The Impact of Emerin and Actin Protein Synthesis Disruption on Muscular Dystrophy Caused by RNA Interference

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Abstract

The purpose of the laboratory investigation was to observe the mutation of Emerin protein, which causes X-linked, Emery-Dreifuss Muscular Dystrophy. Emerin is a nuclear membrane protein that serves the role of skeletal muscle and cardiac muscle development. Additionally, Emerin is a part of the nuclear envelope, a structure that surrounds the nucleus. Furthermore, complementary to the function of Emerin, is a protein found in eukaryotic cells known as Actin. Actin serves as a binding protein that contributes a crucial role in cellular functions, such as, cell morality and the interaction of Filament Actin with myosin that constitutes as the basis of muscle contraction. Henceforth, Emerin and Filament Actin play a pinnacle role in muscle development and movement. However, the investigation was based on the interaction between Emerin, Actin, and RNAi. Throughout the investigation, the relationship between the mutation of Emerin, Actin, IPO9 and RNAi was analyzed. RNAi, referred to RNA interference, initiates the resistance for endogenos parasitic and exogenous pathogenic nucleic acids. Moreover, RNAi ceases the synthesis of protein during the transcription and translation process of DNA.

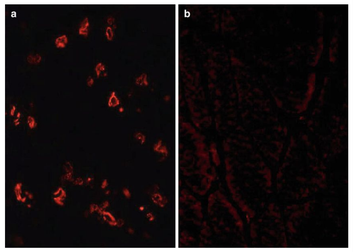
Methods

1. **Cell Lysis:**
   - Fractionation of cell to extract protein for access
2. **BCA Assay:**
   - Ensures sample proteins have equal concentrations
3. **Western Blotting:**
   - Proteins are separated by size
   - Target protein is marked with primary and secondary antibodies
4. **HRP Staining Imaging:**
   - Visualizes protein using CCD camera
   - Protein is stained with Horseradish Peroxidase (HRP) substrates

Muscular Dystrophy

Emery-Dreifuss Muscular Dystrophy (EMDM)

- Genetic degenerative disease
  - Primarily affects skeletal muscle
  - One of nine Muscular Dystrophies
- Inherited, X-linked recessive pattern
- Caused by the absence of Emerin
- A progressive disease with detectable cardiac issues
  - Detectable by age 20
- Joint contractures
  - Elbow
  - Neck tendons
  - Achilles



Emery-Dreifuss Muscular Dystrophy Type 1 - Scientific Figure on ResearchGate. Available from:https://www.researchgate.net/Muscle-biopsy-sections-immunostained-for-emerin-Note-that-in-comparison-with-control_fig1_312731968 [accessed 2 Aug, 2018]

Compared to the muscle in image a, the muscle in image b shows the absence of a reaction after protein staining. This indicated the absence of emerin after immunostaining.

- Immunostaining allows the detection of protein

Patients with Muscular Dystrophy experience difficulties with movement and muscle contraction

Nuclear Extraction and BCA Protein Assay

Performing Western Blot to visualize actin in the nucleus

- Prepared our fibroblast cell samples by extracting them

Completing the nuclear extraction

- Cells were incubated, centrifuged, suspended, and sonicated
- Until cytoplasmic fractions were separated from the nuclear fractions
- Samples were frozen in liquid nitrogen to store

Before running the gel (using gel electrophoresis)

- Needed to determine the sample protein concentrations (BCA Protein Assay)

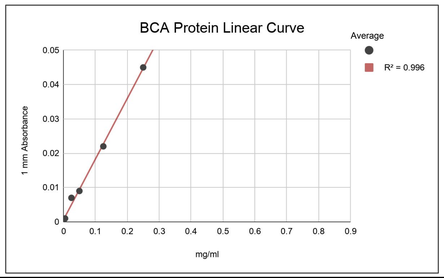
Discovered protein concentrations were too small

- Used an enhanced protocol to compensate for lack of protein concentration
- Used less diluent and stock concentrations for BSA
- Increased the temperature for incubation of the vials

Concentrations determined using spectrometer

	Diluent(uL)	BSA (uL)	Ave. Abs.
A	700	100 stock	0.045
B	400	400 vial A	0.022
C	450	300 vial B	0.009
D	400	400 vial C	0.007
E	400	100 vial D	-0.001
F	400	0(BLANK)	BLANK

