ChondroTargets: Determining Regulators of Chondrogenesis

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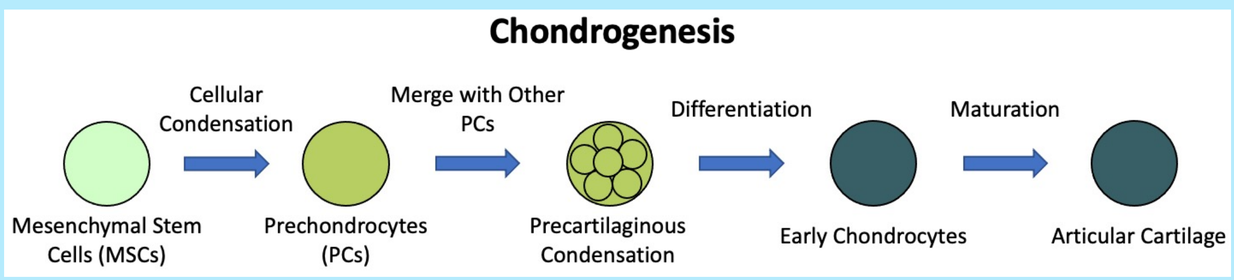
Background

Cartilage is a smooth elastic tissue that is important for long bone formation and joint function. Chondrogenesis is a series of complex procedures that starts from mesenchymal stem cells and leads to the formation of cartilage.[1]
Many genes are associated with chondrogenesis, such as *SOX9*, *COL2A1*, and *ACAN*. SOX9 is the key transcription factor of chondrogenesis, whereas *COL2A1* and *ACAN* are fundamental matrix genes.[2] However, there are still many genes that have not been researched currently. Here, bioinformatically predicted novel regulators of chondrogenesis are investigated by examining gene expression following gene knock–down

