DIFFERENTIATION OF IPS CELLS INTO FIBROBLASTS TO VALIDATE GENE TARGETS FOR DUPUYTREN'S CONTRACTURE

AUTHORS

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All the patients that have contributed to our research.

1. DUPUYTREN'S CONTRACTURE (DD)

- Heritable genetic fibroproliferative disorder affecting the hand
- Genetic cause not known

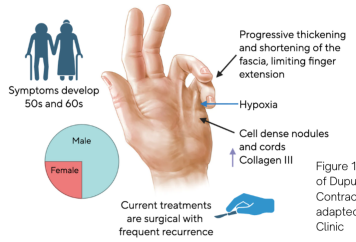


Figure 1. Infographic of Dupuytren's Contracture. Image adapted from Mayo Clinic

Whole exome sequencing (WES) on 4 members of a Western Australian family with DD was used to identify single nucleotide variants (SNV)

- 23 members of the family including non-affected individuals
- Heterozygous, non synonymous SNV in the LRSAM1 gene of chromosome 9 (GRCh37(chr9):g.130265116C>T)
- Cytosine (C) to thymine (T) transition causing an amino acid change from arginine to cysteine

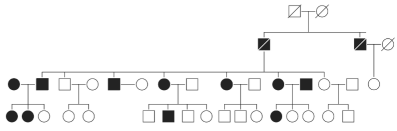


Figure 2. Pedigree of a Western Australian family with autosomal dominant inheritance of Dupuytren's contracture.

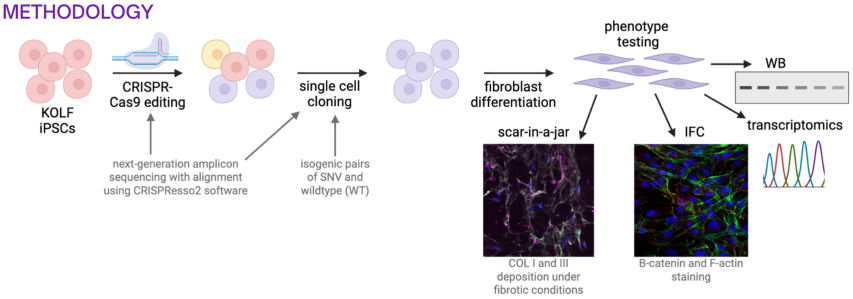
2. HYPOTHESIS

The SNV in LRSAM1 seen in a Western Australian family is a causative SNV for Dupuytren's contracture in this family.

3. PROJECT AIMS

1. Use single cell cloning to achieve a clonal population of isogenic iPS cells containing the WT and DD SNV
2. Differentiate isogenic iPS cells into fibroblasts
3. Phenotypic testing of differentiated cells

4. METHODOLOGY



5. SINGLE CELL CLONING RESULTS

WT iPSCs were selected at 0% HDR and >97% WT

SNV iPSCs were selected at ~50% HDR and ~50% WT or WT with silent mutation

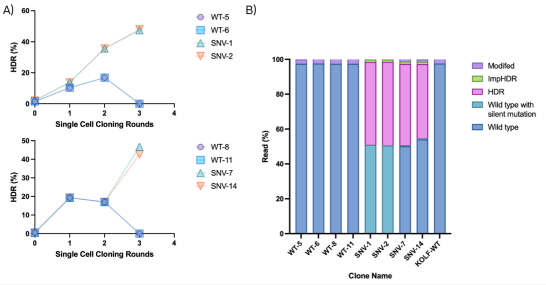
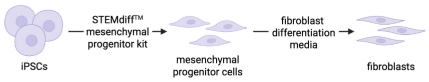
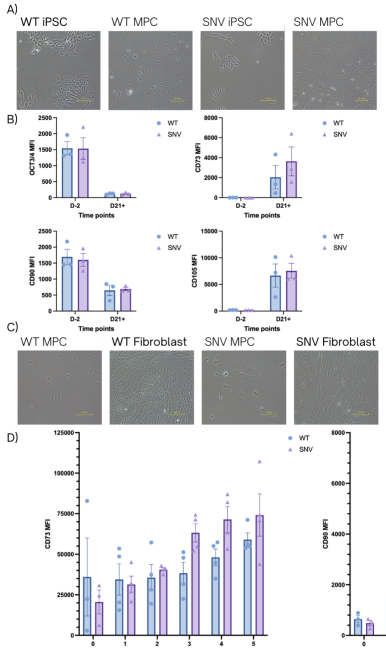


Figure 3. Single cell cloning results of CRISPR edited iPSCs. A), HDR percentage over rounds of single cell cloning. B) Read percentage of isogenic pairs after cloning.

