Effects of p27 Gene Knockout on Skeletal Muscle Development and Post Injury Repair

Alex Hron, Norio Motohashi, and Atsushi Asakura

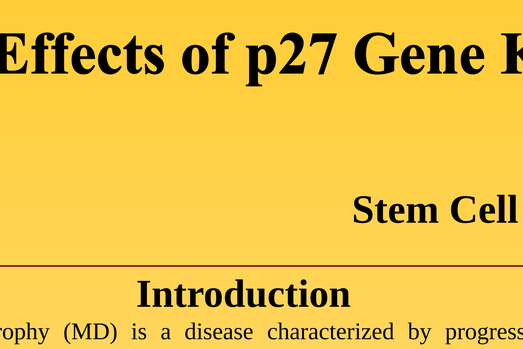
Stem Cell Institute, Department of Neurology, University of Minnesota Medical School

Introduction

Muscular Dystrophy (MD) is a disease characterized by progressive skeletal muscle wasting and degeneration, combined with decreased regeneration capabilities. A possible approach to treating MD, and other diseases which share these characteristics, is to restore muscle repair pathways. Cyclin Dependent Kinases (CDKs) effectively propel the cell through, and are temporally expressed, throughout the cell cycle. CDK inhibitors, such as p27, oppose the role of CDKs, and inhibit cell cycle progression. Through the down regulation or inhibition of CDK inhibitors, it may be possible to partially or fully restore the muscle repair pathway. Results from this research may lead to an improvement in regeneration capabilities in skeletal muscle, paving the way to more effective future treatments for MD and other diseases in which muscle regeneration is impaired.

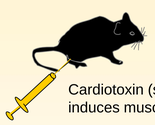
p27 Cell Cycle Regulation

Cyclins are small proteins that bind CDKs and act to drive the cell cycle forward. Cyclins and CDKs are present in varying concentrations throughout the cell cycle depending on the phase the cell is in. CDK inhibitors, such as p27, bind to either the CDK or the CDK/Cyclin complex, and inhibit the CDKs catalytic functions, resulting in the inability to phosphorylate the Retinoblastoma (Rb) protein, resulting in cell cycle arrest due to the inability of the E2A protein to become activated.

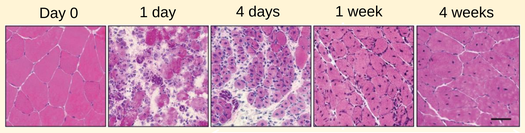


Skeletal Muscle is highly Regenerative

Cardiotoxin (CTX) is a venom from the cobra snake, which has been shown to cause necrosis of skeletal muscle after injection into tibialis anterior (TA) muscle. However, 4 days after CTX injection, muscle fiber regeneration is already observed. In 4 weeks, muscle is completely healed, partially due to the presence of satellite cells, a skeletal muscle stem cell population that provides myogenic cells to contribute to muscle fiber regeneration.

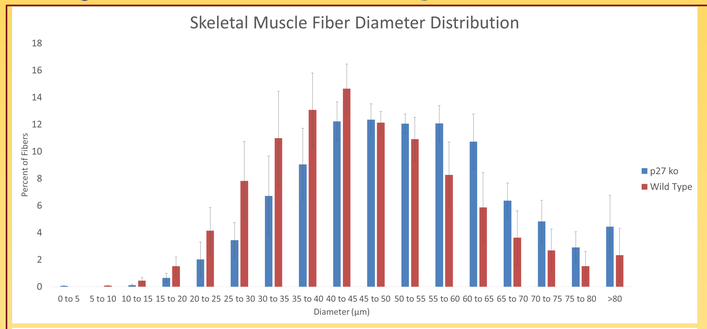


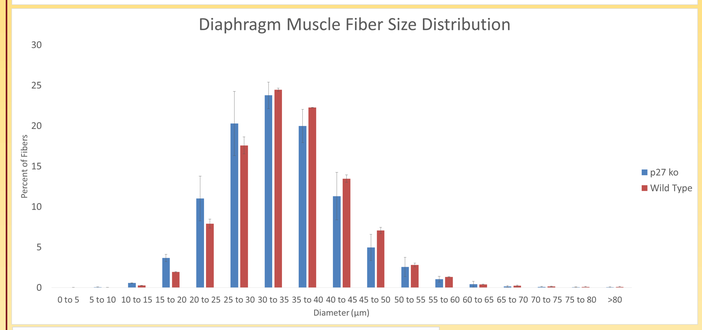
Cardiotoxin (snake venom) induces muscle injury

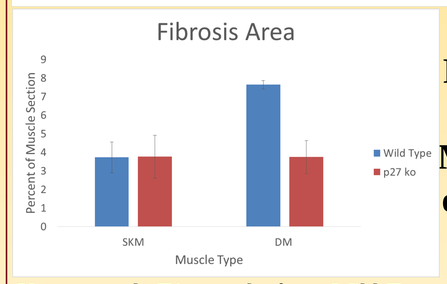


Does p27 Regulate Skeletal Muscle Development and Regeneration?

