Mechanism of Disease-Causing Missense Mutations in Dystrophin

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Introduction

Duchenne muscular dystrophy (DMD) is a fatal, x-linked disease that affects 1 in every 3,500 live born males. DMD is caused by the loss of the protein dystrophin due to genetic mutation. Dystrophin is abundant at the cell membrane of muscle cells, where its function is to stabilize the plasma membrane against contraction-induced membrane damage by binding to cytoskeletal f-actin filaments and the transmembrane protein dystroglycan.[1] (Figure 1) A small percentage of DMD cases are caused by missense mutations where the change in a single amino acid can cause severe disease. This disease can be caused by dystrophin not being able to bind its intracellular partners or by misfolding of the dystrophin protein, which can lead to degradation or insoluble aggregates. I investigated this aggregation as a possible mechanism for the pathogenesis of DMD missense mutations.

I specifically worked with five missense mutations: K18N, L54R, L172H, Y231N, and T279A. These particular mutations are located in the actin-binding domain on the N-terminus of dystrophin. Of the fifteen known missense mutations in DMD patients, nine of them are found in this region. The missense mutations in this area can cause disease in one of two ways. The mutations can cause the protein to fold improperly[2], which leads to the aggregation and the loss of its localization to the membrane. The mutations can also render the protein unable to bind actin properly[2], despite normal folding and transportation of the protein to the membrane.

For this project, I compared the levels of insoluble protein for various types of mutant dystrophin to the levels of wild type (WT) dystrophin (data provided by D.M. Henderson) to determine whether the mutations cause misfolding. I hypothesized that disease-causing missense mutations in the N-terminal actin binding domain of dystrophin cause misfolding and increase the level of insoluble dystrophin in vivo.

Methods

To obtain the mutant protein, I used recombinant baculoviruses that encode for the mutant proteins. The mutant proteins were expressed in insect cells after infection by the mutant viruses. Infected cells were incubated for 72 hours to allow robust expression of mutant dystrophin. The cells were harvested and lysed in detergent liberating the expressed protein. A sample of total protein was taken from this. The rest of the solubilized protein was centrifuged, separating out all the insoluble protein, and a sample of soluble protein fraction was taken. Both the total protein samples and the soluble protein samples were run on a SDS-PAGE gel which was stained with Coomassie Blue so the protein would be visible as distinct bands. The bands corresponding to the overexpressed dystrophin protein were quantified, and the amount of soluble protein was compared to the amount of total protein to determine the level of insoluble protein. Three trials of each mutant were simultaneously conducted per experiment. The number of experiments performed for each mutant varied, ranging from six to nine. The average level of insoluble protein of each different mutant were then subjected to a two sample t-test assuming equal variances comparing it to the level of solubility in WT dystrophin.

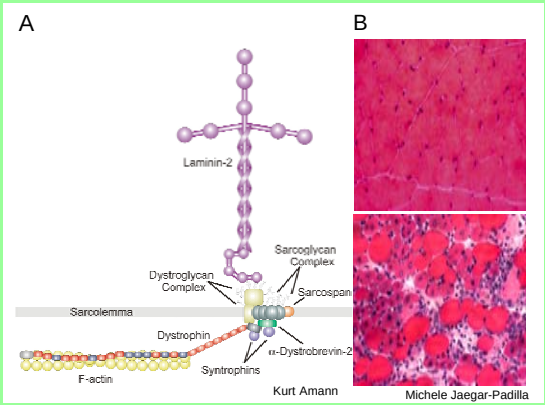

Figure 1. Dystrophin-glycoprotein complex A. Dystrophin is a member of the dystrophin-glycoprotein complex that is localized to the plasma membrane of muscle cells. This complex links the extracellular matrix to the cortical actin cytoskeleton protecting the membrane of muscle cells during contraction. B. H&E stained WT or *mdx* mouse muscle cross sections. *Mdx* mouse muscle lacks dystrophin expression and exhibits severe muscle wasting.

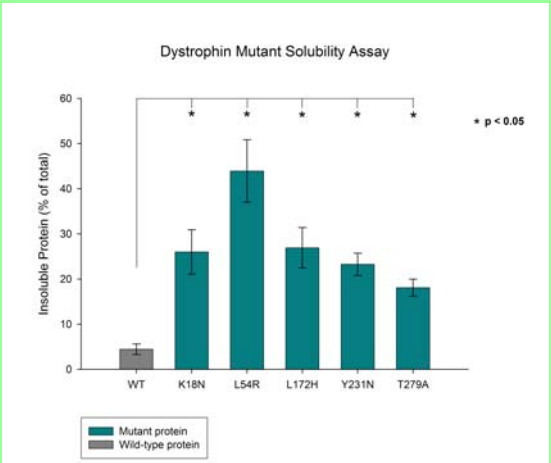

Figure 2. Dystrophin mutant solubility assay The average percentage of insoluble protein for the five mutants are significantly less than for WT dystrophin.

Results and Discussion

The five mutants studied in this project were found to be significantly less soluble than the WT protein. (Figure 2) The insolubility of L172H, K18N, Y231N, and T279A ranged between 16.70% and 26.92% while the percent of insoluble protein for L54R was 47.16%. After conducting a t-test comparing L54R to the other mutants, I determined that L54R was significantly more insoluble than the other mutants. It is likely that higher insolubility is related to the fact that of the five mutants, L54R causes the most severe disease.

Due to the variability of living cells, some trials resulted in data that could not be used, i.e., the soluble fraction was found to be greater than total protein, which is impossible. Experiments with two or more of this kind of result were excluded from the final data.

Because nine of the fifteen known missense mutations are located in the N-terminal actin-binding domain of dystrophin, it is probably very important to the functionality of protein. The results of my project provide evidence that these missense mutations in the N-terminal actin-binding domain are contributing to improper folding of the protein, which leads to its insoluble aggregation in the cell and the loss of its localization to the membrane.

This project is still in progress; four mutants have yet to be evaluated. Additionally, circular dichroism spectroscopy will be performed on truncated versions of the mutant and WT protein to characterize the secondary structure of the N-terminal actin-binding domain and confirm misfolding.

Conclusions

- The mutants K18N, L54R, L172H, and Y231N were all found to be significantly less soluble than the wild-type protein.
- Misfolding of the protein and subsequent aggregation in the cell and loss of localization to the membrane contributes to the mechanism of the disease-causing missense mutations

Literature

1) Dystrophin, its interactions with other proteins, and implications for muscular dystrophy. Ervasti, James M. *Biochim. Biophys. Acta*., 2007 1772(2): p. 108-117.
2) Yue, P., Z. Li, and J. Moult. Loss of Protein Structure Stability as a Major Causative Factor in Monogenic Disease. *J. Mol. Biol.*, 2005. 353(2): p. 459-473.

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