

CD24 induced muscular regeneration: Unraveling the mystery behind satellite cell differentiation

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Introduction

Purpose — To determine CD24 expression in several stages of myogenic differentiation and if study about its effect during muscle regeneration is possible through recombinant DNA.

Hypothesis — CD24 will be expressed in all stages of myogenic differentiation will positively affect muscular regeneration rates

Muscular Dystrophy Disease

- There are 9 types of muscular dystrophy disease but many other illnesses are characterized by similar symptoms
- Muscle degeneration is the universal symptom and varies in severity between the different types

- Duchenne Muscular Dystrophy Disease is incurable and affects 1 in every 3000 male births
- The average lifespan is in the mid 20's due to respiratory illness or internal organ failure
- CD24 has been detected in lower amounts on patients with DMD
- CD24 expression on DMD patients implies that CD24 is related to muscular regeneration

CD24

- A glycoprotein on the surface of B lymphocytes
- Largely unstudied
- Higuchi and Figarella Branger focused on its expression in satellite cells
- Lower expression in DMD patients
- Previously detected on all stages of myogenic lineage 20 years ago

Satellite Cells

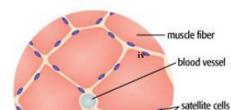
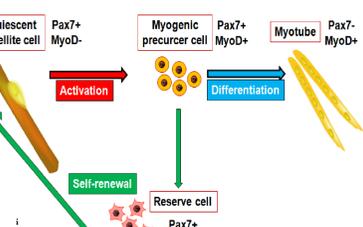
Characteristics – "The Stem Cells of Muscle"

- Express Pax7 and MyoD transcription factors
- Their role is to differentiate along a myogenic lineage to create new muscle cells to repair damaged ones
- Explored as treatment option for DMD
- Quiescent Satellite Cell – Myoblast – Myotube

Satellite cell activation, differentiation and self renewal

Location

-Found between the basal lamina and plasma membrane of muscle fibers



*Figure i-James Thompson ABSU Biology Professor
*Figure ii- http://www.lablife.org/p?a=vdb_view&id=g2.2E5odFCNA.7J04HBZ15yo2U_0

Methodology and Results

1 Quiescent Satellite Cell

- Samples-Nestin CreER iR26R-YFP mice obtained from skeletal muscles.
- Immunohistochemical staining involved primary antibodies of MyoD, CD24 and Laminin.

Results show that CD24 is not expressed in the QSC stage

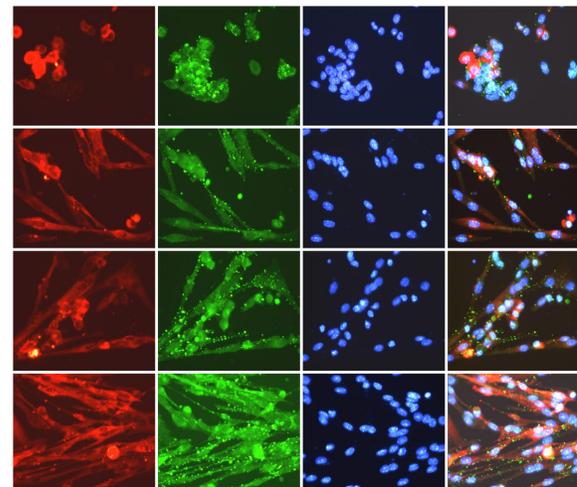
2 Myoblast

- Quiescent satellite cells were isolated with anti-CD45, CD31, Sca-1 antibodies as negative markers and CD24 and Integrin 7 as positive markers.
- Immunohistochemical staining analysis was accomplished with MyoD and Pax7 antibodies to locate activated satellite cells.

Results show that CD24 is expressed in the myoblast stage

3 Overexpression Analysis

- Plat-E cell incubation supernatant was used to infect myoblasts with control pMXs retrovirus vector and CD24 pMXs retrovirus vector
- CD24 positive cells are indicated by green Avidin fluorescence and myosin heavy chain expression is characterized by Alex 568 red fluorescence
- Myosin heavy chain expression identifies the differentiated myoblasts as myotubes



Figures 10-25: Counting method depicted. Row 1: Day 0 Differentiation. Row 2: Day 2 Differentiation. Row 3: Day 6 Differentiation. Row 4: Day 8 Differentiation. Column 1: MHC staining. Column 2: CD24 staining. Column 3: DAPI staining. Column 4: Overlay.

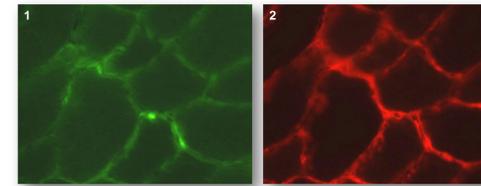


Figure 1: Avidin (green) fluorescence seems to indicate a positive CD24 signal of quiescent satellite cell.

Figure 2: Satellite cells are typically found in the laminin basal area but laminin staining (red) shows that the positive CD24 signals from Figure 1 are not in laminin basal areas. Therefore these signals are not CD24 positive.