6. FIBROBLAST DIFFERENTIATION RESULTS





Validation of differentiations through marker expression

	iPSC	MPC	FIB
OCT3/4	↑↑	↓	-
CD73	↓	↑	↑↑
CD90	↑↑	↑	↑↑
CD105	↓	↑↑	-

Figure 4. iPSCS were differentiated into MPCs then fibroblast like cells and confirmed by flow cytometry. A) Brightfield images of MPC differentiation. B) OCT3/4 and CD90 MFI were used as a iPSC markers, while CD73 and CD105 MFI were used as MPC markers. C) Brightfield images off fibroblast differentiation. D) CD73 and CD90 MFI marker expression over the duration of the fibroblast differentiation up to 100 days. MFI calculated from total live cells minus the unstained MFI.

7. WT AND SNV PHENOTYPE TESTING RESULTS

Fibroblast differentiated WT and SNV phenotypes were tested through SIAJ and transcriptomics.

- SIAJ results
  - Collagen III area
    WT ↓ SNV ↑
  - Collagen III : Collagen I
    WT ↓ SNV ↑
  - Nuclei count
    WT ↓ SNV ↑
- β-catenin results
  - ↑ β-catenin in SNV in WB and IFC
- Transcriptomics results
  - Separation of SNV and WT

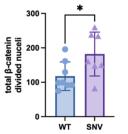


Figure 6. Total β-catenin in WT and SNV differentiated fibroblasts stained for β-catenin in IFC. Total β-catenin was divided by number of nuclei.

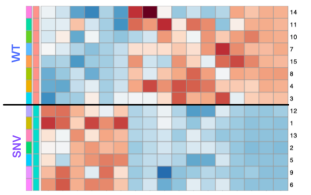


Figure 7. Heat map of WT and SNV differentiated fibroblast segregated by transcriptomes.

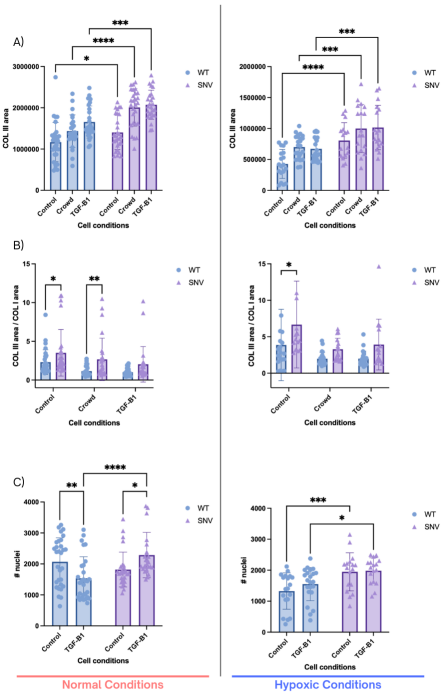


Figure 5. WT and SNV differentiated fibroblast scar-in-a-jar under normal and hypoxic conditions. WT shown in blue and SNV shown in purple. A) Total collagen III area of SNV and WT. B) Ratio of collagen III to collagen I in WT and SNV, C) Nuclei count of WT and SNV.

8. CONCLUSION

- iPSCs were successfully differentiated into MPCs as seen by marker expression and cell morphology
- A novel fibroblast differentiation method was used to differentiate MPCs into fibroblast-like cells with validation through marker expression and cell morphology
- SIAJ showed the SNV had more DD like phenotype under TGF-β1 and hypoxic conditions in comparison to WT
- Increased β-catenin in SNV compared to WT in WB and IFC
- Transcriptomics was able to separate WT and SNV
- Future work includes
  - Completed transcriptomic analysis
  - Completed IFC analysis
- Validating a causative SNV for DD may lead to possible therapeutic drug targets for a disease with common reacurrence

Diagrams made with Biorender