Differential microRNA expression in a mouse model of Duchenne muscular dystrophy

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In Duchenne muscular dystrophy (DMD) it is well known that the lack of dystrophin protein is the primary cause of disease that leads to loss of ambulation, progressive muscle weakness and heart and respiratory complications. However, less is known about how the loss of dystrophin affects other features of the disease including muscle regeneration, inflammation and fibrosis (replacement of muscle with scar tissue). The overall aim of this project is to investigate how a network of small RNAs, called microRNAs (miRNAs), may be involved in changing the levels of proteins that may be involved in some of these disease processes in DMD.

Why are microRNAs important?

MicroRNAs are important for normal function of tissues and organs, including muscle development and maintenance. However their levels have been shown to be changed in many diseases including cancer and neurological disorders. In DMD it is still not well understood which miRNAs are important in the disease although previous studies have implicated a number of roles that these miRNAs may be involved in. Some of these are listed below.

What are microRNAs?

RNA is a carbon copy of DNA that is usually used to make protein. MicroRNAs are small pieces of RNA. These small RNAs act differently in that they can bind to specific genes and when this happens less of the protein product is made.

Aims of Study

• Use a mouse model of DMD (the mdx model) to study the expression of miRNAs in mice lacking dystrophin compared to unaffected control mice. This would be done by measuring the amount of all miRNAs that are known to exist and comparing their levels in heart, quadriceps and diaphragm tissues.

• The miRNAs that are changed the most would be studied further using more accurate assays. Their expression will also be studied across a wider range of tissues to see if different muscles are affected differently with regards their miRNA expression.

• Mdx mice will be treated with an exon skipping strategy to restore dystrophin expression (a similar approach that is being used in clinical trial). miRNA expression will then be studied in these mice to determine if miRNA levels could be a useful indicator if a treatment could have a beneficial effect.

• A large scale study of protein expression in mdx mice vs control mice will be used to determine which proteins are altered due to the lack of dystrophin. It will then be possible to determine if the proteins with the greatest changes are targets of the miRNAs with the greatest changes. Thus we hope to provide direct links between miRNAs and changes in the disease.

miRNA expression in unaffected vs DMD mice

Total RNA was extracted from quadriceps, diaphragm and heart tissues of four unaffected and four DMD mice

A machine measures the levels of miRNA for ~800 miRNAs

The level of each miRNA is compared between the unaffected and DMD mice for each tissue

If miRNA expression in DMD mouse is less than in unaffected then shown as green

If miRNA expression in DMD mouse is increased compared to unaffected then shown in red

Studying miRNAs of interest in greater detail

miRNAs that showed greatest changes from the large study were chosen for further analysis along with miRNAs involved in muscle development (mir-1, mir-133a and mir-206)

A more sensitive assay (called quantitative PCR) was used to study the miRNA expression in a wider range of tissues, including measurement from the blood (serum).

Summary of Findings

• The levels of altered miRNAs in the DMD mouse is different between skeletal muscle, heart and serum samples.

• Generally, the heart had less altered miRNA expression, both in the number of different miRNAs and the level of change of these miRNAs.

• The levels of miRNA in the blood are quite different to that in muscle. In particular, miRNAs involved in muscle development and repair are significantly higher in blood than in muscle (e.g. mir-206 is 100 times higher)

• The highly expressed miRNAs in blood (mir-1, mir-133 and mir-206) may be good markers for studying if a treatment is working by doing a simple blood test rather than an invasive biopsy. We will be testing this theory in future studies.

• The miRNA with the greatest change in muscle was mir-31 which increased 100 fold. This miRNA has recently been shown to reduce dystrophin protein and so we will look at methods to reduce its levels in the body. Thus it is hoped that this approach can be combined with a dystrophin restoration strategy to increase levels of dystrophin in muscle.

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