Fih gene deletion decreases cell proliferation and viability of H9c2 rat cardiomyoblasts during nutrient starvation

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Introduction

Adaption to decreased oxygen availability (hypoxia) is a vital process for cells, enabled by the capability to sense local oxygen levels. Factor inhibiting HIF (FIH) is a major cellular oxygen sensor, regulating the α subunit of the dimeric transcription factor hypoxia-inducible factor 1 (HIF-1). In normoxia, FIH hydroxylates HIF-1α, inhibiting HIF-1-mediated gene transcription. In hypoxia, HIF-1α is no longer hydroxylated by FIH, resulting in the enhanced transcription of HIF-1 target genes. Deletion of FIH in mice ($FIH^{-/-}$) leads to a hypermetabolic state, with increased energy expenditure and enhanced insulin sensitivity. Additionally, FIH knockout (KO) protected mice against high fat diet-induced weight gain, demonstrating the relevance of FIH in regulating energy metabolism. However, it remains unclear if FIH deletion or inhibition may be beneficial for the treatment of diseases, in which the disease progression is accompanied or driven by an altered energy metabolism**. Therefore, we aimed to investigated the effect of nutrient depletion on wildtype and FIH KO rat cardiomyoblasts (H9c2) to gain insights into the potential relevance of FIH in myocardial infarction.**

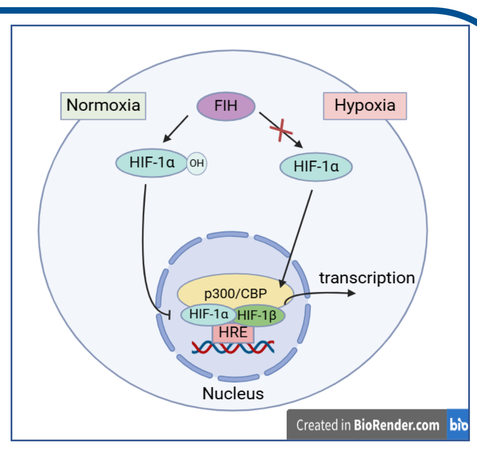

Fig. 1: FIH-mediated regulation of HIF-1α. In normoxia, FIH-dependent HIF-1α hydroxylation inhibits its interaction with transcriptional co-activators. This inhibition is lost in hypoxia due to the inactivity of FIH.

Results

Characterization of FIH KO in H9c2 cells

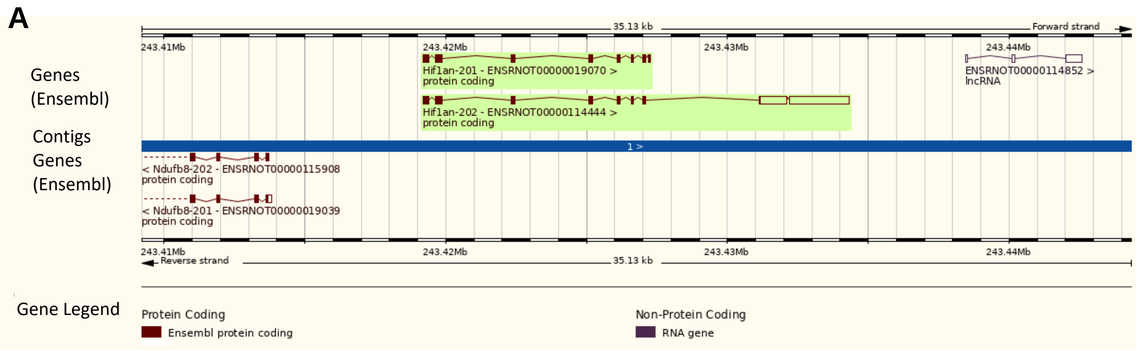

Fig. 2: The cells were purchased from Ubigene (Guangzhou, China). **(A)** *Fih* gene as displayed in the UCSC genome browser (https://genome.ucsc.edu/). **(B)** FIH knockout strategy. The knockout was accomplished with the CRISPR/Cas system by targeting a part of Exon 1 of the *Fih* gene. This resulted in a frameshift and a deletion of the FIH protein. **(C)** Immunoblot-based detection of FIH in wildtype (WT), FIH knockout clone 1 (KO 1) and clone 2 (KO 2) H9c2 cells.