Wild-Type (WT) mice vs **p27 gene knockout (*p27*$^{-/-}$) mice**





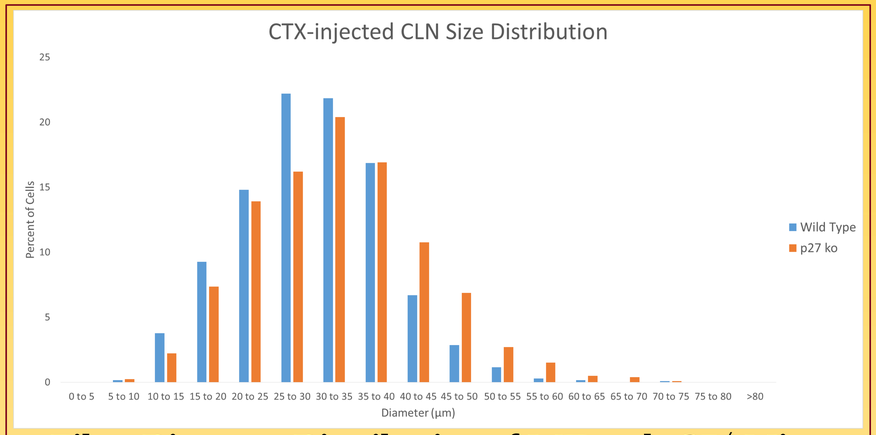


Fiber Size Distribution and Fibrosis in TA Muscle and Diaphragm of Wild-Type and *p27*$^{-/-}$ Mice

Upper panel: TA muscle from Wild-Type (WT) mice (n=3) and *p27*$^{-/-}$ (*p27* knockout) mice (n=3) was sectioned and stained with Hematoxylin and Eosin. Fiber diameters were manually measured using ImageJ. *p27*$^{-/-}$ mice display bigger fibers in TA muscle. Error bars represent SEM.

Middle panel: Diaphragm WT mice (n=2) and *p27*$^{-/-}$ (n=2) were analyzed in the same manner. *p27*$^{-/-}$ mice display smaller fibers in diaphragm. Error in SEM.

Lower Panel: Diaphragm and skeletal muscle were stained with Sirius Red fibrosis stain. Although there is no difference in fibrosis between WT (n=3) and *p27*$^{-/-}$ TA muscle (n=3), fibrosis is more prominent in WT diaphragm (n=2) compared to *p27*$^{-/-}$ diaphragm (n=2). Error in SEM.



Fiber Diameter Distribution of WT and *p27*$^{-/-}$ Mice during Muscle Regeneration

WT (n=1) and *p27*$^{-/-}$ mice (n=1) were sacrificed seven days post-CTX injection into TA muscle. TA muscles were analyzed for centrally located nuclei (CLN: indicative of regenerating muscle fibers). ImageJ was used to manually measure the diameters of fibers with centrally located nuclei. The data show that *p27*$^{-/-}$ mice display larger fiber size in regenerative muscle.

Conclusions

- *p27*$^{-/-}$ mice display hypertrophic fiber phenotype when compared to WT TA muscle fibers.
- *p27*$^{-/-}$ mice display higher muscle regeneration kinetics than WT based on the regenerating muscle fiber diameter.
- *p27*$^{-/-}$ mice display smaller diaphragm muscle fibers and lower fibrosis than WT mice.
- Based on the results of preliminary experiments, there is evidence to support that *p27* plays an important role in muscle development and regeneration.

Future Directions

- *In vitro* experimentation using WT and *p27*$^{-/-}$ myoblasts should be carried out to study their cell cycle and myogenic differentiation kinetics.
- More WT and *p27*$^{-/-}$ mice should be analyzed for fiber diameter experiments.
- CTX data should be gathered at more time points (day 3 and day 14) to determine regeneration kinetics by measuring CLN+ fiber diameters.

Acknowledgements

The authors acknowledge the University of Minnesota Undergraduate Research Opportunity Program (UROP) for partly funding this research

UNIVERSITY OF MINNESOTA
Driven to Discover℠