Understanding New Tumor Suppressors in Cancer

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Introduction

Rhabdomyosarcoma (RMS) is classified into two major subtypes including the embryonal subtype (ERMS), which is the most common form of the disease and the alveolar subtype (ARMS), which is the more metastatic and aggressive subtype. Embryonal subtype usually affects children in their first 5 years of life, but it can occur at older ages as well. ERMS tends to occur in the head and neck area, the reproductive areas, and the urinary system. Alveolar rhabdomyosarcoma typically affects all age groups equally. It makes up a larger portion of RMS in older children, teens, and adults than in younger children. ("What is Rhabdomyosarcoma?",) *Mus musculus* genetic engineering advancement has enabled scientists to explore genetic mutations at a genomic level.

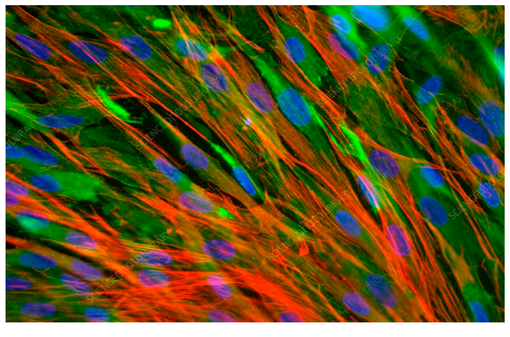
C2C12 cells are myoblast cells from the mouse cell line *Mus musculus.* HEK293 cells are Human Embryonal Kidney cells that are used

Methodology

Polymerase Chain Reaction (PCR)
Gel electrophoresis
Genotyping
Fluorescence microscopy

Genotyping using PCR and Electrophoresis: Polymerase Chain Reaction is a technique that takes a small sample of DNA and amplifies it. This is one of the methods used in this research to determine if mouse models contain the wild type allele or have been successfully knocked out also referred to as DKO mice.

Fluroscence microscopy uses several different dyes, some inserted into cells and some produced by a computer program, such as DAPI, GFP, and FITC.



Results

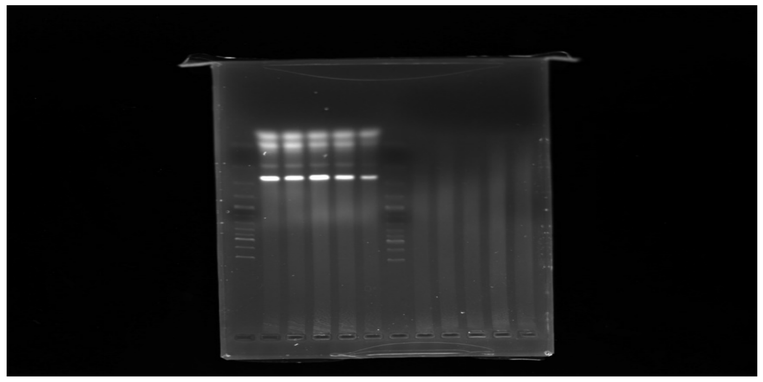


Figure 1. Results of genotyping: Lanes 2 through 6 contain primers encoding for the DNA extracted from cells. Lanes 8 through 12 contain primers encoding for the genotype of a specific sample. DNA amplification was nearly perfect as can be seen by distinct bands. The lanes encoding for genotype weren't as promising as the DNA seeing as no bands or even a streak of where the sample amplification was stopped is not viewable.

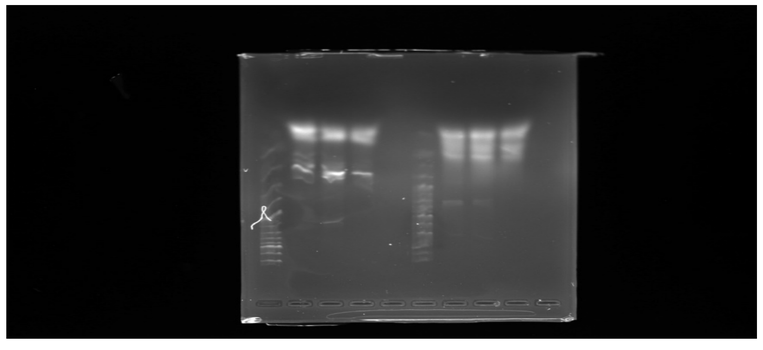


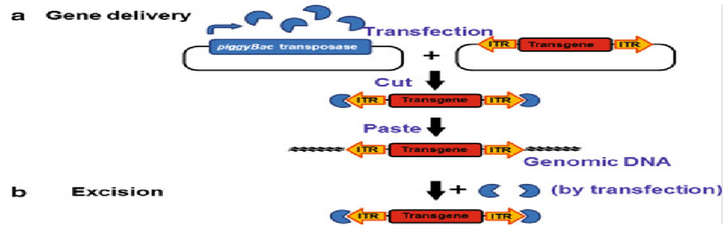
Figure 2. Results of expression between C2C12 cells and HEK293 cells: Lanes 2 and 7 contain primers that encode as a control for comparison to use. Lane 3 contains C2C12 cells at 0 days of differentiation and lane 8 contains C2C12 at 6 days of differentiation. Lanes 4,5,9, and 10 contain HEK293 cells. Lanes 4 and 9 contain cells with 0 days of differentiation and 5 and 10 contain 6 day differentiation cells.

Conclusion

Rhabdomyosarcoma's (RMS) are rare forms of cancers that are known to affect the major muscle groups of the body. Researchers use *Mus musculus* as a model to conduct experiments dealing with the mechanisms and genes that affect Rhabdomyosarcoma. Genetic engineering of mice allows us to generate "knockout mice" which entails that a gene has either been deleted or inactivated. Amplification using polymerase chain reaction (PCR) allows DNA to be multiplied and viewed through the use of gel electrophoresis. This technique, as shown in **Figure 1.**, amplifies the brightness displayed in the samples, substantially, to view and determine if samples are displaying the genotype of a specific mouse and if amplification has worked properly. **Figure 2.,** shows the PCR results between the expressions between C2C12 cells (mouse) and HEK293 cells (human) in comparison to a control group.

Future Directions

- Ensure ways to grow the inducible cells containing the tumor suppressor gene of interest.
- Perform the PiggyBac system to enhance levels of expression inside of mammalian cells. To begin a section to be replaced with by the gene of interest must be removed. This process is done by a transposase which helps in transportation and implementation of the new, desired DNA segment into the bacterial plasmid. ("Gene Delivery and Expression)



References

1. "What Is Rhabdomyosarcoma?" *American Cancer Society*, https://www.cancer.org/cancer/rhabdomyosarcoma/about/what-is-rhabdomyosarcoma.html.
2. "Gene Delivery and Expression Systems in Induced Pluripotent Stem Cells" *ResearchGate*, https://www.researchgate.net/publication/311556058_Gene_Delivery_and_Expression_Systems_in_Induced_Pluripotent_Stem_Cells.

Acknowledgements

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