Exploring the Relationship Between DUX4 and Hypoxia-Inducible Factor (HIF1α)

UMONS RESEARCH INSITUTE FOR HEALTH SCIENCES AND TECHNOLOGY

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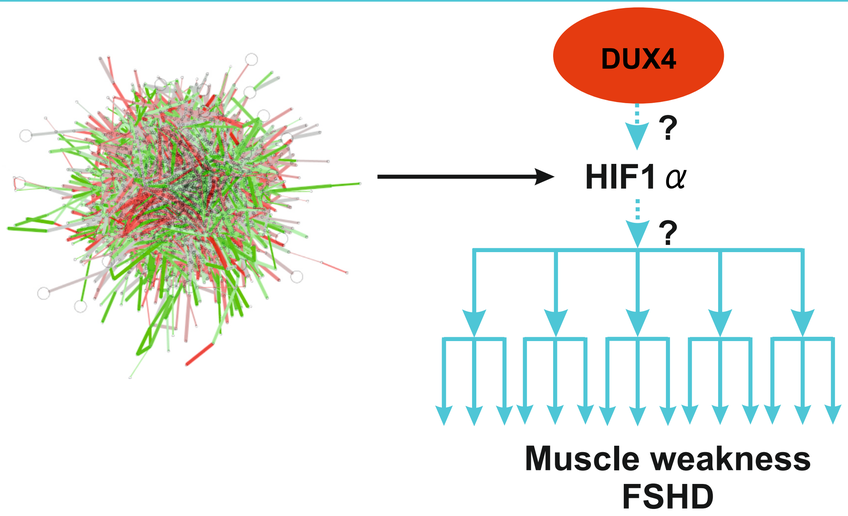
INTRODUCTION

The deregulated molecular network causing FSHD skeletal muscle dysfunction is still a major research topic. Recent meta-analyses (Banerji et al, 2015), highlighted the HIF1 α axis as critically disturbed in FSHD muscles. HIF1 α is a master regulator of oxygen homeostasis and its sustained stabilization in skeletal muscle might affect muscle mass through metabolic disturbances or an increased sensitivity to oxidative stress.

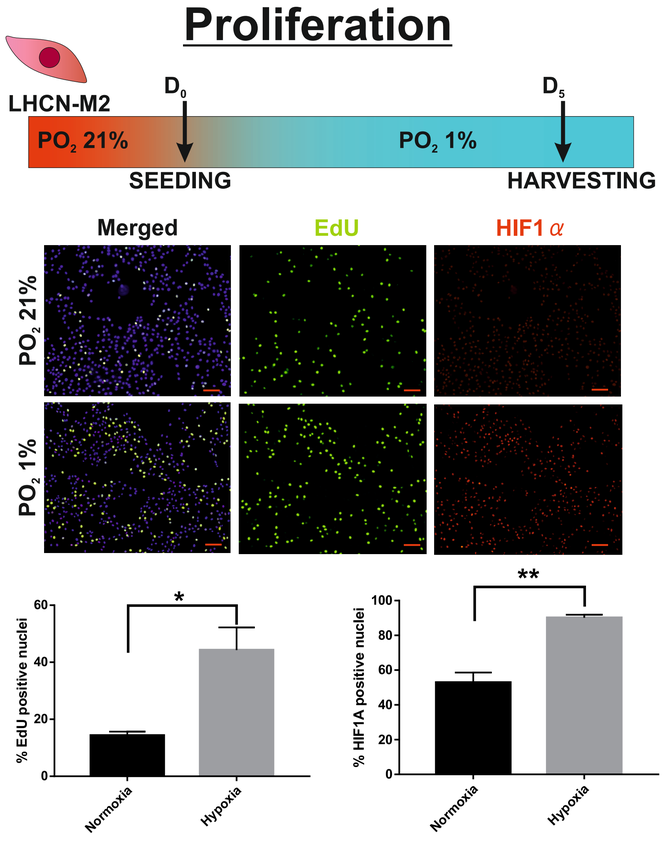


AIM

Our goal is to investigate potential relationships between DUX4 and HIF1 α and its contribution to muscle dysfunction in FSHD.

