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Coupled effect of a miR-155 deficiency and hypoxia conditions leads to the upregulation of SLC2A1 (GLUT1), SLC2A3 (GLUT3) and EGLN1 genes in the leukemic B-cells

Elena Golovina[1], Tomas Heizer[1,3], Lenka Daumova[2], Martin Bajecny[1], Karina Savvulidi Vargova[1]

1 Institute of Pathological Physiology, First Faculty of Medicine, Charles University, Prague, Czech Republic
2 Institute Biocev, First Faculty of Medicine, Charles University, Vestec, Czech Republic
3 Center for Advanced Preclinical Imaging (CAPI), First Faculty of Medicine, Charles University, Prague, Czech Republic

Introduction:

Leukemic cells in hypoxia are forced to reprogram their original transcriptome, miRNome, and metabolome. How the coupling of miRNAs/mRNAs helps to maintain or progress the leukemic status is still not fully described. In the present study, we aimed to uncover the impact of hsa-miR-155-5p (miR-155, *MIR155HG*) on the metabolism, proliferation, and mRNA/miRNA network of human chronic lymphocytic leukemia cells (CLL) in hypoxia conditions. Here we used as a model of CLL the human MEC-1 cell line where we deleted mature miR-155 with the CRISPR/Cas9 tool. However, hypoxia increases the proliferation of MEC-1 cells; the miR-155 deficiency overall reduces cell proliferation. The miR-155 deficiency under hypoxia condition was accompanied by a level-up of apoptosis. Besides the common hypoxia-related genes (*HIF1α, EGLN1, VHL, HK1*, and *HK2*) we also examined glucose-transporters genes *SLC2A1* (*GLUT1*) and *SLC2A3* (*GLUT3*) by qRT-PCR. In hypoxia, miR-155 deficient MEC-1 cells significantly raised the expression of *EGLN1, GLUT1*, and *GLUT3* genes, which points out on possible novel targets of miR-155 in CLL. Consistently, the qRT-PCR and the metabolic assays showed the significant influence of miR-155 on glucose and lactate metabolism. In addition to this, we detected considerable overexpression of hsa-miR-210-5p, a well-known hypoxamiRNA. To conclude, the coupled effect of miR-155 deficiency and hypoxia affects glucose and lactate metabolism that stimulates the expression of *GLUT1, GLUT3*, and *EGLN1* genes in MEC-1 CLL cells. Effective inhibition of key metabolites in the leukemic cells could be one of the novel options in the current treatment of CLL.

Results:

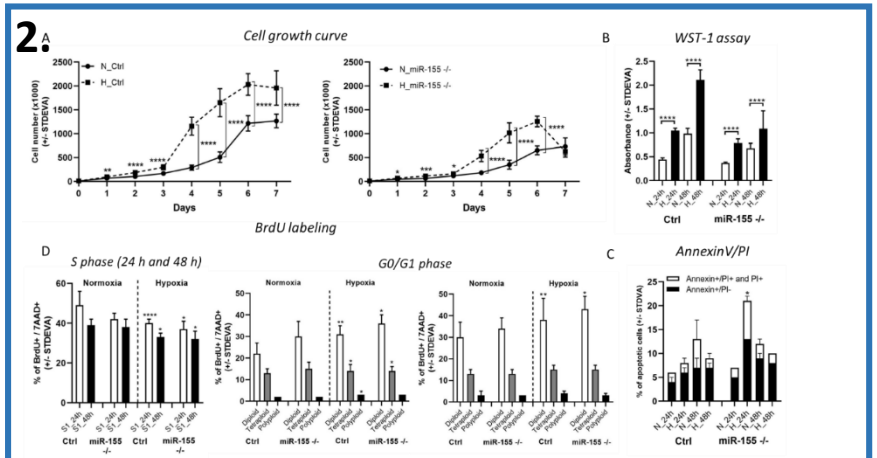

Figure 2. Proliferation and cell viability of MEC-1 cells during hypoxia. A The cell growth curve of MEC-1 cells (ctrl and miR-155 -/-) in normoxia (N) vs hypoxia (H). Cells were counted within 7 days in hemocytometer chamber. **B** Cell viability was measured by WST-1 assay in both normoxia vs hypoxia in 24 h and 48 h time periods. **C** Percentage of apoptotic cells measured by flow cytometry after Annexin V / PI staining. Cells were stained in parallel in normoxia and hy-poxia conditions in 24 h, 48 h. **D** Detection of the cell cycle kinetics by BrdU labelling in normoxia vs hypoxia in 24 h, 48 h. Left graph shows percentage of BrdU/7AAD positive cells. Right graphs depict population of G0/G1. All data are from three independent experiments and for statistics t-test, two tailed, paired was used (* p<0.05; ** p<0.01; *** p<0.001; ****p<0.0001).