FIH KO decreases cellular growth in the presence of nutrients

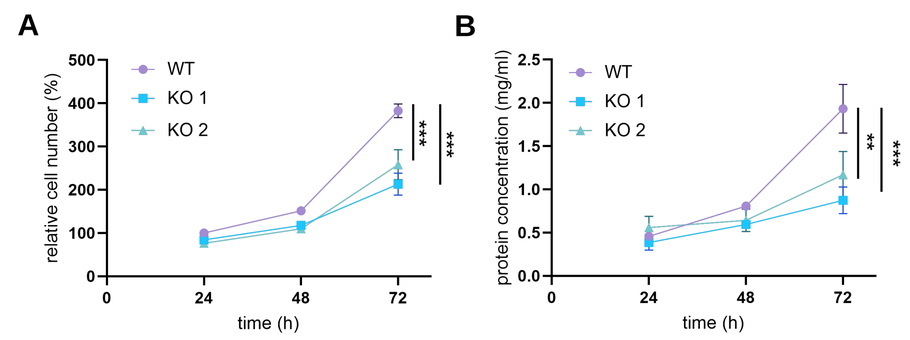

Fig. 3: (A) Cellular growth and **(B)** protein concentration of H9c2 wildtype (WT) as well as FIH KO clone 1 (KO 1) and clone 2 (KO 2) at the indicated time points after seeding. (A). Significance tested with two-way ANOVA followed by Tukey-test. **, $p < 0.01$; ***, $p < 0.001$; n = 3**.** The results indicate that the FIH KO decreseas cellular growth in the presence of nutrients. This could be explained by effects on cell proliferation or cell death. To further investigate this effect, cell proliferation and cell death were analyzed. The results are shown in the following figures.

FIH KO does not effect the ATP levels in nutrient depletion conditions

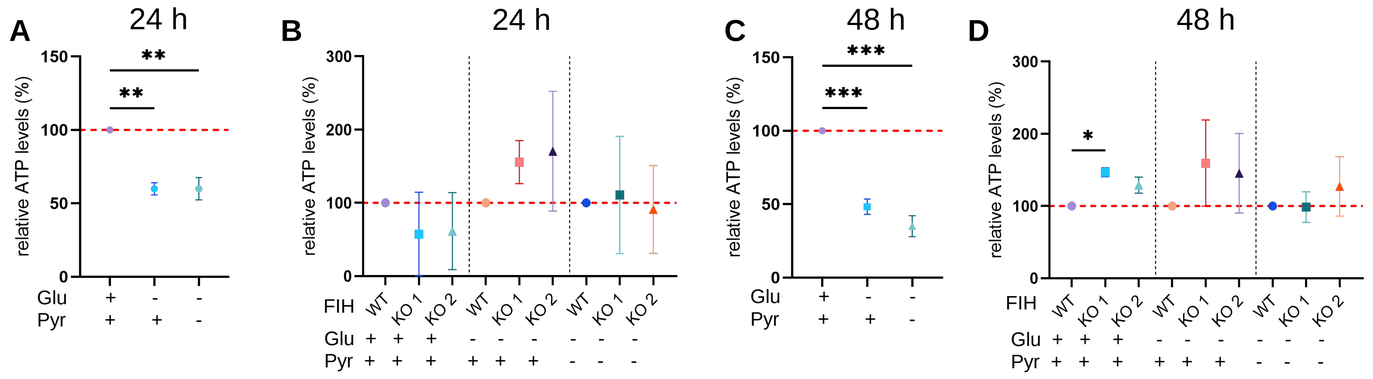

Fig. 4: Effect of the depletion of glucose (Glu) alone or in combination with pyruvate (Pyr) on the ATP concentration in WT cells after **(A)** 24 h or **(C)** 48 h of incubation compared to full growth medium. Relative luminescence was normalized to the corresponding protein concentrations. **(B, D)** ATP levels in WT, FIH KO clone 1 (KO 1) and clone 2 (KO 1) cells after 24 h or 48 h of incubation in the different conditions. Relative Luminescence was normalized to the relative protein concentrations. Values were normalized to the ATP concentrations of H9c2 WT cells in the respective condition. Significance was tested with (B,D) One Sample T-Tests or (A,C) one-way Anova followed by Tukey Test. **, $p < 0.01$; ***, $p < 0.001$, n = 3.

