REPURPOSING METFORMIN FOR THE TREATMENT OF DUCHENNE MUSCULAR DYSTROPHY

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Abstract

Duchenne muscular dystrophy (DMD) is a progressive lethal, X-linked disease of skeletal and cardiac muscles caused by mutations in the dystrophin gene. Loss of dystrophin leads to muscle fiber damage and impairment of satellite cell asymmetric division, which is essential for muscle regeneration. These processes ultimately result in muscle wasting; degenerating muscles are replaced by fibrogenic cells, a process that leads to the generation of fibrotic tissues. Metformin is an FDA-approved oral therapy for type 2 diabetes. Metformin has been also reported to regulate multiple cellular pathways including inflammation, fibrosis and a variety of aging related processes and it is now explored as a potential therapeutic agent in a large number of diseases. Over 2000 clinical trials with metformin are currently being registered in clinicaltrials.gov, including treatment of ALS, cancer and DMD. Metformin acts by activating AMPK In addition to other major signaling pathways. In this study we demonstrated that metformin treatment promoted differentiation of mouse and human myoblasts (from healthy donors and DMD patients) as demonstrated by increases in MyoD and MyHC expression and fusion into myotubes. Additionally, metformin increased the levels of utrophin, a dystrophin homologue and decreased the expression of fibrosis-related genes in DMD muscle cells. Using RNA sequencing of myoblasts from healthy donors and DMD patients, we found that the DMD muscles exhibited decreased levels of H19 and miR-29c and increased let-7 expression compared to healthy donors. We then analyzed the effects of metformin treatment on the expression of non-coding RNAs in muscle cells derived from DMD patients. We identified differentially expressed miRNAs and lncRNAs in the control and metformin treated cells. Metformin treatment upregulated H19, miR-675 and miR-29c expression in the DMD muscle cells, which mediated its myogenic differentiation effects and downregulated the expression of let-7 which targets the 3'-UTR of utrophin. In addition, metformin reduced the expression of miR-21 a major inducer of tissue fibrosis. In conclusion, data from pre-clinical and clinical studies and current results, demonstrating inhibition of muscle fibrosis, while promoting differentiation and utrophin expression, strongly support pursuing metformin as a potential therapeutic agent for DMD and other muscle disorders.

Objectives

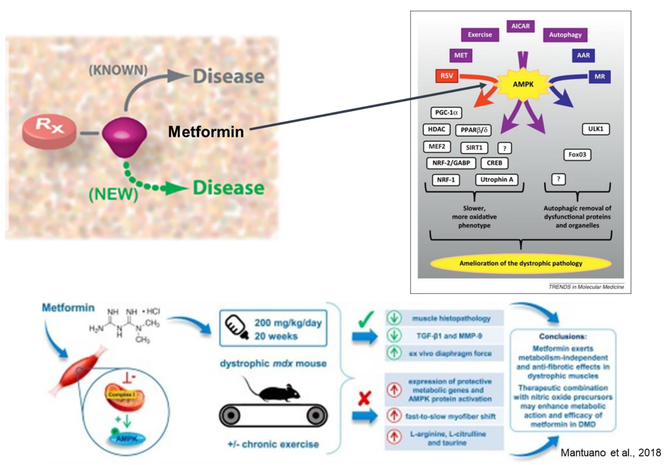
In vitro studies:

- Analyze Metformin effects on myoblast and satellite cell proliferation, differentiation and cell fibrosis
- Analyze molecular and cellular mechanisms of metformin effects focusing on non-coding RNAs

Duchenne Muscular Dystrophy (DMD)

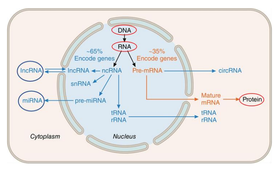
- A severe recessive X-linked form of muscular dystrophy
- Characterized by a rapid progression of muscle degeneration, which leads to loss of ambulation and death
- Diagnosed between the ages of 2-10
- DMD affects mostly males at a rate of 1 in 3,500 births
- There is no cure
- DMD is caused by mutations in the dystrophin gene
- dystrophin-deficient satellite cells have a defect in cell polarity establishment and lack of asymmetric cell division
- Impaired regenerative ability of satellite cells
- DMD is associated with skeletal muscle inflammation and fibrosis

