Estrogen receptor beta is a therapeutic target to improve osteoporotic cortical bone repair in mice: An in-vivo longitudinal study by high resolution microCT

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Introduction:
Orthopedic surgeons are challenged by impaired or delayed fracture repair in osteoporotic bone. Therapeutic strategy should be defined according to identified molecular target. However, the molecular mechanism of osteoporotic fracture repair remains poorly understood. Intramembranous ossification (radial bone growth) and endochondral ossification (linear bone growth) are two important processes in callus hardening during fracture repair. Estrogen-receptor-alpha (ERalpha), mediating the classical estrogen signaling pathway, exerts a positive effect on bone maintenance, but the role of ERalpha in regulation of bone development remains controversial [1, 2]. Previous researches demonstrate estrogen depletion induces a significant increase in Estrogen-receptor-beta (ERbeta) expression in bone [3]. ERbeta signaling participates in inhibiting both intramembranous ossification and endochondral ossification [1, 4]. Therefore, we form the hypothesis that blockade of ERbeta could promote osteoporotic fracture repair.

Methods:
30 ERbeta knockout (KO) and 30 wild type female mice (WT, C57BL/6) aged 3 months were used in this study. All mice were ovariectomised first. 6 weeks after ovariectomy, bilateral 0.8mm-diameter drill holes were made from the posterior to the anterior of the diaphysis of the femur. High resolution micro-CT (VivaCT 40, Scanco) was employed to monitor the repair process at day 0,3,7,10,14 and 21. The bone volume fraction (BV/TV) and bone mineral density (BMD) were evaluated at both defect site and intra-medulla space in two groups. Repeat measure ANOVA and Independent t-test were performed to analyze the data.

Results & Discussion
With regard to microCT measurement, WT and KO mice differed significantly in the pattern of the change in the BMD over time in both defect region and intra-medulla space (P<0.05 for the interaction between time and group by the repeat measure ANOVA) (Fig1,2). In the defect region, the BMD increased to a similar extent from baseline in both WT and KO mice at 3 days post-fracture. At day 7, a significant increase in the BMD over baseline was apparent in both WT and KO mice, but the extent of this increase differed between the 2 groups. (+45% for WT and +78% for KO; P<0.05 versus baseline for both) Thereafter, a continuous increase in BMD to the peak at day 14 and followed by a decline at day 21 was apparent in both groups. The BMD was significantly higher in KO mice compared with WT mice from day 7 to day 21. In the intra-medulla space, the change of BMD showed similar pattern with defect region in both groups from day 0 to day 14, except the BMD at day 21 returns to the baseline level in both groups. In addition, the BV/TV data was consistent with the findings from BMD measurement. A higher BMD within defect region in KO group compared to WT group suggested that osteogenesis was promoted by blockade of ERbeta pathway during osteoporotic bone healing. In both groups, callus remodeling was present after 14 days post-fracture. The returning of BMD to the baseline level within intra-medulla space in both groups at 21 days post-fracture suggested that the blockade of ERbeta pathway didn’t affect remodeling, which was not like delayed remodeling by PTH treatment [5], alendronate treatment [6] or zoledronic acid treatment [7]. The results further suggested blockade of ERbeta could promote osteoporotic fracture repair.

Conclusion:
This is the first study employing high resolution micro-CT to in-vivo monitoring the fracture repair. ERbeta pathway is a potential therapeutic target for promoting osteoporotic fracture repair, highly selective ERbeta antagonist should be investigated as an osteoporotic fracture repair drug.

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References:

Figure 1. Micro-CT 2D (Upper) and 3D (Bottom) images from day 0 to day 21 post surgery showed promoted bone healing in KO group

Figure 2. MicroCT quantification of BV/TV and BMD from day 0 to day 21 post operation in two groups, which suggested promoted early osteogenesis without delayed remodeling in KO group mice.

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