Exploring the Relationship Between DUX4 and Hypoxia-Inducible Factor (HIF1α) in human and murine muscle cells *in vitro* and *in vivo*

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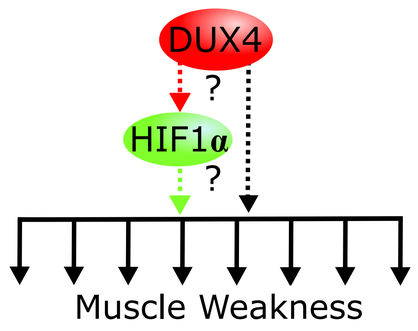
INTRODUCTION

The cellular and molecular network causing FSHD skeletal muscle dysfunction is still a major research topic. Recently, cellular pathways involved in hypoxic response and oxidative stress (Heher and et al., 2022,doi.org/10.1016/j.redox.2022.102251) have been highlighted by transcriptomic analyses (Banerji et al., 2017,doi:10.1038/s41467-017-01200-4) and genome-wide CRISPR-Cas9 screening (Lek et al., 2020,doi: 10.1126/scitranslmed.aay0271). Particularly HIF1α, a master regulator of oxygen homeostasis, shows an aberrant or sustained stabilization in FSHD (Reviewed in Nguyen et al., 2021, doi: 10.3390/ijms22137220). However, the potential link between DUX4 and HIF1α is still unclear.

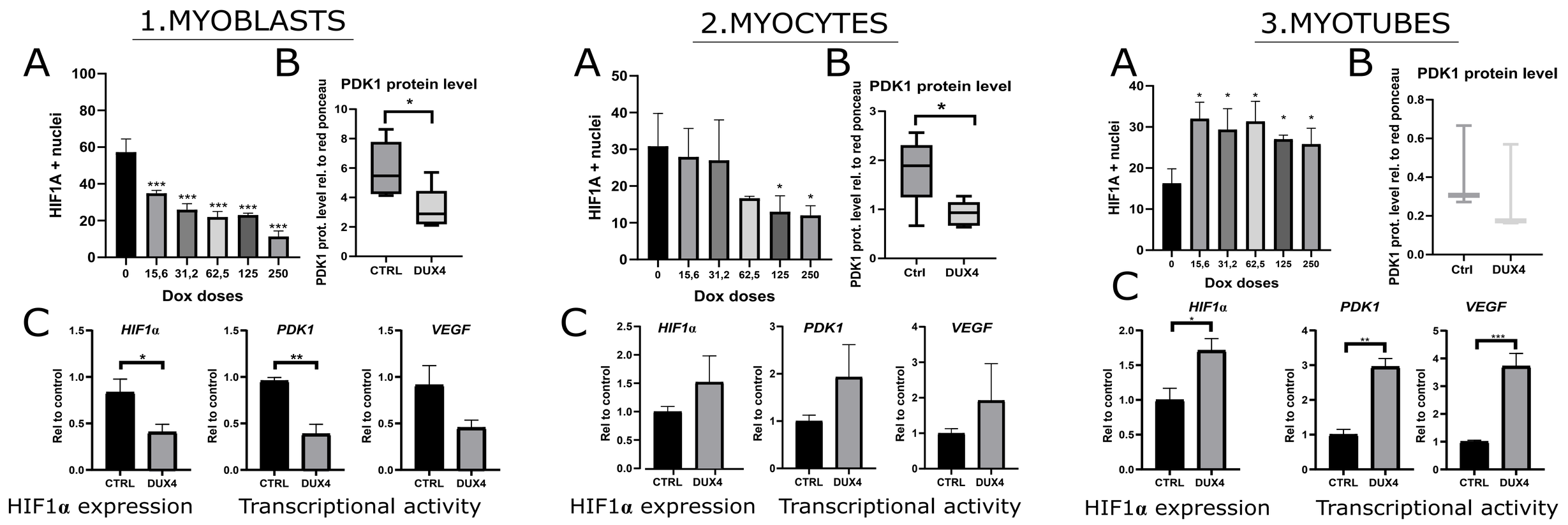


AIM

Investigate **DUX4** - **HIF1α** crosstalk and unravel its contribution to muscle dysfunction in FSHD

