AGEING, SARCOPENIA AND TROPHIC FACTORS – POSTER PRESENTATIONS

T.P.34
FHL1 reduces muscle degeneration in dystrophin-deficient mdx mice through the sarcolemmal recruitment of utrophin
1 Department of Biochemistry and Molecular Biology, Monash University and The Alfred Hospital, Melbourne, Australia; 2 Monash University, Biochemistry and Molecular Biology, Clayton, Melbourne, Australia; 3 Alfred Hospital, Anatomical Pathology, Prahran, Melbourne, Australia; 4 University of Melbourne, Basic and Clinical Myology Laboratory, Department of Physiology, Melbourne, Australia; 5 IGBMC, Translational Medicine and Neurogenetics, Illkirch, France

Duchenne muscular dystrophy (DMD) is caused by dystrophin gene mutations, resulting in loss of dystrophin from the sarcolemma. Utrophin is a therapeutic target for DMD due to its functional compensation for dystrophin, by forming the protective utrophin–glycoprotein complex (UGC) at the sarcolemma. In adult muscle fibres utrophin localizes to the neuromuscular junction, therefore the challenge is to identify factors which increase utrophin expression and sarcolemmal recruitment. Calcineurin/NFATc1 is a utrophin regulatory pathway and we reported that FHL1 coactivates NFATc1. Crossed our skeletal muscle FHL1 transgenic mice with the mdx model of DMD. FHL1 ameliorated degeneration in multiple mdx muscles at 4- and 16-weeks of age. Relative to mdx mice, mdx/FHL1 mice also exhibited decreased serum creatine kinase (2.5-fold) and a reduction in muscle fibres stained for serum IgM, further indicating the protective effect of FHL1. Chronic myofibre damage in DMD and mdx mice induces pronounced muscle infiltration with macrophages, which were reduced in mdx/FHL1 mice. The benefit of FHL1 was sustained in older mdx/FHL1 mice (9 months) as shown by a significant reduction (2-fold) in diaphragm fibrosis compared to mdx mice. In functional studies the tibialis anterior from mdx/FHL1 mice was also protected against contraction-induced injury, indicating FHL1 stabilizes muscle fibres during repetitive muscle contractions. We identified the mechanism by which FHL1 reduced muscle degeneration; luciferase assays revealed FHL1 potentiated NFATc1-activation of the utrophin A promoter and utrophin mRNA and protein were increased in mdx/FHL1 mice. Immunofluorescence analysis revealed utrophin localized to the sarcolemma in adult muscle fibres of mdx/FHL1 mice, where it directed formation of the UGC by recruiting α- and β-dystroglycan, α- and γ-sarcoglycan and syntrophin. This study identifies FHL1 as a utrophin regulatory protein and therefore a potential therapeutic target for DMD.

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T.P.35
FHL1 is beneficial in reducing muscle wasting in dystrophic FRG1 mice
S.J. Feeney 1, M.J. McGrath 1, C.E. D’Arcy 1, A. Sriratana 1, R. Tupper 2, J.T. Price 1, C.A. McLean 1, C.A. Mitchell 1
1 Monash University, Biochemistry and Molecular Biology, Melbourne, Australia; 2 University of Massachusetts Medical School, Program in Molecular Medicine, Worcester, United States; 3 Alfred Hospital, Anatomical Pathology, Melbourne, Australia

Four and a half LIM 1 (FHL1) is a protein highly expressed in skeletal muscle, and we have previously reported that FHL1 can promote myoblast fusion in vitro and skeletal muscle hypertrophy in vitro by activating the calcineurin/NFATc1 pathway. Therefore FHL1 has potential as a therapeutic target for muscle diseases that display a myoblast fusion defect and/or progressive muscle wasting. The focus of this study was to determine if FHL1 can reduce muscle wasting in the dystrophic FRG1 mouse. FRG1 is one of several candidate genes for Facioscapulohumeral muscular dystrophy and FRG1 transgenic mice show resemblance to this disease clinically and pathologically. The dystrophy in FRG1 mice is characterised by progressive muscle wasting and is accompanied by spinal kyphosis caused by weakness of the trapezius muscle. We generated myoblast cell lines overexpressing FRG1 that exhibited a myoblast fusion defect, and as FHL1 enhances myoblast fusion we generated FRG1 mice over-expressing FHL1 by crossing our FHL1 skeletal muscle-specific transgenic mice with the dystrophic FRG1 mouse model. X-ray images of 6-week old mice revealed that FHL1 reduced the kyphosis of FRG1 mice. Analysis of muscle weights in mice revealed an increased muscle mass in FRG1/FHL1 mice relative to FRG1 mice, indicating FHL1 reduces muscle wasting. Histological analysis of muscle further revealed an increase in the average myofibre cross-sectional area and increase in the proportion of larger myofibres in FRG1/FHL1 mice compared to FRG1 mice. This beneficial effect of FHL1 was present in 6-week old FRG1/FHL1 mice and sustained into adulthood (12-weeks). FRG1/FHL1 mice also exhibited a reduction in fibrosis and fat accumulation in muscle further supporting the sustained benefit of FHL1 in reducing the dystrophic pathology of FRG1 mice. This study confirms that FHL1 is sufficient to reduce the dystrophy in FRG1 mice and is a potential future therapeutic target in treating muscle diseases.

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T.P.36
Hepatocyte growth factor reverses atrophy by inducing protein synthesis in mice
S. Hauserslev, J. Vissing, T.O. Krag
Neuromuscular Research Unit, Department of Neurology, Rigshospitalet, Copenhagen, Denmark

We investigated if hepatocyte growth factor (HGF) and leukemia inhibitory factor (LIF) can enhance muscle regeneration and thus increase muscle mass in a hypoxia-induced atrophy mouse model. B10 mice were exposed to 2 weeks of hypoxia to induce muscle atrophy before starting treatment. Hypoxia was continued during treatment with HGF/LIF (N = 13) or placebo (N = 14). Treatment was given as intraperitoneal injections every second day alternating between HGF and LIF for 2 weeks. Hypoxic exposure resulted in an average drop of 30% in body weight and 20% in muscle weight. Dividing satellite cells were twofold increased in the treatment group compared to control and the PI3K/Akt pathway significantly induced to a 100-fold activation of p70S6K, known for its implication in protein synthesis. We found that HGF treatment lead to activation of the Akt/mTOR/p70S6K protein synthesis pathway, up-regulation of the myogenic transcription factors MyoD and myogenin and subsequently the negative growth control factor, myostatin and atrophy markers MAFbx and MuRF1. Furthermore, we found that HGF/LIF treatment of muscle atrophy models lead to an increase in lean muscle mass: Tibialis anterior weight increased by 9% and extensor digitorum longus by 18%. Finally we demonstrate that myostatin regulates satellite cell activation and myogenesis in vivo following HGF/LIF treatment, consistent with previous findings in vitro. Our results suggest a novel in vivo pharmacological treatment with HGF, directed specifically at activating the satellite cells. This treatment could potentially be applied to many conditions that feature muscle wasting to increase muscle bulk and strength.

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