Inhibition of Gremlin 1 as a Novel Prophylaxis and Treatment for Aseptic Loosening

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INTRODUCTION:

Aseptic loosening is one of the leading cause of failures of total joint replacements (TJRs) and current treatment requires revision surgery which resulted in additional morbidity and mortality for patients. Regardless of the etiology of loosening or the type of implants used (cementless or cemented), fibrotic tissue instead of bone ubiquitously forms at the bone-implant/cement interface. Transcriptomic analysis of $LepR^+$ cells isolated from murine peri-implant fibrous tissue and bone showed upregulation of Gremlin1 (*Grem1)*, inhibitor of bone morphogenic protein (BMP) -2, 4, and 7[1]. We aimed to create novel prophylaxis and treatment for aseptic loosening by using neutralizing antibody against Gremlin1.

METHODS:

All experiments were approved by local IACUC.

Model of peri-implant fibrosis: 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. The tibia intramedullary canal is over-drilled, resulting in implant micromotion and thus inducing osseointegration failure and formation of fibrotic tissue. The implant is then inserted into the mouse's tibial medullary canal[2].

Intraarticular injection of anti-Gremlin1: We performed intra-articular administration of a neutralizing antibody against gremlin1 (anti-gremlin1) everyday for 14 days starting at postoperative day 0 (prohphylaxis experiment) or starting at postoperative day 14 (treatment experiment). For each administration, we injected 10 µL of 10 µg/mL rhGREM1 solution diluted in PBS, 10 µL of 50 µg/mL gremlin1–neutralizing antibody (LS-C125371, LSbio) solution diluted in PBS, or 10 µL of 50 µg/mL isotype antibody as a isotype control. We divided n=20 mice into 2 experimental groups: First group (prophylaxis experiment, n=10) received either anti-gremlin 1 (n=5) or isotype antibody (n=5) starting at postoperative day 0. Second group (treatment experiment, n=10) received either anti-gremlin 1 (n=5) or isotype antibody (n=5) starting at postoperative day 14.

Statistical Analysis: Statistical analysis was performed using Student's t-test. $p<0.05$ was considered as significant.

RESULTS:

MicroCT and histological analysis of mice receiving anti-gremlin1 antibody immediately after surgery has less peri-implant fibrosis and increased peri-implant bone formation (Figure 1). Similarly, microCT and histological analysis of mice receiving anti-gremlin1 antibody 2 weeks after surgery demonstrated significantly less peri-implant fibrosis and increased peri-implant bone (Figure 2). In both the prophylactic and treatment experiments, there were less leptin receptor lineage progenitor cells in the peri-implant region of the mouse receiving anti-gremlin1 than in control group.

DISCUSSION AND CONCLUSION:

To our knowledge, this is the first study that shows prevention and reversal of aseptic loosening without the necessity of revision surgery. Inhibition of gremlin-1 through administration of intraarticular gremlin-1 successfully prevent and remodel per-implant fibrosis.

SIGNIFICANCE/CLINICAL RELEVANCE: Result of this study showed the feasibility of using pharmaceutical agent to both prevent and treat aseptic loosening. This study is the first study to demonstrate that it might be feasible to treat aseptic loosening with pharmaceutical agent instead of revision surgery.

REFERENCES: 1, Canalis E, et al., *J Cell Physiol*, 2012, 227:269-277. 2, Kuyl EV et al., *Bone Joint J*, 2021, 103-B: 135-144.

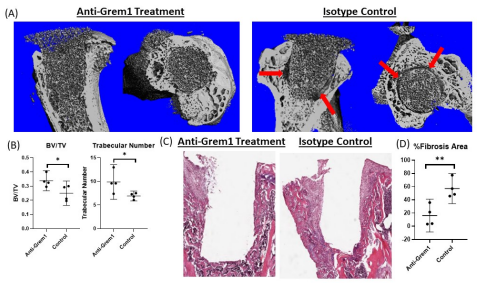

Figure 1. Administration of intrarticular anti-grem1 starting immediately after surgery successfully prevent peri-implant fibrosis. (A) Representative microCT images of the tibia receiving anti-gremlin1 and isotype control. Red arrow marks fibrous-tissue filled gap between implant and host bone. (B) microCT quantification demonstrated significantly higher bone volume/total volume (BV/TV) and higher trabecular number in mice receiving anti-grem1 than isotype control. (C) Representative histology of the tibia receiving anti-gremlin1 and isotype control. (D) Histology quantification demonstrated significantly less peri-implant fibrosis in mice receiving anti-gremlin1 as compared to control.

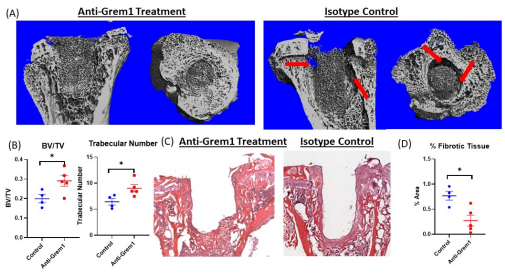

Figure 2. Administration of intraarticular anti-grem1 after the formation of peri-implant fibrosis can successfully reverse peri-implant fibrosis. (A) Representative microCT images of the tibia receiving anti-gremlin1 and isotype control. The red arrow marks a fibrous-tissue-filled gap between the implant and host bone. (B) microCT quantification demonstrated significantly higher bone volume/total volume (BV/TV) and higher trabecular number in mice receiving anti-grem1 than in isotype control. (C) Representative histology of the tibia receiving anti-gremlin1 and isotype control. (D) Histology quantification demonstrated significantly less peri-implant fibrosis in mice receiving anti-gremlin1 as compared to control.