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PB1787 PLZF-RARA ENHANCES METABOLIC PLASTICITY AND ROS SCAVENGING IN AML CELLS

Topic: 3. Acute myeloid leukemia - Biology & Translational Research

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Background:

PLZF-RARα (ZBTB16-RARα) fusion protein binds retinoic acid response elements (RARE) to repress transcription of genes essential for myeloid maturation and differentiation, driving a rare acute promyelocytic leukemia (APL) variant (2% of APLs). Metabolic reprogramming is the hallmark of cancer: it confers increasing flexibility in nutrient uptake and supports cell growth and malignant progression

Aims:

We studied PLZF-RARα bearing cells' metabolism in order to define possible targets for tailored therapeutic strategies.

Methods:

To define PLZF-RARα induced metabolism features via Seahorse Bioscience XFe96 analysis, we used an inducible cellular system: U937-B412 cells (B412) containing a $ZnSO_4$ inducible construct, and control U937-MT (MT) cells.

To carry out the metabolomic profiling of lysates and supernatants samples of B412 and MT cells, we used ^{1}H-NMR spectroscopy. The NMR spectra were acquired in a 600 MHz spectrometer, and they were analyzed with the Chenomx NMR suite 9.0 software. The statistical analysis was performed using the MetaboAnalyst 5.0 platform (https://www.metaboanalyst.ca). In addition, we carried out the ROS MitoSOX-based assay and analyzed the ROS and glycolytic related NRF2, PFKP, and HK2 by Western blot and Immunofluorescence analysis.

Results:

PLZF-RARα expression induces: (i) mitochondrial respiration enhancement [basal oxidative phosphorylation (OXPHOS) (MT: 95±6 B412: 135±14 (pmol/min)/6x104 cells), p<0.0001), respiratory reserve (MT: 93±41 B412: 155±55 (pmol/min)/6x104 cells), p=0.01) and ATP production (MT: 65±20 vs B412: 96±23 (pmol/min)/6x104 cells), p=0.008)]; (ii) glycolysis reduction (MT: 665±55 B412: 574±47 (pmol/min)/6x104 cells), p=0.01). These results were confirmed by enhanced mitochondrial ATP production and reduction of glycolytic ATP (XF Real-Time ATP Rate Assay); (iii) enhanced substrate flexibility (pyruvate, glutamine and fatty acids).

By NMR, we identified around 50 metabolites in the lysates and supernatants. The heat map highlights differences between the metabolic composition of lysates and supernatants. The analysis of the metabolome data is in progress. However, preliminary results show an increased concentration of TCA intermediates in B412 vs MT lysates, which is in line with an enhanced mitochondrial respiration for B412. Regarding the supernatants, in B412, we detected a differential uptake of some amino acids for B412 vs MT. For instance, the increased consumption of Threonine and Asparagine, which could be converted to Acetyl-coA and Oxaloacetate, respectively, is in accordance with increased mitochondrial metabolism. Also, the higher consumption of Glycerol in B412s can be

supporting lipid synthesis. 2-OH-Butyrate, a by-product of glutathione production which provides an indication of the antioxidant activity of glutathione, is more secreted into the medium in the presence of PLZF-RARα. Despite NRF2 whole cellular levels are decreased in the presence of PLZF-RARα, its nuclear fraction at six hours increases. Importantly, after ascorbic treatment (3 mM), PLZF-RARα expressing cells increased their ROS scavenger capacity, abating ROS quantity with respect to control cells. These results are in line with the increased resistance to therapy showed by PLZF-RARa AML patients

Summary/Conclusion:

Our results show how PLZF-RARα influences cellular metabolism, explaining its capability to induce flexibility and resistance to therapy.