FIH KO increases cell death

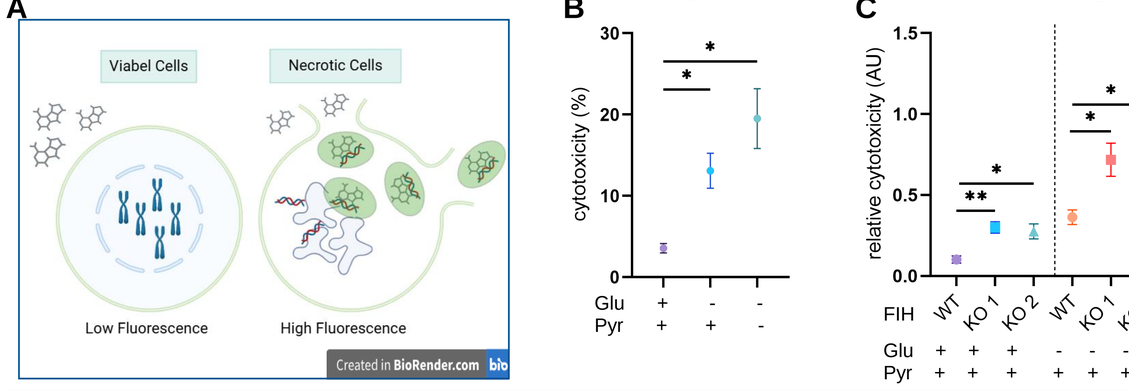

Fig. 5: (A) Method of the cytotoxicity assay. Effect of the depletion of glucose (Glu) alone or in combination with pyruvate (Pyr) on the cell death of H9c2 WT cells compared to complete medium after incubation for **(B)** 24 h or **(D)** 48 h. **(C,E)** Cell death analysis after 24 h or 48 h, as indicated. Relative cell death is displayed in arbitrary units (AU), normalized to the sum of H9c2 WT cell death in the respective medium. Significance tested with students t-test. *, $p < 0.05$; **, $p < 0.01$; n=3.

FIH KO decreases cell proliferation

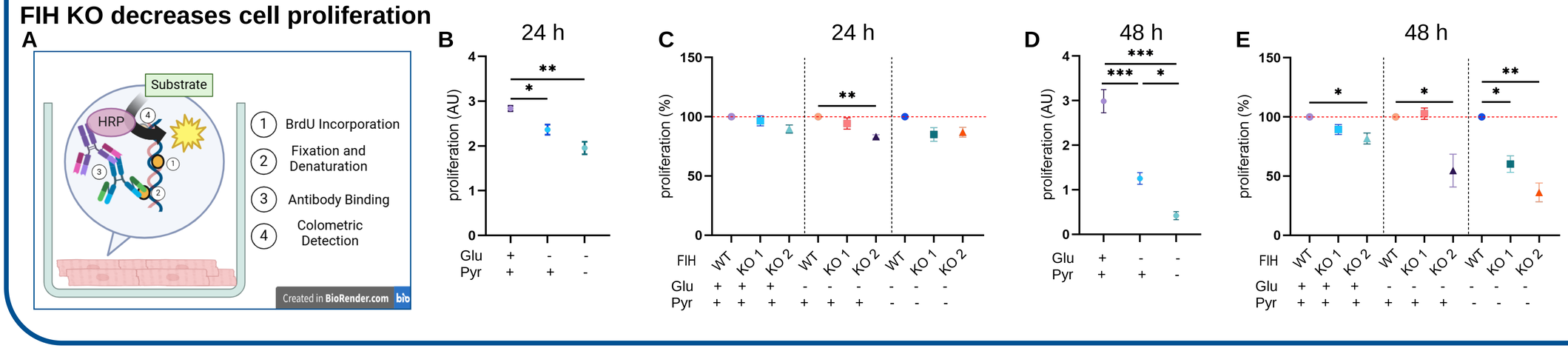

Fig. 6: (A) Method of the proliferation assay. Effect of the depletion of glucose (Glu) alone or in combination with pyruvate (Pyr) on the proliferation of **(B, D)** H9c2 WT and **(C, E)** H9c2 WT vs. FIH KO clone 1 (KO 1) and clone 2 (KO 2) at the indicated time points. The proliferation is displayed in percent relative to H9c2 WT cells in the respective medium. Significance tested with one sample t-test; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; n=3. AU, arbitrary units.

Conclusions

FIH KO in H9c2 cardiomyoblasts:

- does not affect ATP levels
- decreases cellular growth even in the presence of nutrients
- decreases cell proliferation during nutrient starvation
- increases cell death in all tested nutrient condition

→ **FIH activity is necessary to maintain appropriate cell growth in H9c2 cells**

Outlook

- Extention of the myocardial infarction model (pH-value and hypoxia)
- Investigation of the effect of *Fih* KO in different cardiomyoblast cell lines
- Comparison of the effect of a PHD-selective with a pan-hydroxylase inhibitor
- Analysis of the effect of a FIH-selective inhibitor
- Heart infarct model in *Fih* KO mice