Gene expression analysis of the *Dlk1-Dio3* non-coding RNA locus in C2C12 cells

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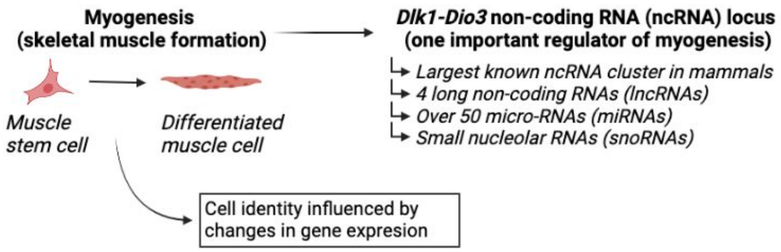
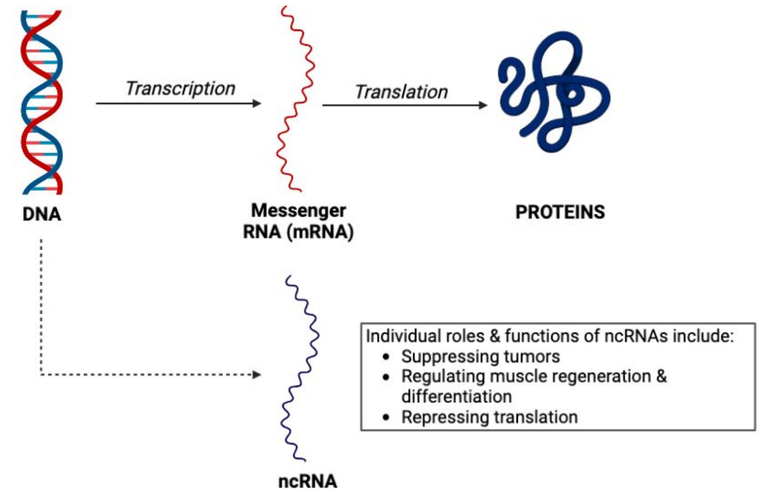
Introduction

- Skeletal muscle is crucial to one's long-term health and quality of life.



- ncRNAs are produced during transcription (RNA synthesis) but are **not translated into proteins**.



- Individual roles of *Dlk1-Dio3* ncRNAs have been studied.
- **Collective role** of the *Dlk1-Dio3* ncRNA cluster remains poorly understood.
- To better **understand the effects of a promoter deletion** on expression of the *Dlk1-Dio3* ncRNAs, we aim to explore **changes in gene expression of the *Dlk1-Dio3* ncRNAs** at multiple time points between **control and mutant C2C12 cells**.