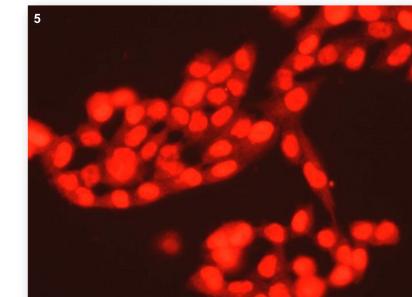


Figure 5: Red fluorescence indicates positive Pax7 expression. Almost all of the cells exhibit bright fluorescence and thus confirm that they are activated satellite cells.

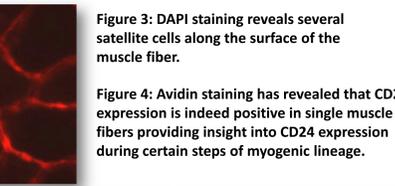


Figure 3: DAPI staining reveals several satellite cells along the surface of the muscle fiber.

Figure 4: Avidin staining has revealed that CD24 expression is indeed positive in single muscle fibers providing insight into CD24 expression during certain steps of myogenic lineage.

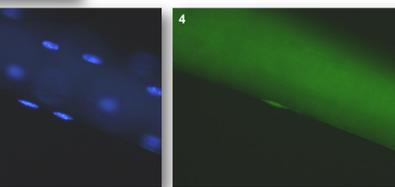


Figure 6: Green fluorescence indicates positive MyoD expression. Almost all of the cells exhibit bright fluorescence and thus confirm that they are activated satellite cells.

Day 0 Differentiation

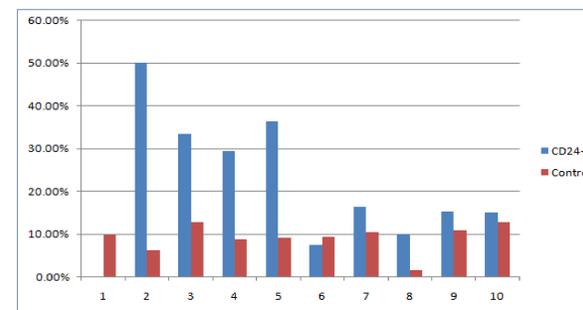


Figure 7: Depicts a number set of the percentage of CD24+ /MHC+ compared to the negative pMXs control group of CT/MHC+ already reveals a seemingly large difference in differentiation rates for the formation of myotubes after 4 days of incubation.

Day 2 Differentiation

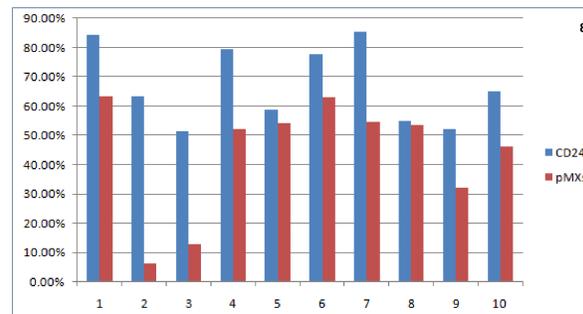


Figure 8: Depicts a number set of the percentage of CD24+ /MHC+ compared to the negative pMXs control group of CT/MHC+ already reveals a seemingly large difference in differentiation rates for the formation of myotubes after 6 days of incubation.

CD24	Day 0 Ave.	Day 2 Ave.	Day 6 Ave.	Day 8 Ave.
(MHC+ & CD24+)/CD24+	21.33%	67.21%	86.13	90.89%
pMXs	Sample1	Sample 2	Sample 3	Sample 4
MHC+/CT	9.21%	43.76%	64.05	77.69%
P =	0.0256	0.00653	0.00536	0.00191

Table 9: Depiction of Average differentiation rates among CD24 overexpressed cells and negative control cells. A comparably greater number of CD24 overexpressed cells differentiated into myotubes than the negative control.

Discussion

- CD24 expression has been determined as a byproduct of differentiation proving a causal over correlational relationship
- Differences between these results and past research can be attributed to more knowledge of satellite cell function now
- In the stages where CD24 is not expressed, the question is raised about what exactly controls CD24 expression
- Success in the use of recombinant DNA to overexpress CD24 in myoblasts is an important step in creating new research plans that can specifically address how CD24 can affect the role of differentiating and proliferating satellite cells.

Possible sources of error:

- Immunohistochemical staining includes user error
- Fluorescence microscopy introduces user error
- Contamination

Future Work

Areas of further research include:

- What controls CD24 expression during differing stages of myogenic differentiation
- What functions can be discovered in myoblasts with overexpressed CD24 expression
- New analysis via different techniques to confirm CD24 expression
- Determine CD24's ligand

Conclusion

Overall we see that the goal of this project to test the following three hypothesis have been fulfilled:

- 1) Is CD24 expressed only in distinct stages of muscle regeneration
- 2) In what amounts is CD24 prevalent in satellite cells
- 3) CD24 overexpression affects satellite cell differentiation

- CD24 is only expressed in certain stages of muscle development, specifically during activated satellite phase as well as myoblasts
- CD24 expression through immunohistochemical staining is rather small and thus symbolizes a weaker expression and signal some stages while overall it can vary.
- CD24 increases proliferation and differentiation rates of satellite cells when introduced in an overexpression vector

*All photographs taken by Evan Chen using LEEDS Olympus BX51 fluorescence microscope with DP70 Olympus attachment used for digital pictures unless otherwise noted