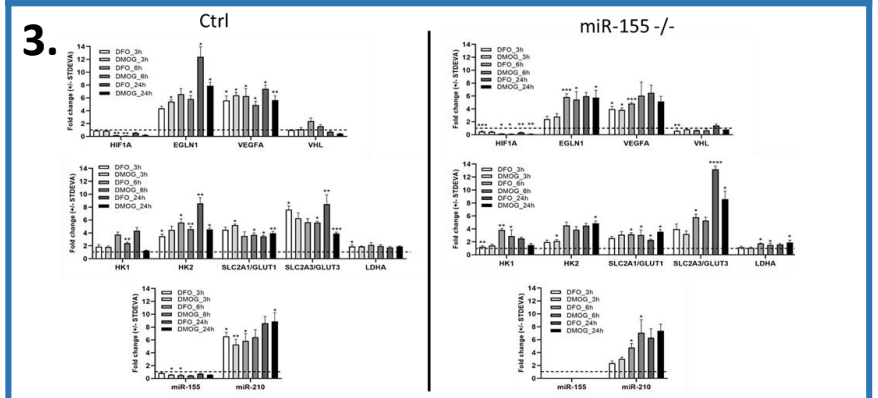

Figure 3. Chemically induced hypoxia *in vitro* in MEC-1 cells. MEC-1 cells were treated with chemicals DFO and DMOG for 3 h, 6 h, 24 h and in parallel in normoxia (NONE). Gene expression profile contains the same set up of genes as in hypoxia experiments performed in hypoxia glove box. In all graphs, expression data were normalized to expression level in normoxia and set as 1. Data are from three independent experiments. Statistics t-test, two tailed, paired was used, (*p<0.05; **p<0.01; *** p<0.001; ****p<0.0001).

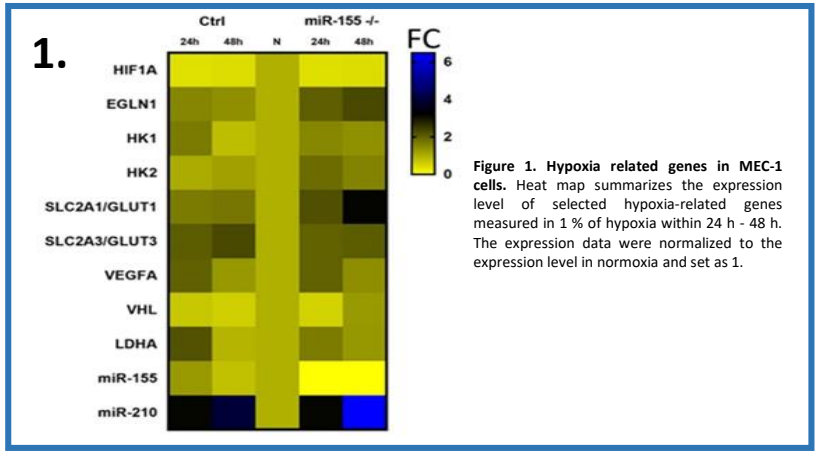

Figure 1. Hypoxia related genes in MEC-1 cells. Heat map summarizes the expression level of selected hypoxia-related genes measured in 1 % of hypoxia within 24 h - 48 h. The expression data were normalized to the expression level in normoxia and set as 1.

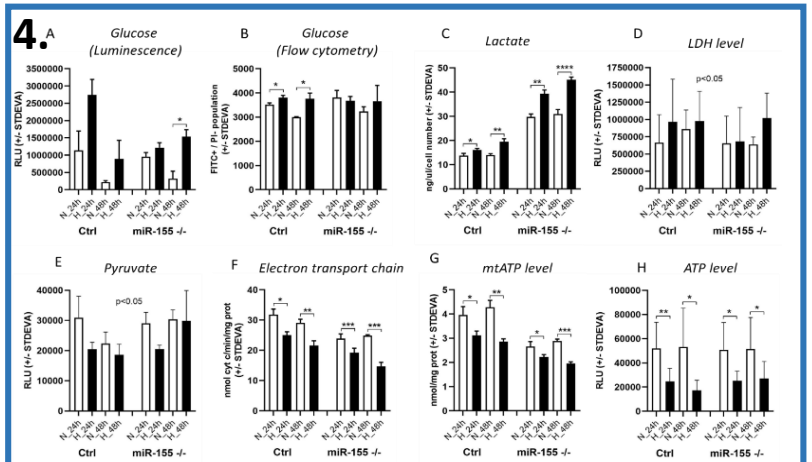

Figure 4. Measurement of metabolism in MEC-1 cells in normoxia and hypoxia. For the assessment of glucose uptake in MEC-1 cells (ctrl and miR-155 -/-) in normoxia vs hypoxia we used either Glucose Uptake-GloTM Assay **A** or 2-NBDG fluorescent molecule **B**. **C** Shows the concentration of Lactate measured by colorimetric assay. **D** LDH level was measurement by LDH-Glo™ Cytotoxicity Assay. **E** Pyruvat concentration was measured by Pyruvate Assay Kit. **F** The electron transport chain form com-plex I to complex III (ETC) was measured by following the protocol in [Wibom R et al, 2002]. **G** mtATP level was measured by Adenosine 5′-triphosphate (ATP) Bioluminescent Assay Kit. **H** ATP level was measured by CellTiter-Glo® 2.0 Cell Viability Assay. All data are from three independent experiments. Statistics t-test, two-tailed, paired was used, (*p<0.05; **p<0.01; ***p<0.001; ****p<0.0001).

Conclusion:

Deletion of miR-155 inhibits cell growth of MEC-1 cells in normoxia and also hypoxia. Hypoxia-related genes such as *EGLN1, VEGFA, HK1, HK2, LDHA* and especially *GLUT1, GLUT3* are upregulated in hypoxia. MiR-155 deficiency and hypoxia results in downregulation of glucose uptake, upregulation of lactate uptake, downregulation of ATP production.

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