What role do mechano-modulatory pathways play in embryonic bone formation, and can these pathways be targeted for therapeutic treatment of osteoarthritis?

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Osteoarthritis is a chronic, degenerative disease of which the cause and progression are not completely understood. Even though it is the most common cause of pain and disability in adults, there are currently no therapeutic treatments, and the only options are to manage the pain, loss of function, and limit further progression of the disease [1].

Osteoarthritis progression involves a return to aspects of embryonic bone formation. During foetal development, bones form by endochondral ossification. Within the early limb bud, cells lay down a cartilage template for bone. The cartilage is then calcified before it is used as a pattern for bone formation [2]. This is a very simplified description that belies the complexity and coordination required during endochondral ossification. When this process is inappropriately activated in the adult, it may result in osteoarthritis [3].

Mechanical cues, such as those exerted during embryo movement, play a vital role in limb skeletal development [4]. The mTOR (mammalian Target of Rapamycin) signalling pathway plays an important part in sensitisation to mechanical stimuli and regulation of endochondral ossification [5]. NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) and mTOR pathway interact with each other and exhibit necessary roles in developmental and mature physiological processes [2]. These mechano-modulatory pathways contribute to embryonic bone formation and have been implicated in pathologic processes including osteoarthritis.

A lot that remains unknown about the role of mechanical cues in limb skeletal development. If local regulators of endochondral ossification were identified, there is potential for therapeutic treatment to restrict bone formation in disease such as osteoarthritis, or to accelerate bone formation to aid fracture healing.

Focusing on known regulators of the mTOR and NF-κB pathways, this project seeks to better define the roles of mTOR, NF-κB, and mechanical stimuli in endochondral ossification. Known activators and inhibitors for each of the chosen pathways were administered to chick embryos *in-ovo*, and tibiotarsal bones were analysed by micro CT and histology.

Methods

Chick embryos were used as in-vivo models, as they are the gold standard for studying development and are easy to treat and monitor. Between development days E7 - E15, chick embryos were treated twice weekly directly to the chorioallantois with PBS (control), leucine, rapamycin, betulinic acid, or SC-514. For the mTOR pathway, leucine is an activator and rapamycin is an inhibitor. Similarly, for the NF-κB pathway betulinic acid is an activator and SC-514 is an inhibitor. Embryos were euthanized on day E15 and both entire hind limbs were collected for micro computed tomography (micro CT) imaging and histology.

Micro CT scans were taken using a Skyscan 1172 micro-CT System (Figure 1). 3D scans were reconstructed using NRecon and analysed using CTan software. 2D and 3D statistical analysis was performed using GraphPad Prism 7. D'Agostino-Pearson normality test, one-

way ANOVA, and two variable comparison t-tests were used. P values lower than 0.05 were considered statistically significant.

Histology samples were processed and stained according to Haemotoxylin and Eosin staining protocol (Figure 2). No analysis of histology samples was performed during the duration of this project.

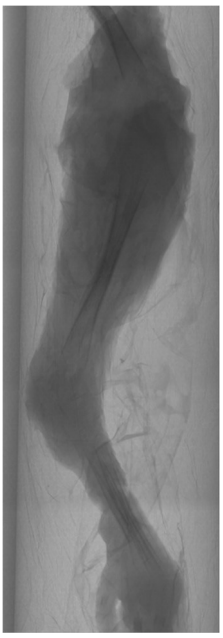

Figure 1: Micro CT scan of a chick embryo E15 hind limb

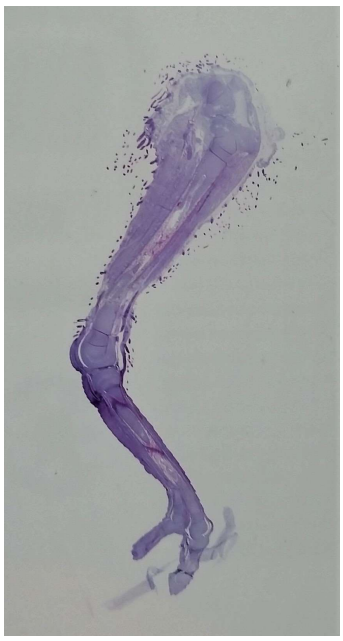

Figure 2: H&E stained section of chick embryo E15 hind limb

Results and Conclusions

Given the short duration of this project, it is difficult to assert any definitive findings. Of the pharmacological regulators used, leucine appears to have the most dramatic and consistent effect on the development of the chick tibiotarsal bones. Treatment with leucine, an mTOR activator, resulted in a decrease in tibiotarsal length, tissue volume, and bone volume. A decrease in percentage bone volume to tissue volume reveals decreased relative bone volume as well as total bone volume. The number and size of trabeculae was the same as the control, though trabecular separation decreased with leucine treatment. This implies that though the tibiotarsal is smaller and has relatively less bone volume, the bone is denser and possibly better consolidated.

In activating mTOR, leucine may allow the embryonic bone to form in a way that is more mechanically sound at the cost of increased length. This is an exciting finding that complements the knowledge that fast growth results in reduced consolidation of cortical bone and therefore reduced response to mechanical loading [6].

Further study will explore the effect of leucine on bone formation, and the role mechanical input has in this complex coordination of development. Then we will look to the use of leucine as therapeutic treatment for bone pathologies in adults.

Working on this project was a unique opportunity for me to immerse myself in a research environment and practice techniques and protocols I would not otherwise experience. The studentship was made possible by the symposium to celebrate the career of Professor AR Michell. I am grateful to Soraia Silva and Professor Andrew Pitsillides, without whom I would not have this opportunity. Thank you.

References

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