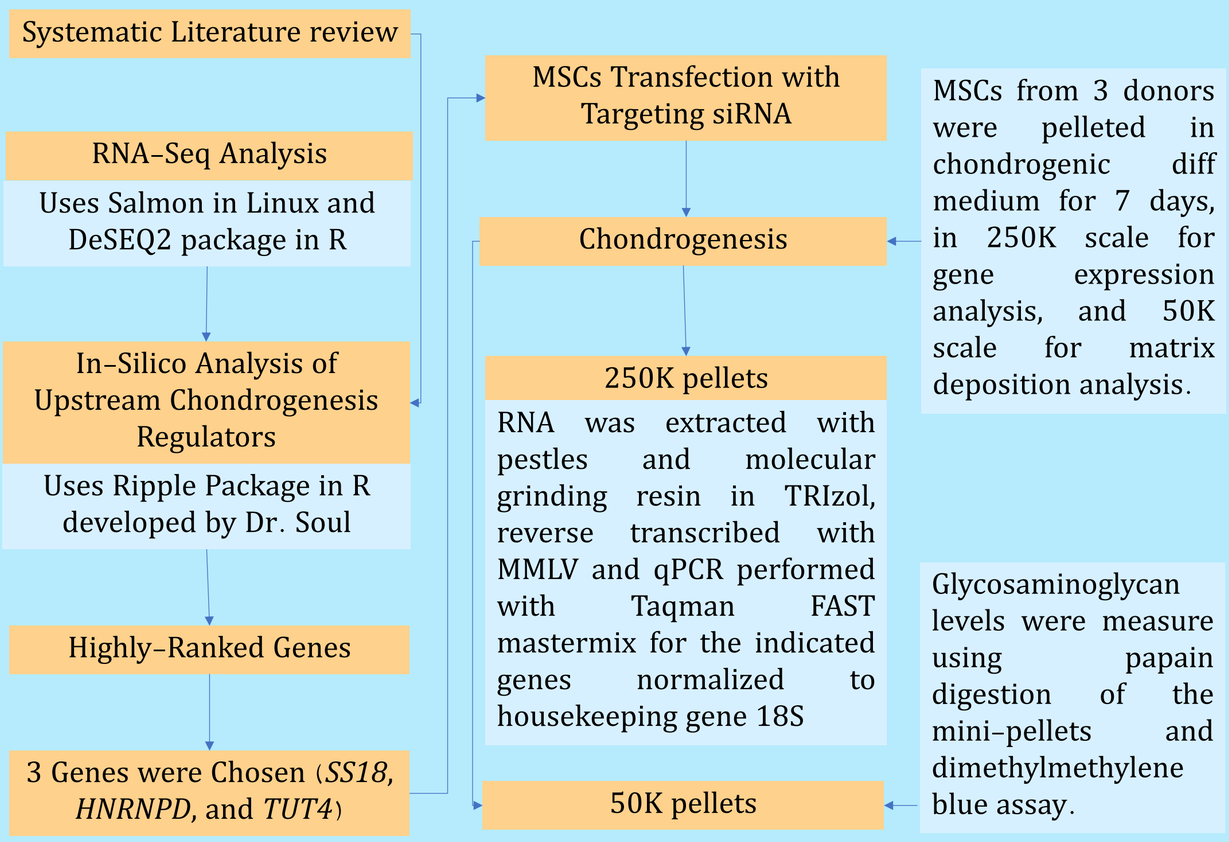
Chondrogenesis



Aims

To identify novel regulators of chondrogenesis and demonstrate their role in chondrogenesis.

Methods



Results and Discussion

- A systematic literature review of PubMed was performed using selected search terms related to chondrogenesis and gene perturbations (e.g. RNAi).
- Around 900 publications containing genes that regulate chondrogenesis after perturbation were collected and serial checked by several individuals.
- 391 unique genes associated with chondrogenesis were included in a ChondroTargets Database and used in *in–silico* analysis.

Table 1 – *In–silico* analysis of upstream chondrogenesis regulators

Gene Name	Perturbation Type	Sign	Rank
USP34	Knock Down	–1	1
YAP1	Overexpress	–1	2
CEMP1	Overexpress	1	3
SS18	**Knock Out**	**1**	**4**
SOX9	Knock Down	1	5
LATS1	Knock Down	–1	6
KDM1A	Knock Down	–1	7
SIRT1	Knock Out	1	8
P2RY2	Knock Down	–1	9
MECOM	Knock Down	–1	10
CYR61	Overexpress	–1	11
EZH2	Knock Down	–1	12
TUT4	**Knock Down**	**–1**	**13**
SOX10	Knock Down	–1	14
HNRNPD	**Knock Down**	**1**	**15**
PAEP	Knock Down	–1	16
AATF	Knock Down	–1	17
POU5F1	Overexpress	1	18
HNRNPD	Overexpress	1	19
SLC4A11	Knock Down	–1	20

An RNA–seq and *in–silico* analysis experiment using MSC–chondrogenesis data (day 0 – day 7) revealed twenty genes that when perturbed (in publicly available data) gave a gene expression signature that matched that occurring during chondrogenesis.

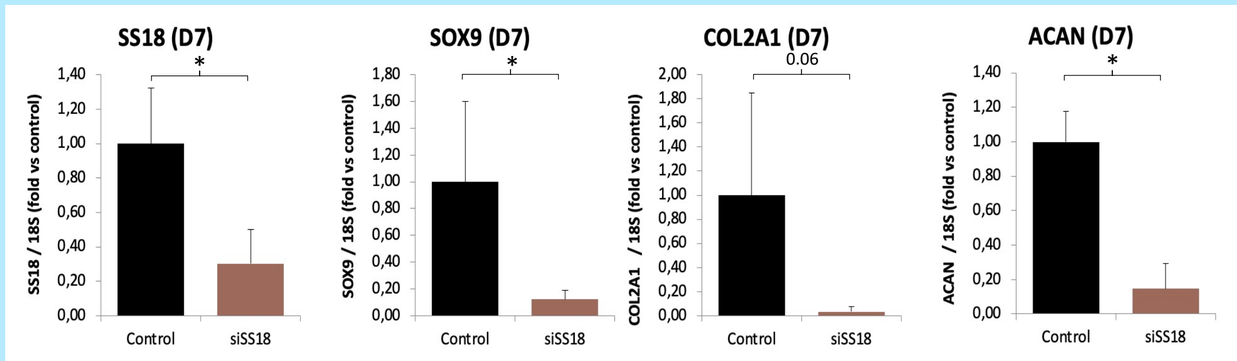
We hypothesised that these genes are regulators of chondrogenesis.