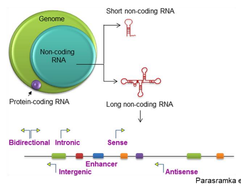
Metformin



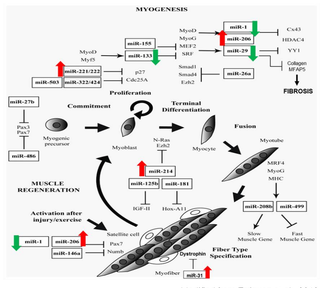
Mantuano et al., 2018

Non-coding RNAs in DMD and BMD

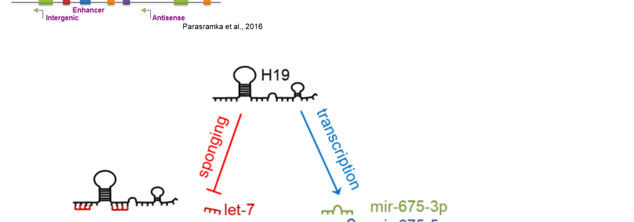


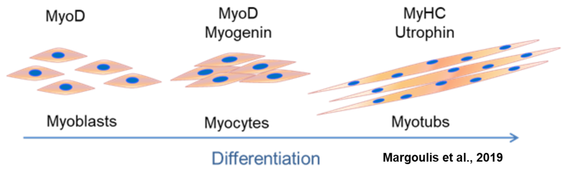


Parasramka et al., 2016



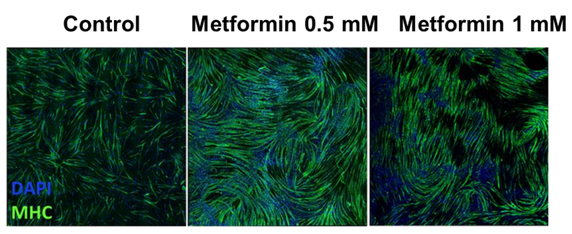
Modified from Erriquez et al., 2013

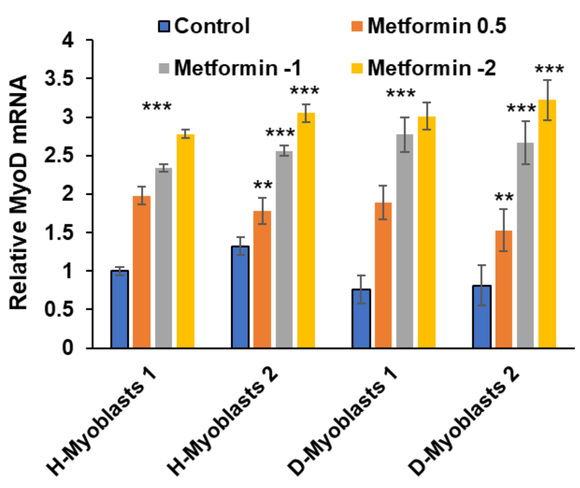


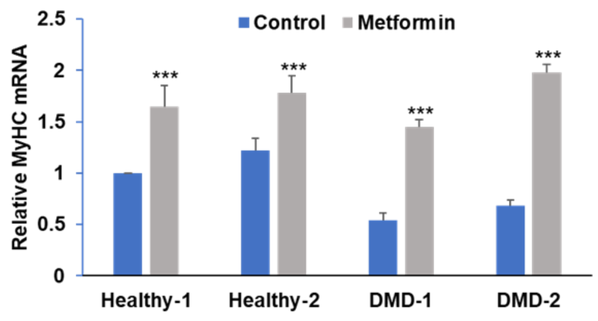


Margoulis et al., 2019

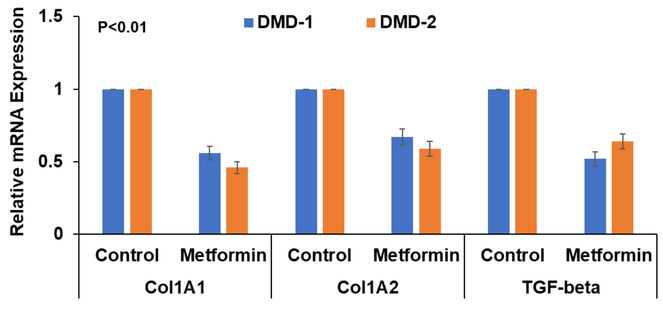
Metformin increases the differentiation of human and mouse myoblasts



