia of Lepr-Cre; tdTomato; Adgfr5$^{fl/fl}$ (Lepr$^{cre/+}$; Adgrf5$^{f/f}$) and Lepr-Cre; tdTomato (Lepr$^{cre/+}$) at postoperative day 14. (C) Ablation of Adgrf5 in LepR lineage cells resulted in higher peri-implant bone than control mice as measured by bone volume/total volume (BV/TV). (D) Host bone-implant failure load of Lepr$^{cre/+}$; Adgrf5$^{f/f}$ and Lepr$^{cre/+}$ mice at postoperative day 28. ***p<0.001. (E) Lepr-tdtomato (red) and ACTA2 (green) expressing cells in murine peri-implant fibrotic tissue. (F) Immunofluorescent quantification of peri-implant tissue of control and flox mice at postoperative day 14 demonstrated ablation of ADGRF5 in LEPR lineage cells resulted in a reduction in tdTomato+ cells and ACTA2-expressing LepR lineage cells. ***p<0.001.