Xyloside Restructuring of the Glycocalyx

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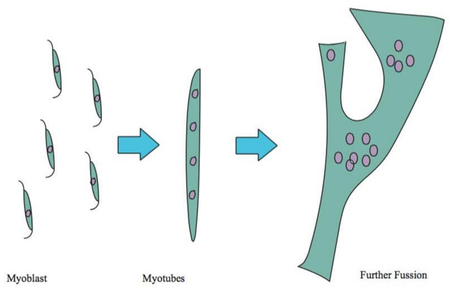
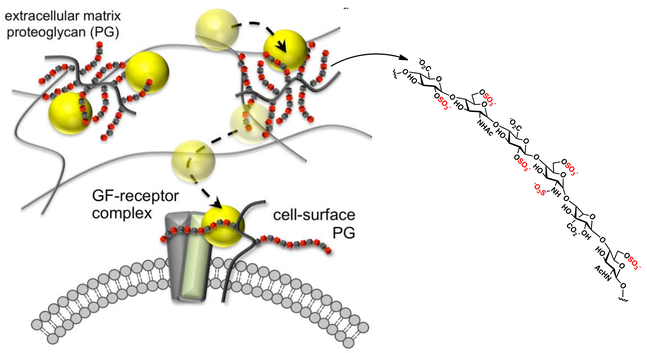
Introduction

Muscular dystrophies are genetic disorders characterized by progressive muscle wasting and weakness. They begin with microscopic changes in the muscle, including the heart and respiratory muscles, and can lead to death.[1] In order to better understand and potentially develop treatments, there has to be a fundamental understanding of the differentiation process (Fig 1).

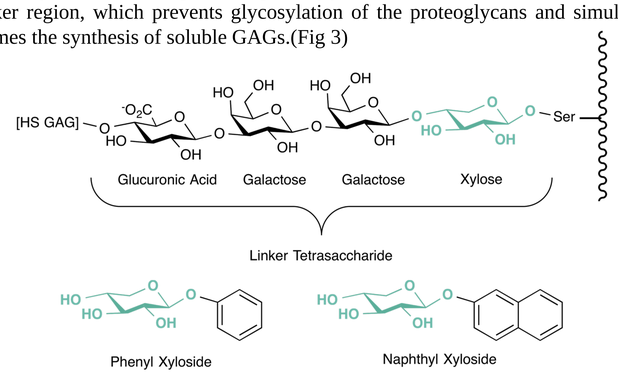


A critical factor in differentiation and fusion process are due to interactions on the cell surface. Cell surface interactions are mediated by the glycocalyx which is a network of glycosylated proteins on the cell surface.(Fig 2).

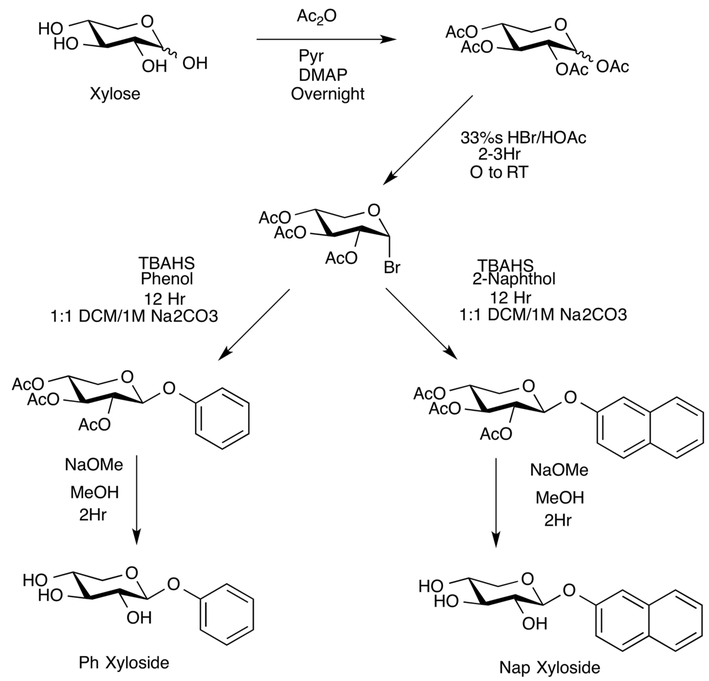


Adding small molecule inhibitors to C2C12 cells (mouse muscle progenitor cells) disrupts the glycosylation on the cell membrane, which can affect differentiation. This has been done utilizing small molecules called xylosides which have been shown to outcompete native proteoglycans for the extension of glycosaminoglycan (GAG) chains.[2] This experiment seeks to determine the effect that this change in cell surface glycosylation has on the ability of myoblasts to differentiate into myotubes.