Three genes (***SS18***, ***HNRNPD***, and ***TUT4***) were selected based on several criteria including expression level and novelty. *SS18* works with RNA–binding proteins as a transcription coactivator. *HNRNPD* is involved in homologous recombination–mediated repair. *TUT4* regulates miRNA strand selection.

Future Work

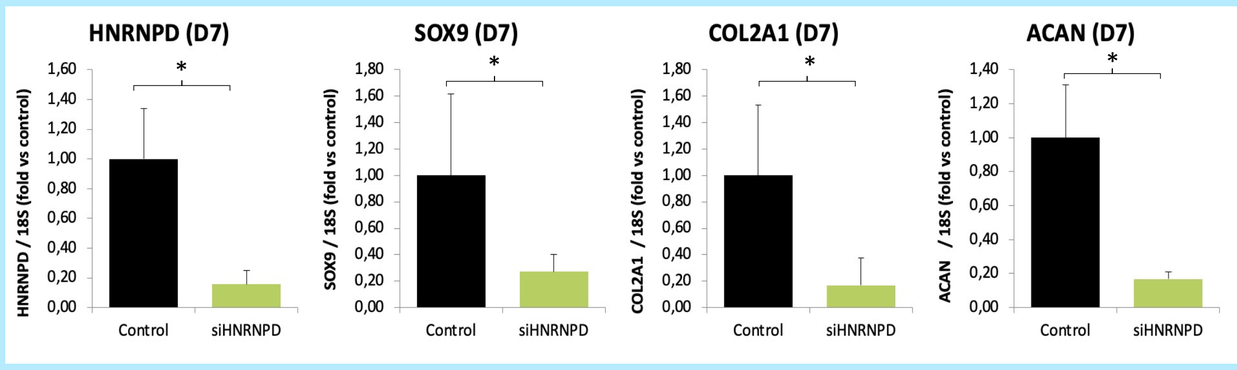
SS18, *HNRNPD*, and *TUT4* are genes that contribute to RNA stability. Our data support the hypothesis that RNA stability regulates chondrogenesis. Discovery of the pathway used by these genes to regulate chondrogenesis might open–up new association between RNA stability and chondrogenesis. More samples and timepoints are also recommended for better results.

Fig. 1 – *SS18*–knock–down cells display lower levels of chondrogenic markers after seven days of chondrogenesis



During day 7 of MSC–chondrogenesis gene expression analysis (*SS18*, *SOX9*, *COL2A1*, and *ACAN*) revealed significant suppression of *SOX9* and *ACAN* ($p < 0.05$) and not very significant suppression towards *COL2A1* ($p = 0.06$) following SS18 depletion.

Fig. 2 – *HNRNPD*–KD cells display suppression of chondrogenic markers after seven days of chondrogenesis



Similar to the experiment using *SS18*–KD MSCs, significant suppression of *SOX9*, *COL2A1*, and *ACAN* ($p < 0.05$) can be clearly seen in HNRNPD–KD cells compared to the WT control. The depletion of all three chondrogenic markers suggest that chondrogenesis might be suppressed in both *SS18*–KD MSCs and *HNRNPD*–KD MSCs. This result implied that *SS18* and *HNRNPD* might be critical upregulators of chondrogenesis.