RESULTS

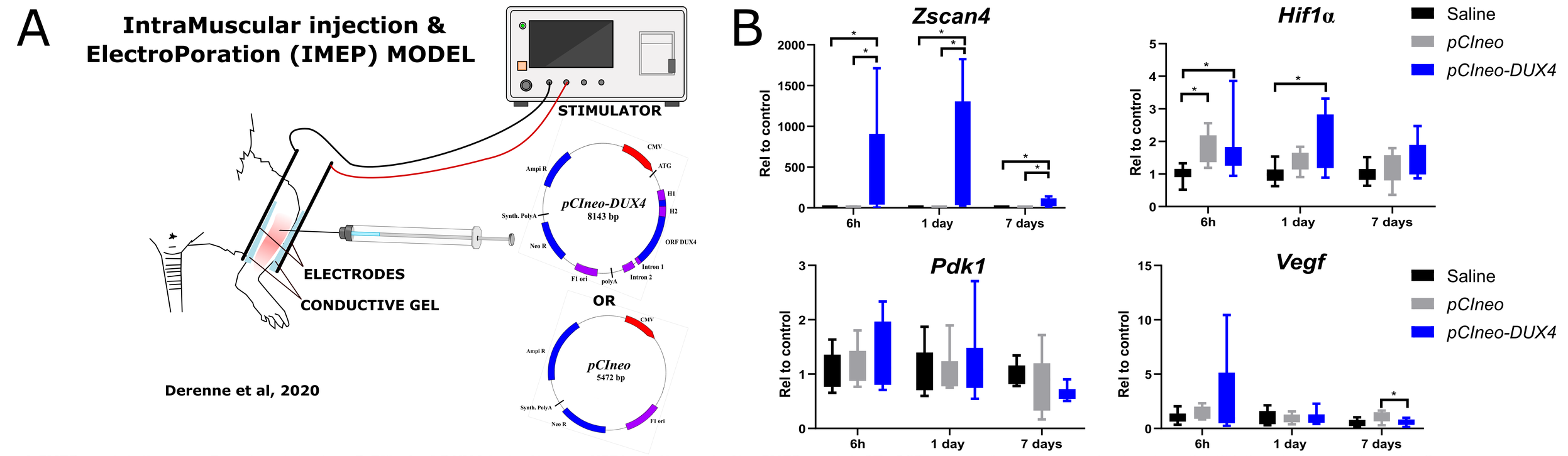
Effect of DUX4 expression on HIF1α pathway *in vitro*



Effect of DUX4 induction on HIF1α pathway in human 1. LHCN-iDUX4 in proliferation (24h of DUX4 induction) 2. in D2 of differentiation (48h of DUX4 induction between D0 and D2) 3. in D4 of differentiation (48h of DUX4 induction between D2 and D4). A. Immunofluorescence and quantification of HIF1α positive nuclei normalized to the total number of nuclei (DAPI). N=3, mean +/- SEM, *p<0.05, ***p<0.001, One way ANOVA with Holm Sidak post hoc test vs control (0 ng/ml). Experiments were performed on 3 independent cultures, each in triplicates (n=3). **B.** HIF1α target gene, PDK1 protein level determined by Western Blot. DUX4 expression was induced with 62,5 ng/µl dox. N=3, *p<0.05, Rank sum test. **C.** Expression of *HIF1α* and its target genes *PDK1* and *VEGF* normalized to *RPLP0* (RT-qPCR). DUX4 expression was induced with 62,5 ng/µl dox. N=4 for myoblasts and N=3 for myocytes and myotubes, mean +/- SEM, *p<0.05, **p<0.01, T-test.

➡ **UPON DUX4 EXPRESSION, HIF1α PATHWAY IS REPRESSED IN PROLIFERATING MYOBLASTS BUT INDUCED IN MYOTUBES**

Effect of DUX4 expression on HIF1α pathway *in vivo*



A.IMEP model diagram. Experimental set up **B.Effect of DUX4 induction on HIF1α pathway in the IMEP model.** RT-qPCR were performed on proximal and medial *tibialis anterior* muscle parts. Gene expressions were normalized to RPLP0. Kruskal Wallis followed by a Dunn's post-hoc test, *p<0.05. N=8. 8 week old mice were injected and electroporated in the *tibialis anterior* with either, *pCIneo-DUX4* plasmide (DUX4 expressing vector), *pCIneo* (control plasmid) or saline solution.

➡ **UPON DUX4 EXPRESSION, HIF1α PATHWAY IS INCREASED AT EARLY TIME POINTS IN MATURE MYOFIBERS**

CONCLUSION

HIF1α pathway is modulated upon **DUX4** expression but these modifications are dependent on the differentiation stage of muscle cells. Indeed, **HIF1α** pathway is inhibited in human myoblasts and activated in human myotubes and murine mature myofibers.

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