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Impaired mitochondrial metabolism drives T–cell dysfunction in chronic lymphocytic leukemia patients

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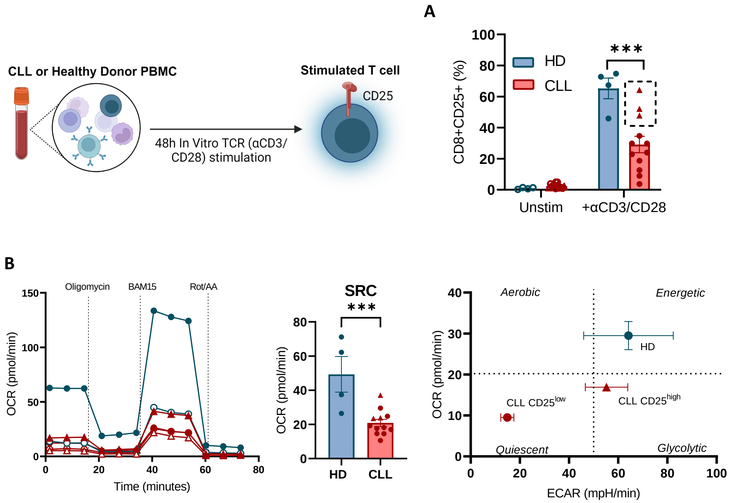
Background

Chronic lymphocytic leukemia (CLL) remains incurable despite implementation of novel targeted therapies. Successful autologous cell-based anti-cancer therapies require functionality and longevity of effector cells, features that highly depend on complex metabolic processes. Our group previously demonstrated a disease-specific dysfunction in CLL T-cells including signs of impaired metabolic plasticity (Van Bruggen, 2019 Blood). However, in-depth analysis of the metabolic phenotype of CLL T cells and mechanisms underlying the dysfunction are lacking, preventing development of therapeutic strategies aiming to overcome dysfunction by metabolic reprogramming.

1. Upon activation, CLL T cells fail to induce mitochondrial metabolism

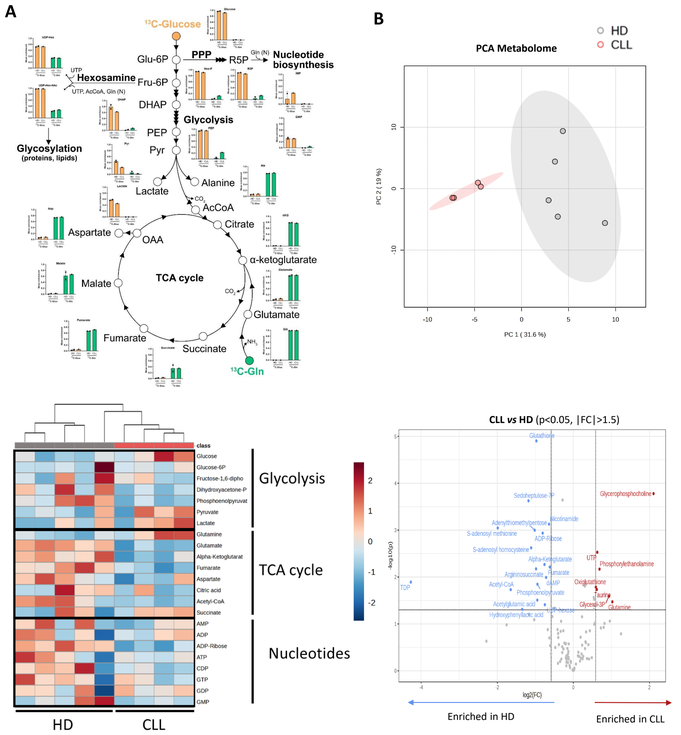
T cell activation levels are variable and correlate with glycolytic activity, while mitochondrial metabolism is decreased in all CLL patients.



Activation and real-time metabolic activity of T cells from healthy individuals (HD) and CLL patients. PBMCs from HD or CLL patients were stimulated for 48h. A) T-cell activation was measured by CD25 surface expression. B) Extracellular flux analysis was performed on isolated T cells with the T-cell metabolic profiling test on a Seahorse XF HS. A representative measurement of oxygen consumption rate (OCR) over time together with the spare respiratory capacity (SRC) of all measured samples. Additionally, OCR and extracellular acidification rate (ECAR) are represented together in an energy map.. Data are presented as mean ± SEM and differences were analyzed with two-way ANOVA or unpaired t test (*** p < 0,001).