EFFECT OF HIF1α ACTIVATION IN HUMAN MYOBLASTS IN PROLIFERATION, DIFFERENTIATION AND FUSION

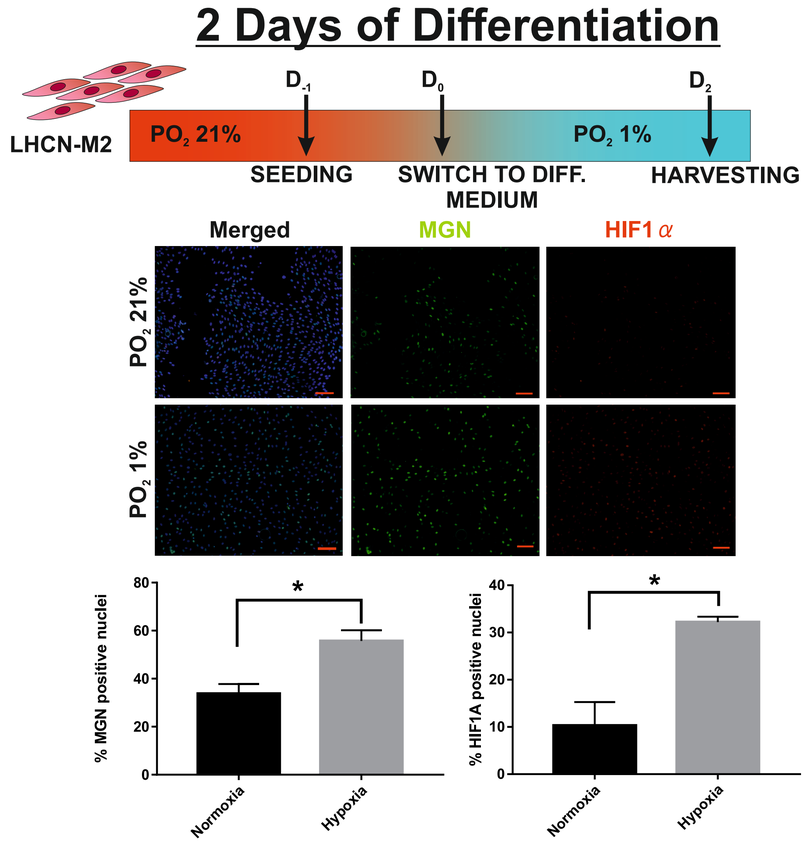
Proliferation



Effect of Hypoxia on myoblast proliferation after exposure to 1% or 21% PO_2 for 5 days. Upper panel. Localization of HIF1 α and EdU staining by Immunofluorescence. Scale bar: 100 µm. **Lower Panel.** Quantification of EdU and HIF1 α positive nuclei normalized to the total number of nuclei (DAPI). T-test, p<0,05, mean +/- SEM, n=3

Hypoxic conditions increase HIF1 α protein level with a concomitant increase in proliferation rate

