METABOLOMIC CHARACTERIZATION OF HUMAN MULTIPLE MYELOMA CELL LINES TO STUDY TUMOR RESISTANCE TO DIFFERENT CLASSES OF THERAPEUTIC AGENTS

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Introduction

Multiple myeloma (MM) is the second most common hematological malignancy, characterized by the abnormal accumulation of plasma cells in the bone marrow. Although the latest treatments, have greatly improved patient survival, a residual subset of cells remains resistant to therapies and usually causes relapses. Among the factors influencing the resistance of cancer cells, the "metabolic plasticity" of the tumor and, therefore, its ability to adapt to stress conditions is a mechanism increasingly studied in recent years in cancer. Although measuring mitochondrial metabolism has been identified as a major factor influencing response to treatments in several cancers, few studies have been documented in MM. Here, we aim to characterize the metabolic profile of a panel of 20 Human MM cell lines (HMCLs) representative of the molecular heterogeneity found in MM patients.

Materials and methods

A.

Name	HMCL	MM Sub-group
AMO1	HMCLs	CD2L; t(12;14)
JJN3	HMCLs	MF; t(14;16)
KM12BM	HMCLs	CD2L; t(11;14)
L363	HMCLs	MF; t(20;22)
LP1	HMCLs	MS; t(4;14)
MM1S	HMCLs	CD2L; t(14;16)
MOLP8	HMCLs	CD2L; t(11;14)
OPM2	HMCLs	MS; t(4;14)
RPMI 8226	HMCLs	MF; t(14;16)
SKMM2	HMCLs	CD-1; t(11;14)
XG1	Proprietary HMCLs	CTA/FRBZ; t(11;14)
XG11	Proprietary HMCLs	CD-1; t(11;14)
XG16	Proprietary HMCLs	CTA/FRBZ
XG2	Proprietary HMCLs	CTA/FRBZ; t(12;14)
XG20	Proprietary HMCLs	MS; t(4;14)
XG24	Proprietary HMCLs	MS; t(4;14)
XG28	Proprietary HMCLs	MS; t(4;14)
XG30	Proprietary HMCLs	MS; t(4;14)
XG6	Proprietary HMCLs	MF; t(16;22)
XG7	Proprietary HMCLs	MS; t(4;14)

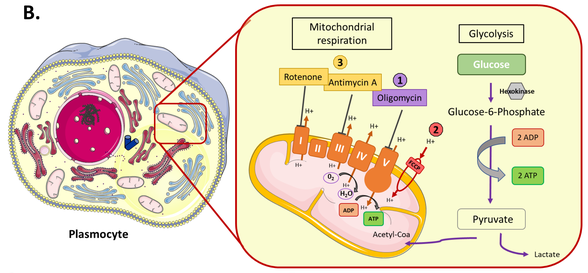

Figure 1 : Materials and Methods. **A.** Table of human myeloma cell lines used in the study representative of the molecular heterogeneity found in MM patients. **B**. Schematic overview of the two major energy-producing pathways in the cell : Glycolysis and mitochondrial oxidative phosphorylation (Oxphos) with the mitoStress Assay principle in MM. **C**. Detection of the mitochondrial respiration by measurement of the Oxygen consumption rate (OCR) with the Seahorse XF96 Mito stress kit using the optimal cell seeding density **D.** Detection of the glycolytic activity by measurement of the Extracellular acidification rate (ECAR) with the Seahorse XF96 Mito stress kit using the optimal cell seeding density

Heterogeneity of the metabolic profiles in HMCLs

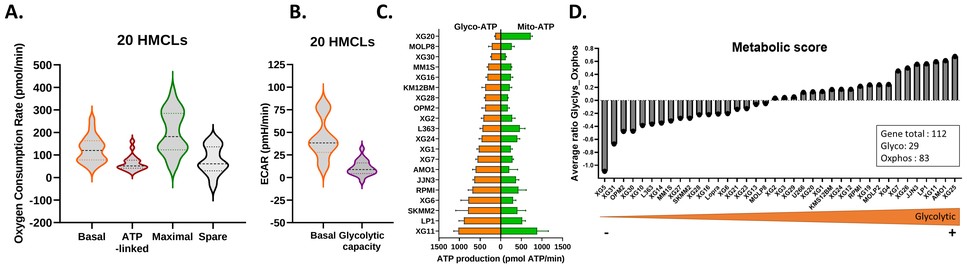

Figure 2 : Heterogeneity of the metabolic profiles in HMCLs
A. Basal, ATP-linked, Maximal and Spare OCR are heterogeneous in the 20 HMCLs. **B**. Basal ECAR as well glycolytic capacity are heterogeneous in the 20 HMCLs . **C**. Calculated glycolytic and mitochondrial ATP-production of the 20 HMCLs. **F**. Generation of Metabolic score in the HMCLs based on 112 genes including 29 glycolytic genes and 83 Oxphos genes.