2. Reduced mitochondrial metabolites and antioxidants in CLL T cells

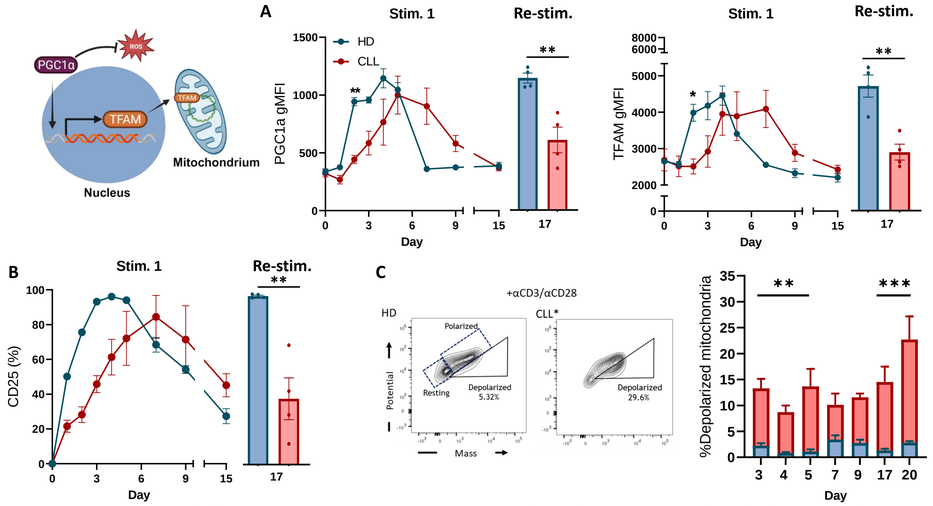
CLL T-cells exhibited distinct metabolomes, including reduced TCA cycle intermediates and nucleotides, alongside higher oxidized/reduced glutathione ratios indicating mitochondrial dysfunction and increased ROS.



13C isotope tracing and metabolomics analysis of stimulated T cells from HD and CLL patients. PBMCs from healthy individuals (HD) or CLL patients were stimulated for 48h. CD4 and CD8+ cells were FACS-sorted and A) subsequently cultured in 13C-glucose (11mM 13C6-glucose, 2mM unlabeled Gln: in orange) or 13C-Gln (11mM unlabeled glucose, 2mM 13C5-Gln: in green) for 4h and pelleted for LC-MS isotope analysis. Data are shown as mean enrichment (fraction of 13C-labelled molecules relative to the total amount of a given metabolite within each sample). B) Cells were pelleted right after sorting for LC-MS metabolomics analysis. Metabolite abundance was normalized by total metabolome pool in each sample and auto scaled. Principal component and statistical analysis, as shown by volcano plot comparing metabolite abundance in CLL T cells vs HD T cells, were performed on total metabolome. Heatmap of pathway-specific metabolites was generated with MetaboAnalyst 5.0.

3. Impaired expression of mitochondrial regulators and polarization along CLL T cell response

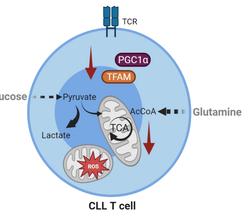
Upregulation of both PGC1a and TFAM upon stimulation was delayed in CLL T cells compared to healthy T cells, and especially impaired after the second stimulation. Concurrently CLLT cells show increased mitochondrial depolarization during the whole T cell response.



Mitochondrial regulators and (de)polarization during T cell response to stimulation (stim. 1) and re-stimulation (stim. 2). A) Expression of Peroxisome proliferator-activated receptor Gamma Coactivator 1-alpha (PGC1α) and Mitochondrial Transcription factor A (TFAM) was measured over time using Flow Cytometry. B) Concurrently we measured activation with CD25 levels and C) Mitochondrial depolarization by stainingwith MitoTracker Green and MitoTracker Orange to identifiy cell populations with a decreased ratio of mitochondrial potential compared tp mass upon activation. Data are presented as mean ± SEM and differences were analyzed with two-way ANOVA or unpaired t test (*p < 0,05; **p < 0,01; ***p < 0,001).

Discussion and future directions

- CLL T cells in patients show impaired mitochondrial activity and function, upon activation.
- Key mitochondrial regulators exhibit delayed expression during (re)stimulation
- Compromised mitochondrial function can significantly impact T cell effector and memory development.
- Future research aims to elucidate the mechanisms of mitochondrial dysfunction in CLL T cells and devise strategies for enhancing mitochondrial function.



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