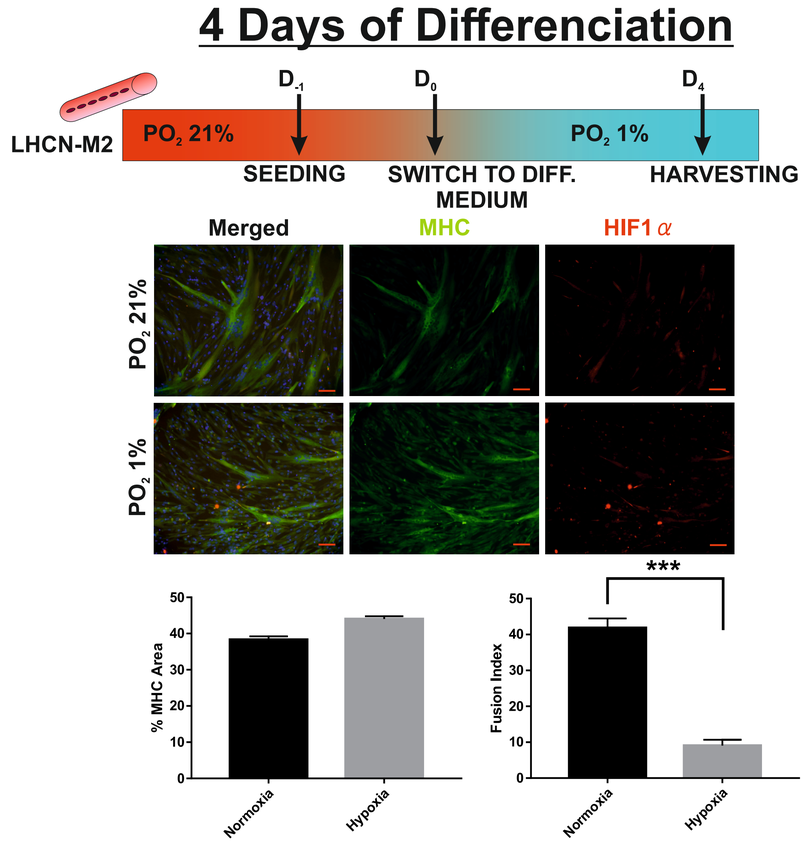
2 Days of Differentiation



Effect of Hypoxia on myocyte differentiation after exposure to 1% or 21 % PO_2 during the 2 days of differentiation. Upper panel. Localization of HIF1 α and Myogenin (MGN) by Immunofluorescence. Scale bar: 100 µm. **Lower Panel.** Quantification of MGN and HIF1 α positive nuclei normalized to the total number of nuclei (DAPI). T-test, p<0,05, mean +/- SEM, n=3

Hypoxia induces early myogenic differenciation but reduces myoblast differentiation into multinucleated myotubes

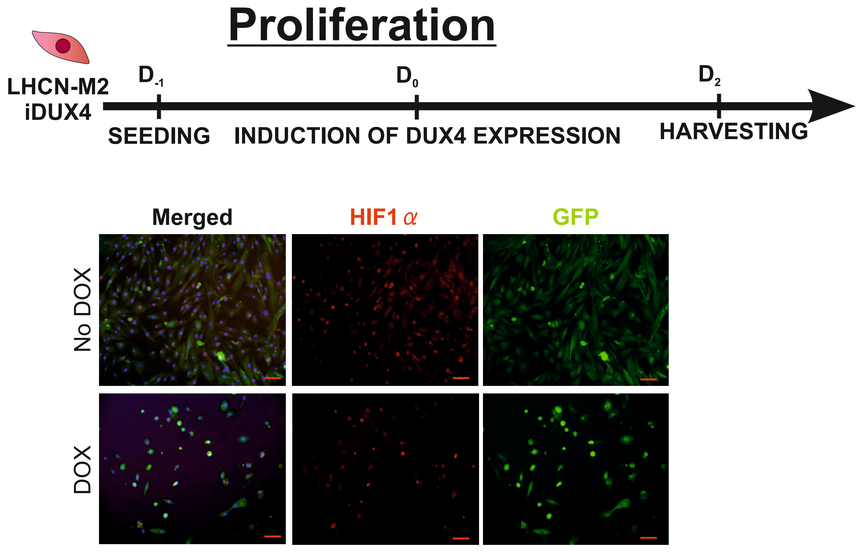
4 Days of Differenciation



Effect of Hypoxia on myotubes after exposure to 1% or 21 % PO_2 after 4 days of differentiation. Upper panel. Localization of HIF1 α and Myosin Heavy Chain (MHC) by Immunofluorescence. Scale bar: 100 µm. **Lower Panel.** Quantification of MHC area and Fusion Index. T-test, p<0,05, mean +/- SEM, n=3

