The Role of mTORC1 in Postnatal Skeletal Development

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Introduction

• mTOR (mammalian target of rapamycin) is a serine-threonine kinase that plays a central role in a number of key cellular pathways that have been previously implicated in bone formation. mTOR mediates these diverse roles by forming two multi-protein complexes, mTORC1 and mTORC2, each of which is defined by unique proteins raptor and rictor respectively.

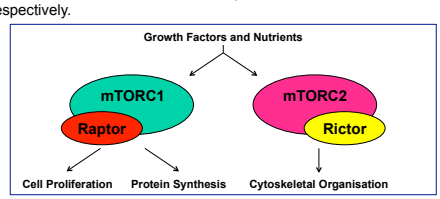

Fig 1: Growth factors and nutrients stimulate mTORC1 and mTORC2 to promote cell proliferation, protein synthesis and cytoskeletal organisation.

• Studies from our laboratory have demonstrated that inhibition of mTOR increases the osteoblastic potential of MSCs and increases mineralised matrix production while simultaneously inhibiting adipogenic differentiation, suggesting that mTORC1 inhibitors might be used therapeutically in diseases characterised by low bone mass.[1]

• To determine the effect of mTORC1 on the formation of the skeleton, we have utilised the Cre-*loxP* system to generate mice with targeted deletion of *raptor* in pre-osteoblast cells. This was achieved by crossing mice expressing the Cre recombinase, under control of the pre-osteoblast specific *Osterix* promoter (tTA:OSX:Cre), with mice harboring floxed raptor genes.

Aim of study

To determine the role of mTORC1 in skeletal development through the conditional knock out of Raptor in osteoblasts *in vivo*

Methods

• Male Raptor$^{OB-/-}$ (hom), Raptor$^{OB-/+}$ (het) and cre recombinase expressing Raptor$^{OB+/+}$ (cre) mice were analysed at 4, 8 and 12 weeks of age and growth statistics (e.g. weight, spinal length, tibial length) collected.

• Tibiae were taken from the animals and 3D trabecular micro-architecture was evaluated using micro-computed tomography (μCT; Skyscan 1174). μCT was further used to examine the tibial growth plate and 3D micro-architecture of the calvaria.

• Following μCT, tibiae were fixed in 10% formalin and embedded in methyl methacrylate. Sections (5μm) were stained using Toluidine Blue.

Raptor$^{OB-/-}$ show minor phenotypic differences compared to Cre controls

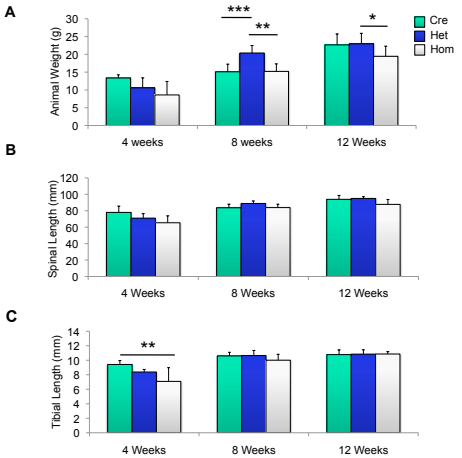

Fig 2: KO animals display no gross phenotype when compared to cre controls. A: Body weights, **B:** spinal lengths and **C:** tibial lengths of male animals at 4, 8 and 12 weeks of age. (n=8 per group) *p<0.05, **p<0.01 and ***p<0.001, ANOVA.

Raptor$^{OB-/-}$ mice have decreased tibial bone volume

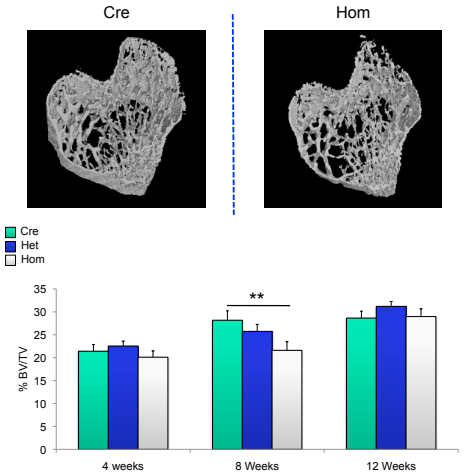

Fig 3: A significantly decreased bone volume (BV/TV) was observed at 8 weeks. 3D micro-architecture was evaluated in a length-normalised region of the proximal tibia using μCT. Percentage bone volume (BV/TV) was calculated using CTAn (SkyScan) and normalised to weight. (n=8 per group) **p<0.01, ANCOVA.