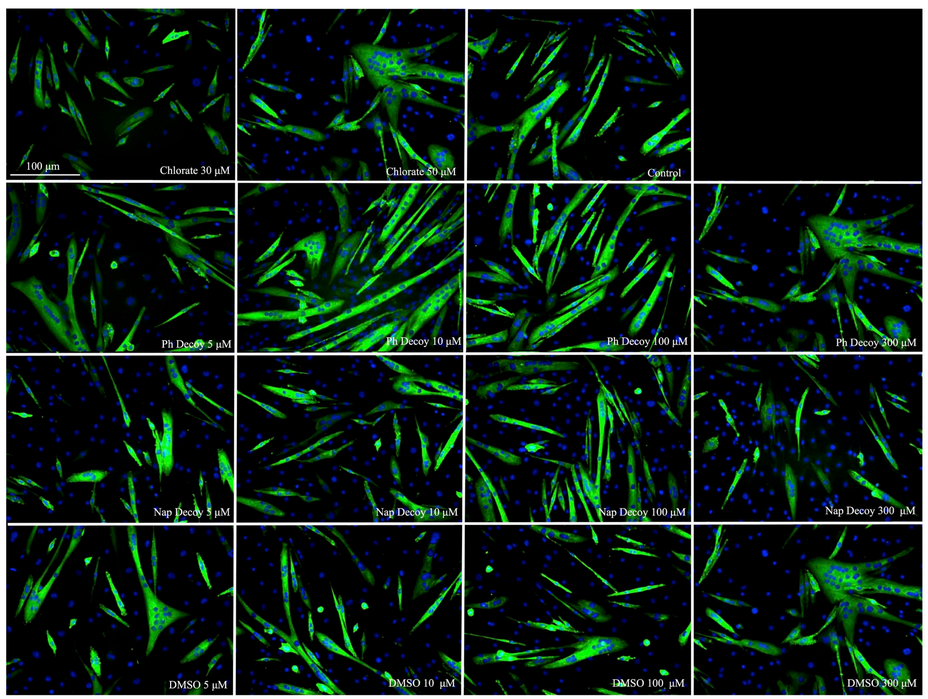
These xylosides outcompete the proteoglycans for extension of the tetrasaccharide linker region, which prevents glycosylation of the proteoglycans and simultaneously primes the synthesis of soluble GAGs.(Fig 3)



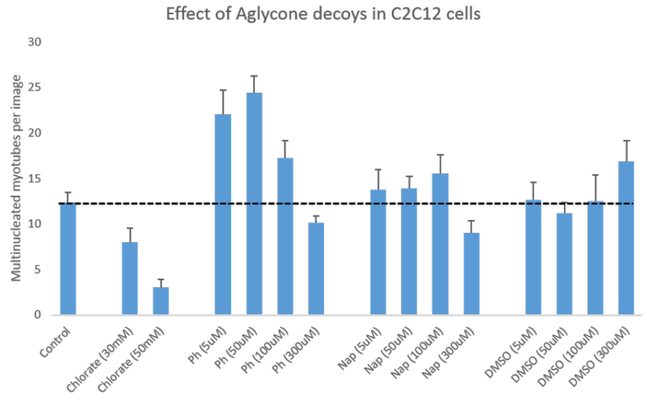
Synthesis of Ph and Nap Xylosides



Effects of Xylosides *in vitro*



Results



Conclusions

Judging by the results of the experiment, the greatest effect was seen by the Phenyl (Ph) decoy which resulted in the highest count of multinucleated myotubes. As a negative control, chlorate was shown to be concentration dependent, with a decrease in myotube formation at higher concentration. At lower concentrations, Ph increases myotube formation whereas Nap appears to have no effect. At high concentrations of both xylosides, myotube formation decreases. Though high concentrations were used, minimal cell death was observed.

Chlorate inhibition of myotube formation was expected and has been previously noted.[3] Chlorate is a reversible inhibitor of proteoglycan sulfation.

The exact mechanism of Ph xyloside promotion of differentiation has not yet been elucidated. In CHO cells Ph xyloside has been shown to not only inhibit cell surface GAG formation, but prime synthesis of soluble Chondroitin Sulfate (CS). Based on this data, we speculate that an increase in soluble CS concentration, in combination with a decrease in GAGs on the cell surface, effectively sequesters growth factors away from their receptors on the cell surface, altering signaling pathways to favor differentiation.

Further Research

Research that would build upon this experiment could be testing how different types of molecules affect cell differentiation. Xyloside decoys were used in this experiment but in future research, inhibitors, such as fluoro-xyloside[4] could be investigated.

Another useful experiment would be determining the mechanism of the Ph decoy in the differentiation process. Once the mechanism is known, future experiments could be designed with more specific inhibitors to better affect the differentiation.

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