Average absorbance dictates the average concentration of light that has been absorbed by the sample using a spectrometer (NanoDrop)



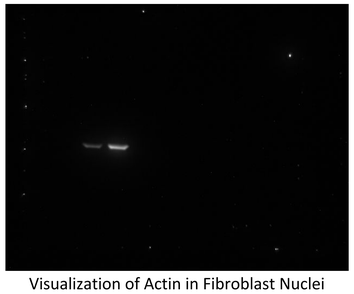
Western Blotting and Imaging

Gel electrophoresis separated our proteins based on molecular weight.
Electrophoretic transfer allowed proteins to move to the PVDF membrane
Unspecific binding was prevented using a blocking buffer 5% milk in Tris Buffered Saline(TBS).
To detect actin we incubated the blot with the anti-actin primary antibody.
After the antibody incubation we used the HRP enzyme and chemiluminescence solution
- detection of actin
  - Fibroblast RNAi Nuclear
  - Fibroblast Nuclear
- imaging using a CCD camera

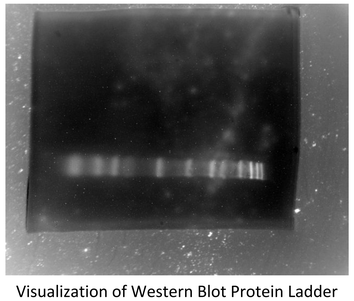
Our results showed that the weights did not deviate much from each other. With the images we conducted the quantitative analysis that led us to conclude that there was not much of a difference with RNAi being introduced into the nuclear envelope.

On the other hand, during other Western Blots that we conducted, we were unable to detect the actin.
Possible causes include:
- quality of the antibody
- the use of the lysis buffer as diluent in BCA assay instead of PBS.
- duration of rinses could have interfered with the expression of the bands on the membrane.

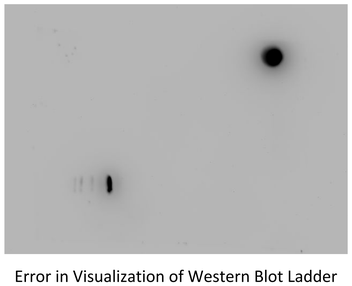
Alternative to the Western Blot procedure
- dot blot detects proteins
- simple procedure
- though, does not determine molecular weight
- our dot blot showed that actin could be detected on nuclear samples of the fibroblast cells.



Visualization of Actin in Fibroblast Nuclei



Visualization of Western Blot Protein Ladder

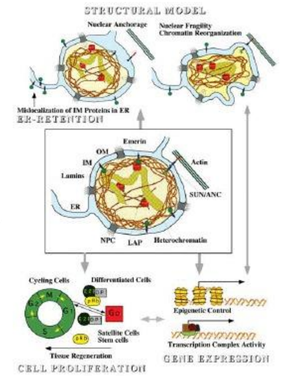


Error in Visualization of Western Blot Ladder

Future Plans

In the near future, we plan to continue our investigation by:

- Understanding how the nuclear organization of emerin impacts Muscular Dystrophy,
- Investigating how lamins play a role in chromosome regulation and gene expression.
- Comparing the expression of actin, emerin, and lamin to determine connection of nuclear envelope organization to other muscular dystrophies.
- Investigating the mutations of Emerin and Lamin proteins and their connection to the development of muscular dystrophy.



Summary

In conclusion, it was revealed that RNA interference (RNAi) disrupted the synthesis of Emerin and Filament Actin protein during DNA replication and mRNA transport. By interfering with the synthesis of Emerin and Filament Actin, the production and binding of muscle was also disturbed. We know that Emerin and Actin are present on the nuclear membrane of mammalian cells. Thus, it could be concluded that IPO9 was also involved in the development of Emery Dreifuss Muscular Dystrophy, as IPO9 (Importin 9) functions as a nuclear protein import, also known as a nuclear transport receptor. However, for patients with Emery Dreifuss Muscular Dystrophy, there is a lack of Emerin to develop skeletal and cardiac muscle, so the presence of Actin and the possibility of mutation is still unknown. Albeit, from the Western Blot, it was revealed that Actin is present. On the contrary, we have to determine the variance of our results in order to estimate our accuracy.

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3. Mr. Bautista
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6. BUGS staff

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