EFFECT OF DUX4 ACTIVATION ON HIF1α IN DUX4 INDUCIBLE HUMAN MYOBLASTS

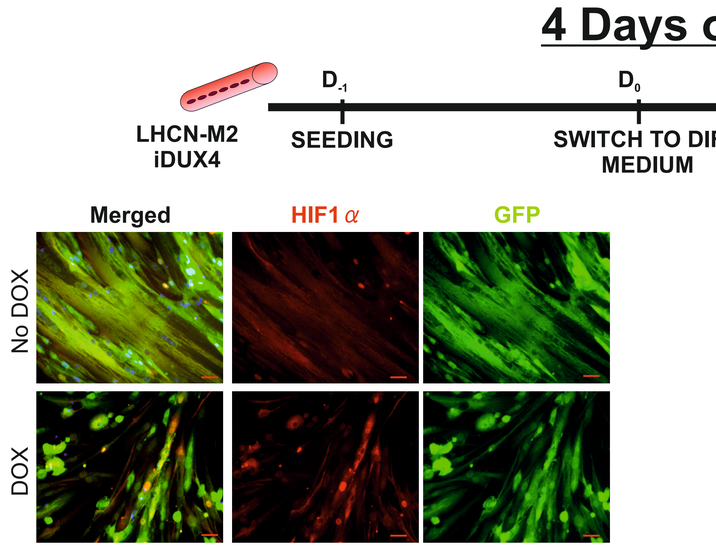
Proliferation



DUX4 inhibits HIF1 α nuclear protein level in proliferating myoblasts

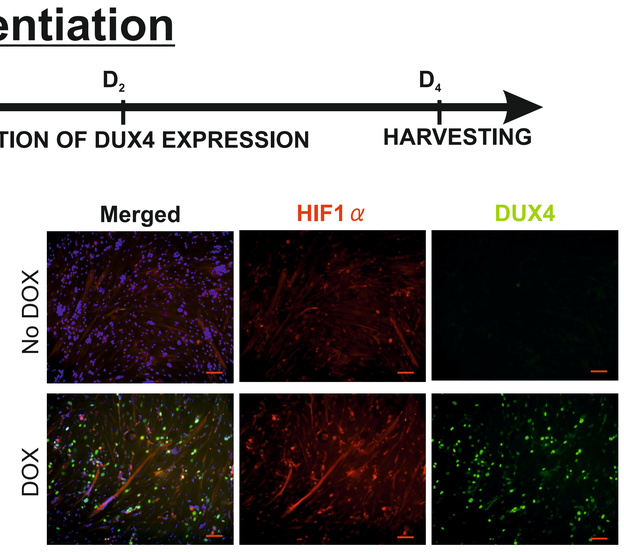
Effect of DUX4 on HIF1 α on Doxycycline-DUX4 inducible myoblasts in proliferation after 48h of DUX4 induction. Upper panel. Localization of HIF1 α staining by Immunofluorescence. Scale bar: 100 µm. **Lower Panel.** Quantification of HIF1 α positive nuclei normalized to the total number of nuclei. T-test, p<0,05, mean +/- SEM, n=3

4 Days of Differentiation



DUX4 increases HIF1 α nuclear protein level in myotubes

Effect of DUX4 on HIF1 α on Doxycycline-DUX4 inducible myoblasts after 4 days of differentiation (48h of DUX4 induction). Upper panel. Localization of HIF1 α staining by Immunofluorescence. Scale bar: 100 µm. **Lower Panel.** Quantification of HIF1 α positive nuclei normalized to the total number of nuclei. T-test, p<0,05, mean +/- SEM, n=3



52 % of HIF1 α positive nuclei were DUX4 positive

Effect of DUX4 on HIF1 α on Doxycycline-DUX4 inducible myoblasts after 4 days of differentiation (48h of DUX4 induction). Upper panel. Colocalization of HIF1 α and DUX4 staining by Immunofluorescence. Scale bar: 100 µm. **Lower Panel.** Quantification of HIF1 α positive nuclei normalized to the total number of nuclei. T-test, p<0,05, mean +/- SEM, n=3

CONCLUSION

FSHD is linked to a greater sensitivity of muscle cells to oxidative stress. Using **transcriptomic studies**, we have found that **HIF1 α** signalling is deregulated in FSHD. Expression of DUX4 in human myoblasts associates with HIF1 α signalling, and we are investigating this association with the **DUX4-induced phenotype.**

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