GEP-based metabolic score is representative of the metabolic activities in MM cells

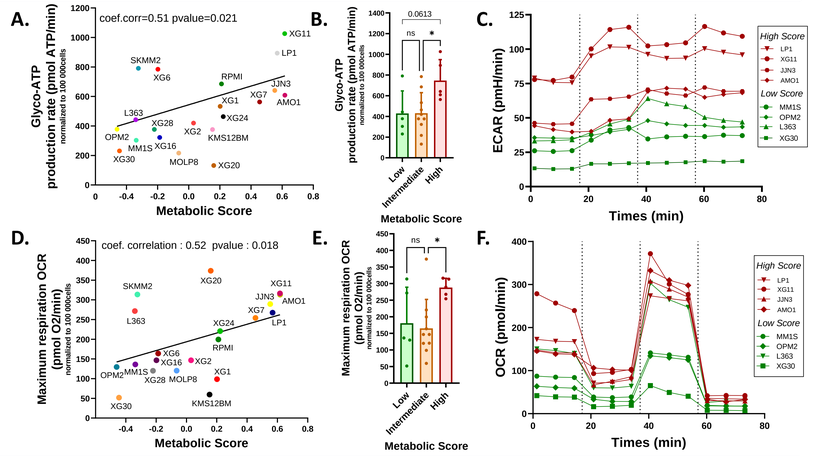

Figure 3 : GEP-based metabolic score is representative of the metabolic activities in MM cells. **A.** Correlation between Glycolytic ATP production rate and the genomic score. **B.** HMCLs were classified by the quartile method into low, intermediate and high score. HMCLs with a higher metabolic score produce more ATP from glycolysis than HMCLs with in lower score. **C**. ECAR representation of the 5 HMCLs with the higher score compared to the 5 HMCLs with the lower score. **D**. Correlation between Mitochondrial ATP production rate and the genomic score. **E.** HMCLs were classified by the quartile method into low, intermediate and high score. HMCLs with a higher metabolic score produce more ATP from mitochondria than HMCLs with in lower score. **F**. OCR representation of the 5 HMCLs with the higher score compared to the 5 HMCLs with the lower score. "ns" present for no significant and *p < 0.05 analyzed by one-way ANOVA test followed by Tukey's test (means ± SD).

High metabolic score values are associated with a poor outcome in MM patients

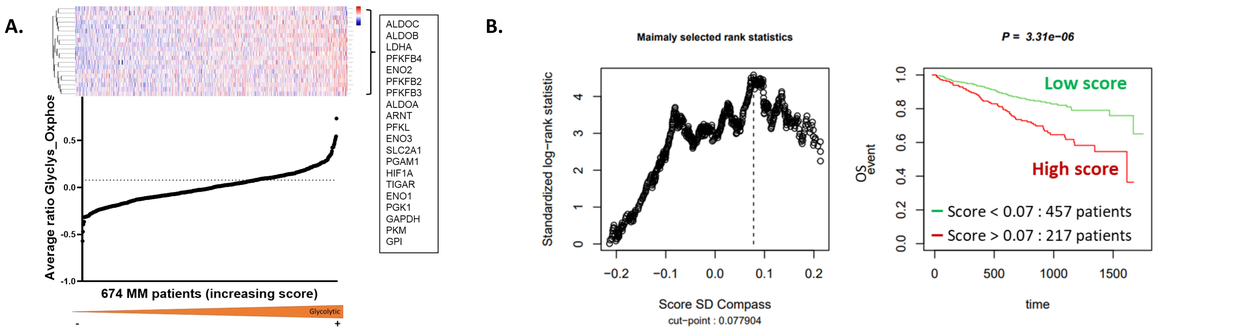

Figure 4 : High metabolic score values are associated with a poor outcome in MM patients. A. Representation of the Metabolic score in the patient from the CoMMpass Cohort based on 112 genes including 29 glycolytic genes and 83 Oxphos genes. **B.** Patients of the CoMMpass cohort (n = 674) were ranked according to the increased metabolic score. The maximum difference in OS was obtained using MaxStat with a cut off -0.07, splitting patients into high-risk (n = 217; red curve) and low-risk (n = 457; green curve) groups.

High mitochondrial ATP production is associated with Carfilzomib resistance in MM cells

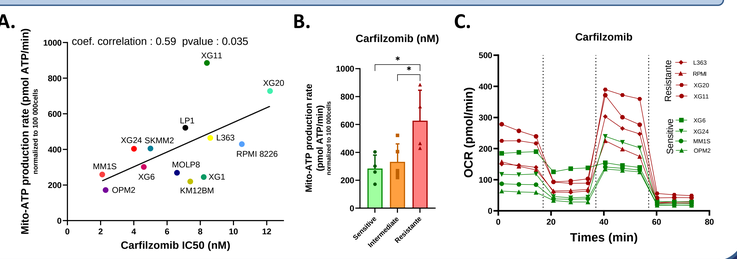

Figure 5 : High mitochondrial ATP production is associated with Carfilzomib resistance in MM cells. **A.** Correlation between Mitochondrial-ATP production rate and the increased of Carfilzomib IC50. **B.** Carfilzomib resistant HMCLs produce more Mitochondrial ATP than Carfilzomib sensitive and intermediary HMCLs. **C.** OCR representation of the most 5 Carfilzomib resistant HMCLs compared to the most 5 Carfilzomib sensitive HMCLs. *p < 0.05 analyzed by one-way ANOVA test followed by Tukey's test (means ± SD).

CONCLUSION

The metabolic activities were shown very heterogeneous in HMCLs. By integrating the HMCL's metabolic profiles with their respective transcriptomic data (RNAseq), we defined a metabolomic score to classify the HMCL into different groups representing of their glycolysis level. First, high significant correlations between the HMCL's functional metabolic profiles and their calculated metabolic score were identified. Secondly, the gene-based metabolomic score calculated in the MMRF CoMMpass cohort (newly diagnosed MM patients, n=674) confirmed metabolic heterogeneity in the patient with segregation of the cohort into two groups with a significantly different outcome. Thirdly, significant correlation between a high mitochondrial ATP production and the resistance to proteasome inhibitor (P = 0.035, n= 13) were observed.

=> Altogether, we demonstrated that metabolomic deregulation could participate in drug resistance in MM. Inhibitors targeting metabolic activities may be of therapeutic interest to overcome drug resistance in MM.