SOHO ANNUAL MEETING 2024

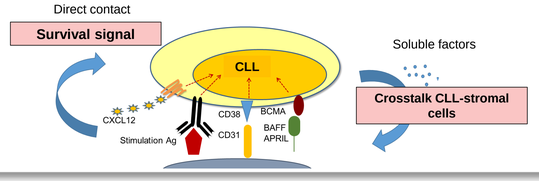
METABOLIC REPROGRAMMING OF BONE MARROW STROMAL CELLS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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INTRODUCTION

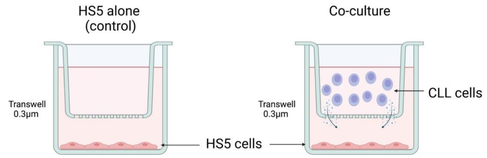
In chronic lymphocytic leukemia (CLL) tumor cell survival depends on a multitude of supportive signals delivered by the immune and stromal surrounding cells of the microenvironment. Through cell-to-cell direct contact or soluble factors production, the microenvironment promotes chemotaxis, homing, survival and proliferation of CLL cells within the lymph nodes and the bone marrow by activating pathways in the tumor cells.
On the other hand, CLL cells are able to educate the surrounding immune and stromal cells to set up a protective environment leading to tumor evasion and production of soluble factors by the latter, which highlights the importance of the crosstalk between the tumor cells and the microenvironment.



AIM

This study aimed to understand **how CLL cells alter stromal cells via soluble factors**.

METHOD

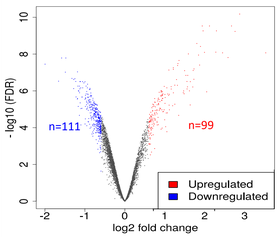


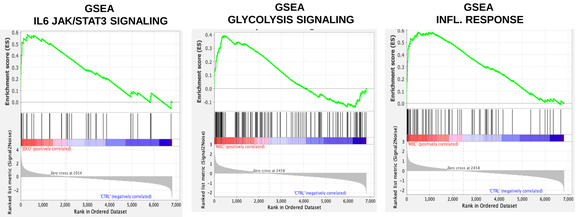
RNA-seq and **mass spectrometry** were performed in the bone marrow mesenchymal stromal cell line HS5, as a surrogate of the microenvironment in co-culture with the CLL cell line MEC1. HS5 and MEC-1 cells were co-cultured separated by a transwell to avoid direct contact in order to analyze specifically the effect on the stromal cells of soluble factors produced by CLL cells. After 24 hours of co-culture, both RNA and proteins were extracted from HS5 cells for transcriptome and proteome analyses.

The **cellular oxygen consumption rate (OCR)** and the **extra-cellular acidification rate (ECAR)** was assed using the Seahorse platform to **quantify the mitochondrial respiration** and the **glycolysis respectively**. HS5 were either alone or cultured with conditioned media (CM) issued from primary CLL cells culture.

RESULTS

Gene expression profiling of stromal cells





Analysis in HS5 co-cultured with MEC vs HS5 alone shows that **CLL cells produce soluble factors** that specifically **modify the gene expression profile of stromal cells**

Gene set enrichment analysis (GSEA) of these differentially expressed genes revealed a significant enrichment of the **IL6-dependent JAK/STAT3 pathway** (Normalized enrichment score NES=2.2), the **inflammatory response** (NES=2.5), the **glycolysis** (NES=1.9) (all with False Discovery Rate <0.001).

Proteomic analysis of stromal cells

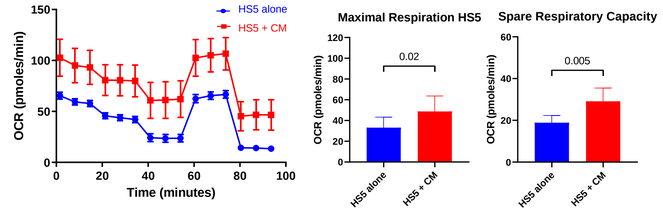
Analysis in HS5 co-cultured with MEC vs HS5 alone shows the presence of **mitochondrial** and **metabolism proteins among the top upregulated proteins** :

Protein	Function
AKR1B1	Mitochondrial protein involved in the respiratory chain
PLOD1	Mitochondrial protein involved in the respiratory chain
MCAD	Acetyl CoA-dehydrogenase
UFA10	Subunit of the NADH dehydrogenase

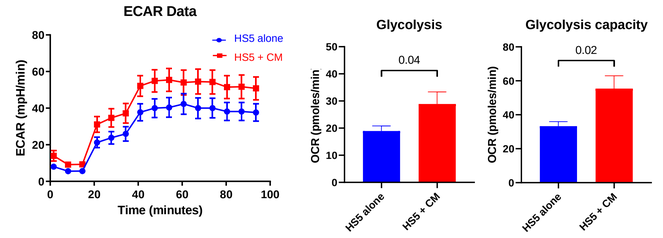
Annotation Cluster 1	Enrichment Score: 1.05			Count	P_Value
GOTERM_BP_DIRECT	oxidation-reduction process	RT		4	2.1E-2
UP_KEYWORDS	Oxidoreductase	RT		3	1.0E-1

Clustering and pathway analysis of the proteomic data showed a protein **signature related to the oxidation-reduction process.**

Metabolic profiling of stromal cells



Analysis of the OCR showed a **significant increase of the maximal respiration** and of **the spare respiratory capacity** in **HS5 cells with CLL CM** (conditionned media) as compared to HS5 cells alone.



Analysis of the ECAR also revealed a **significant increase of the glycolysis** and **the glycolysis capacity in the HS5 cells upon exposure to CLL CM** (conditonned Media).

CONCLUSIONS

- These results demonstrate that **CLL cells increase the production of energy in surrounding stromal** cells by **enhancement of the glycolysis** and by **promoting mitochondrial-based oxidative phosphorylation**.
- This bioenergetic shift induced by CLL cells **could enhance stromal cells fitness**, which therefore will **benefit to tumoral cell survival**.
- We are currently investigating how this crosstalk interferes with the effect of **BCR pathway inhibitors or BH3-mimetics**. Targeting this axis may represent a therapeutic strategy of interest.

CONTACT INFORMATION

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