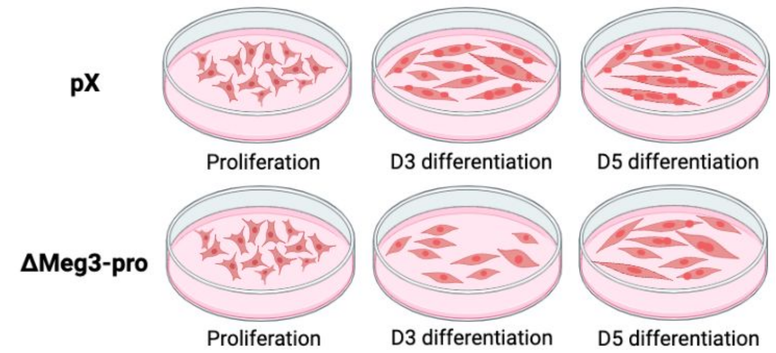
Methods

Control versus CRISPR-Cas9 mutant

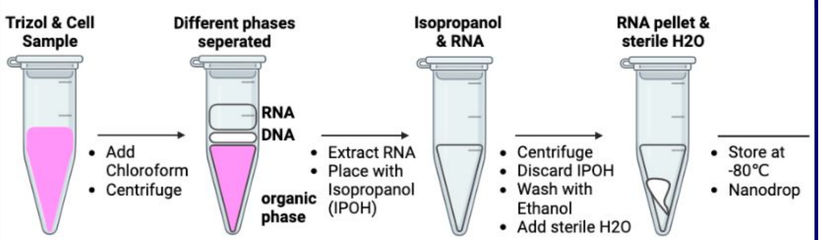
- Control C2C12 cells (**pX**): C2C12 cells transfected with pX458 pSpCas9 expression plasmid (circular DNA) without guide RNAs (no mutation occurred).
- CRISPR-Cas9 mutant C2C12 cell line (**ΔMeg3-pro**): 302 base pairs CRISPR-Cas9 induced deletion of the *Meg3* proximal promoter in C2C12 cells.

pX & 2-4 Cell Culture

- Triplicates of each of these six groups (n=3), were prepared in 6-cm diameter cell plates as follows.



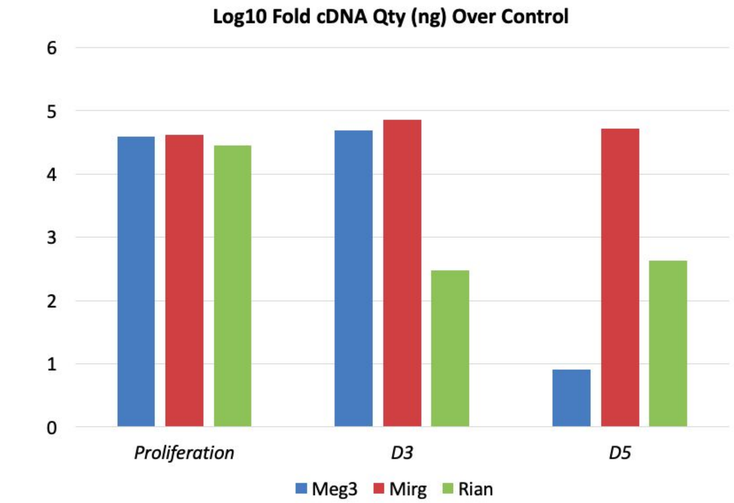
RNA extraction & cDNA amplification



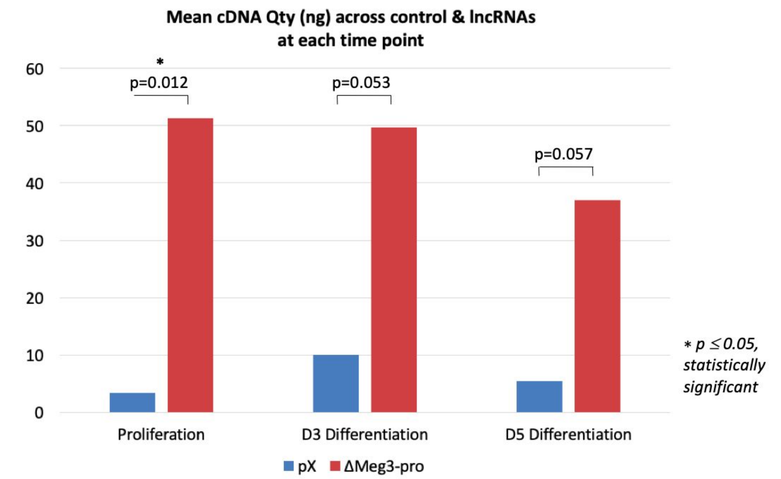
- Synthesis of complementary DNA (cDNA) from RNA.
- qPCR measured the quantity of cDNAs in both cell types.
- t-test performed to compare mean cDNA quantity amplified between control and mutant cells.

Results & Discussions

- **pX:** low lncRNA expression at all time points.
- **ΔMeg3-pro**: increased and sustained upregulation of lncRNA expression at all time points.



- Two sample t-test results showed that cDNA Qty difference is statistically significant at proliferation but not at differentiation.



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Conclusion

Deletion of sequences in the promoter region of the *Dlk1-Dio3* ncRNA locus causes an **increased and sustained overexpression of all the lncRNAs on the locus**. Collectively, this in turn **affects cell growth and differentiation**.