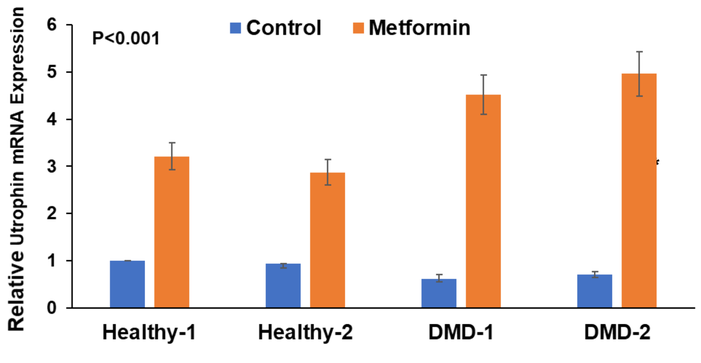




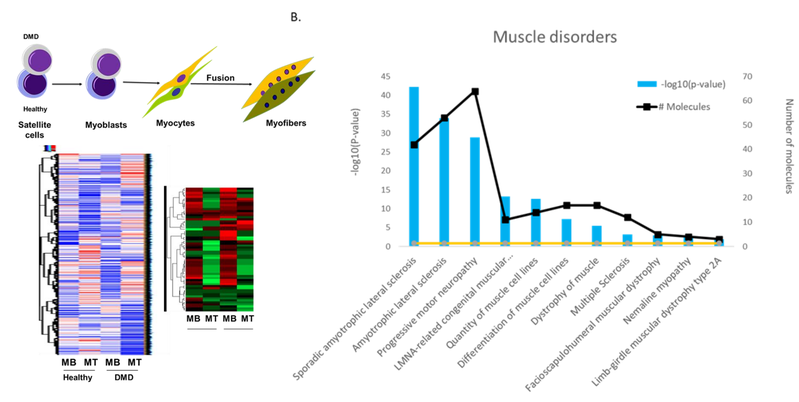
Metformin decreases fibrosis-related gene expression in DMD muscle cells



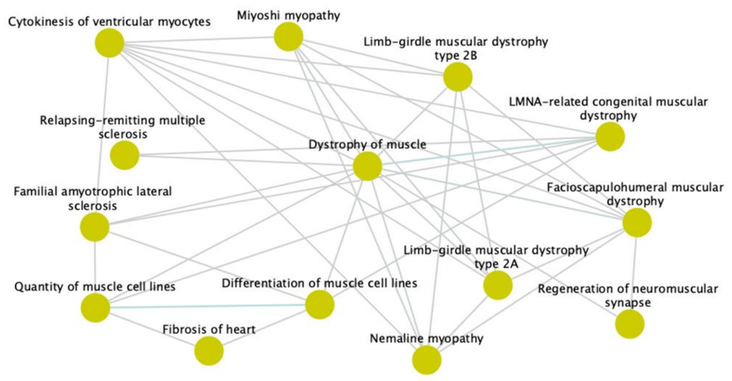
Metformin increases utrophin expression in human muscle cells



Metformin changes the expression of specific miRNAs in DMD muscle cells



Pathway and disease specific clusters in metformin treated DMD muscle cells



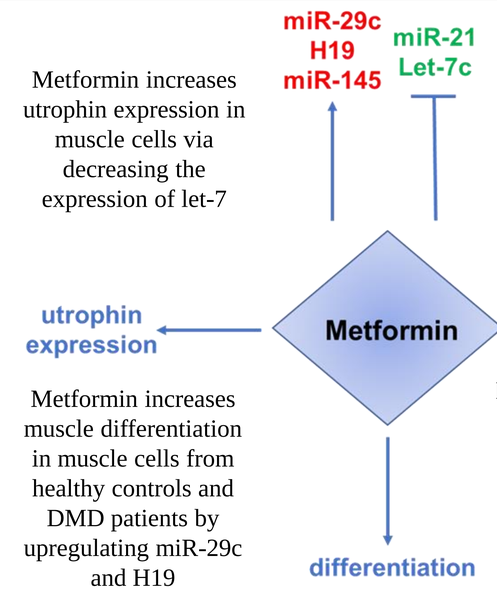
Conclusions

Metformin increases utrophin expression in muscle cells via decreasing the expression of let-7

Metformin increases muscle differentiation in muscle cells from healthy controls and DMD patients by upregulating miR-29c and H19

Using CRISPR/Cas 9 and overexpression experiments, the roles of the specific non-coding RNAs in metformin effects were validated.

Metformin decreases the expression of fibrosis-associated genes in DMD muscle cells by downregulating miR-21 and upregulating miR-29c expression



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