UCONN HEALTH

Cdc73/Parafibromin in Mesenchymal Progenitors and Tumorigenesis

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Introduction

- Hyperparathyroidism-Jaw Tumor Syndrome (HPT-JT) is a tumor predisposition disorder where patients develop multiple parathyroid tumors, ossifying and/or cementifying fibromas of the jaws, and other tumor types

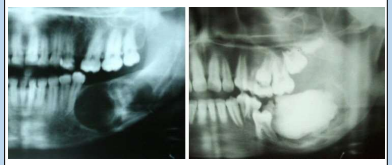

Figure 1: Ossifying fibroma in the jaw

- An ossifying fibroma is a mixed cell type tumor, with both a fibrous component and a mineralized component resembling bone and/or cementum
- Previous data showed that the cell of origin may be a mesenchymal progenitor cell capable of differentiation into both bone and cementum-producing cells
- Loss of the *CDC73* gene is seen in patients with HPT-JT
- *CDC73* is a tumor suppressor gene that encodes for parafibromin

Aim

Examine the role of *Cdc73*/parafibromin in mesenchymal progenitor cell cultures

Methods

Generation of Cell Cultures

- Mesenchymal stem cell (MSC) cultures will be made from femurs extracted from *Cdc73*-floxed/Ai9 mice
- Tumorigenesis after *Cdc73*/parafibromin loss affects the craniofacial skeleton differently than the appendicular skeleton, so functions of parafibromin may also differ between the craniofacial and appendicular skeleton
- Mandibles and hind limbs are dissected out and overlying tissue is removed
- The ends of the bone are cut off to access the bone marrow
- Marrow containing the MSC's are flushed out, filtered and collected

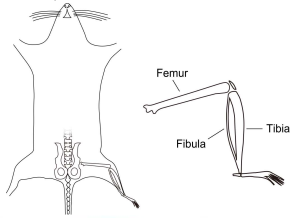

Figure 2: Diagram of femur bone extraction from mice

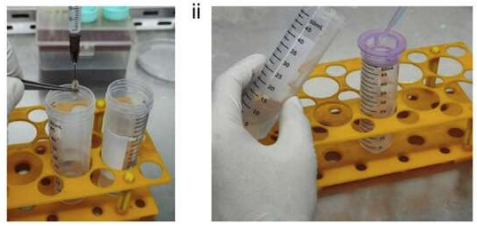

Figure 3: Bone marrow is flushed out to access MSC's

- Cells are incubated and non-adherent (non MSC, such as hematopoietic cells or dead cells) are removed through media change

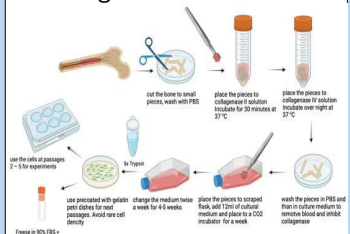

Figure 4: MSC's are plated, media is changed to remove non-adherent cells, and cells are split to avoid over-confluency

Results

- MSC's were successfully extracted from femur bones
- MSC's grew in cell cultures and proliferated
- RNA was extracted from one set of cultures

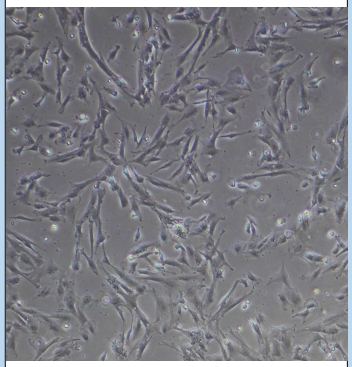

Figure 5: MSC's proliferating in cell culture

Future Directions

- Cells will be infected with an adenovirus containing Cre-recombinase which will remove the loxP sites from the DNA, effectively removing *Cdc73*
- The Ai9 mice that we use have 2 loxP sites surrounding *Cdc73* that Cre-recombinase cuts out to generate the *Cdc73*-null cultures
- Recombination is confirmed by fluorescence then confirmed again by PCR

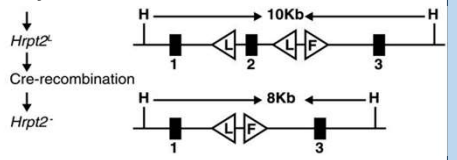

Figure 6: Deletion of Cdc73: Cdc73-floxed mice already contain 2 loxP sites flanking exon 2 of the Cdc73 gene, delivery of Cre-recombinase will catalyze deletion of exon 2

- Total RNA will be extracted and transcriptome analysis performed
- Changes in gene expression between *Cdc73*-replete and *Cdc73*-null cultures will be represented through quantitative rt-PCR

Acknowledgements

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References

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