Fig. 3 –Glycosaminoglycan (GAG) assay analysis on HNRNPD–KD cells display suppressions of cartilage formation after seven days of chondrogenesis

After seven days of chondrogenesis, *HNRNPD*–KD cells revealed suppression of cartilage formation compared to WT controls suggesting that suppression of *HNRNPD* might hinder chondrogenesis. GAG assay analysis was only done on *HNRNPD*–KD because cartilage does not form on both the control and experimental sample of both *SS18*–KD and *TUT4*–KD. Repetition of the experiment might be needed to be done to obtain the GAG assay analysis result from the remaining genes.

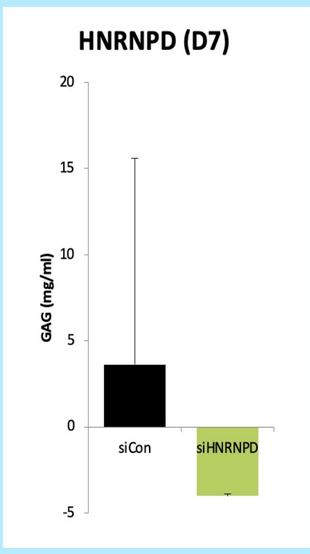
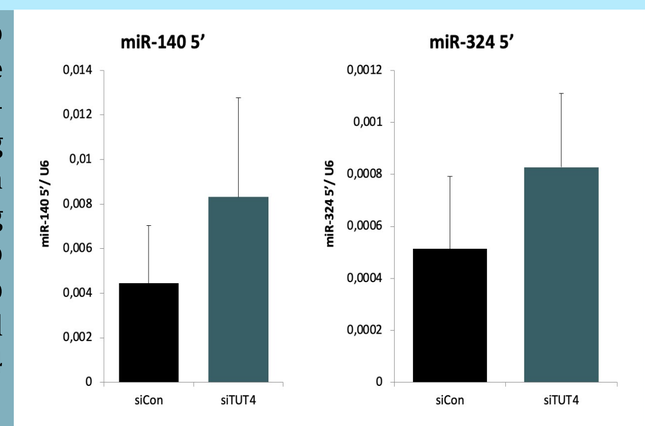


Fig. 4 – *TUT4*–KD SW1353 cells display higher levels of miR–140–5p and miR–324–5p after transfection

Due to unexpected use of siTUT4 leading to cell death, we use SW cells to measure the expression of miR–140–5p and miR–324–5p because TUT4 is known for regulating miRNA strand selection. In addition, both the microRNAs are known for regulating skeletal development. qPCR was used to examine the expression of miR–140–5p and miR–324–5p. *TUT4*–KD cells revealed mild increase of mir–140 and mir–324 expression compared to WT controls.



Conclusion

- In silico analysis of RNA seq data indicated that *SS18*, *HNRNPD*, and *TUT4* as novel regulators of chondrogenic gene expression.
- Depletion of *SS18* and *HNRNPD* suppressed chondrogenic gene expression suggesting that these genes might contribute and promote differentiation of MSCs into cartilage.
- Depletion of *TUT4* enhanced the expression of miR–140–5p and miR–324–5p indicating TUT4 might regulate miRNA strand (5p vs. 3p) selection.

References

1. Goldring MB. Chondrogenesis, chondrocyte differentiation, and articular cartilage metabolism in health and osteoarthritis. Ther Adv Musculoskeletal Dis. 2012 Aug;4(4):269–85.
2. Yi SW, Kim HJ, Shin H, Lee JS, Park JS, Park KH. Gene expression profiling of chondrogenic differentiation by dexamethasone–conjugated polyethyleneimine with SOX trio genes in stem cells. Stem Cell Research & Therapy. 2018 Aug; 9(1):1–13.