Raptor$^{OB-/-}$ mice have an altered trabecular micro-architecture

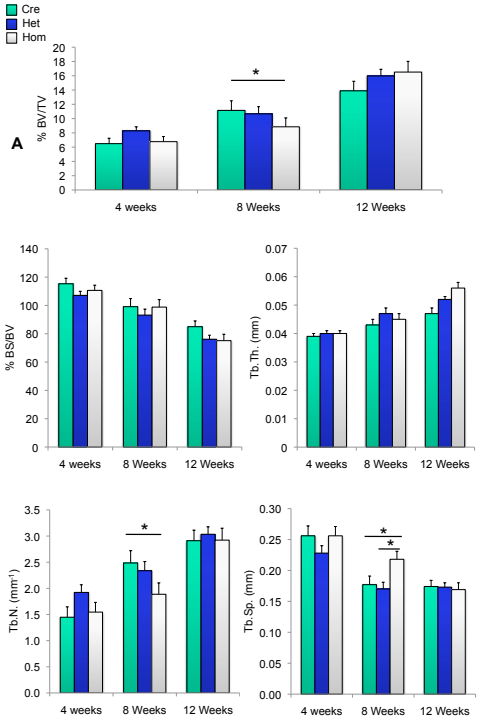

Fig 4: KO animals show a decrease in trabecular bone caused by a decrease in trabecular number at 8 weeks. 3D trabecular micro-architecture was evaluated in a length-normalised region of trabecular bone using μCT. **A:** Trabecular bone volume (BV/TV), **B:** bone surface fraction (BS/BV), **C:** trabecular thickness (Tb.Th), **D:** trabecular number (Tb.N) and **E:** trabecular spacing (Tb.Sp) were calculated using CTAn (SkyScan) and normalised to body weight. (n=8 per group) *p<0.05 ANCOVA.

Raptor$^{OB-/-}$ mice have a thinner growth plate at 4 weeks

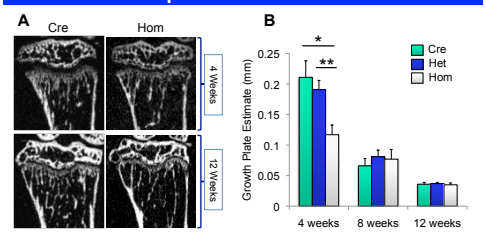

Fig 5: At 4 weeks the proximal tibial growth plate is significantly thinner in male KO animals compared with cre controls. μCT images were used to determine the width of the cartilaginous tibial growth. **A:** μCT images of the proximal tibial growth plate for cre and hom animals at 4 and 12 weeks. **B:** Average growth plate width for male animals at 4, 8 and 12 weeks normalised to tibial length. (n=8 per group) *p<0.05 and **p<0.01 ANCOVA.

Raptor$^{OB-/-}$ mice have impaired calvarial formation

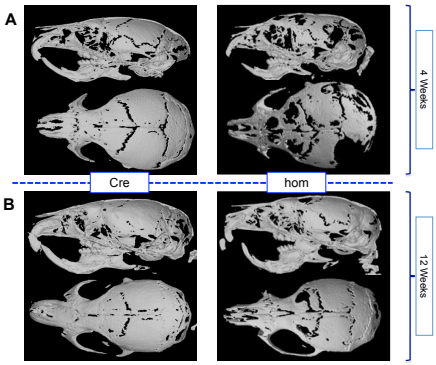

Fig 6: 3D reconstructions of calvariae reveal a decrease in mineralisation of the calvarial plates and impaired suture fusion in KO animals compared with cre controls at 4 and 12 weeks. 3D reconstructions were created using ANT (SkyScan). Representative images of cre and hom animals at **A:** 4 weeks and **B:** 12 weeks.

Raptor$^{OB-/-}$ mice have increased intramedullary adiposity

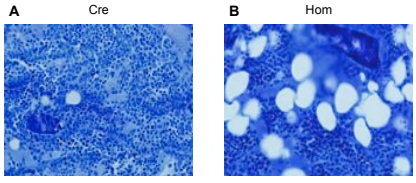

Fig 7: Histological analyses reveal increased intramedullary adiposity in KO animals compared with cre controls. Tibiae were embedded in plastic, sectioned (5μm) and stained with Toluidine Blue. Representative images indicating bone and marrow space in blue and adipocytes in white (20x) of **A:** cre and **B:** hom animals at 4 weeks.

Conclusions

• uCT analysis reveals a decrease in tibial and calvarial bone in KO animals suggesting a role for mTORC1 in endochondral and intramembranous ossification.

• Growth plate analyses suggest OB-specific raptor KO interferes with endochondral ossification in early skeletal development.

• Histological analyses reveal an increase in intramedullary adiposity in KO animals, suggesting that OB-specific raptor KO promotes the the production of adipocytes.

References

1. MARTIN, S. K. et al. (2010). *Journal of Bone and Mineral Research, 25, 2126-2137.*