Analysis of the Metabolic State of Residual Multiple Myeloma Plasma Cells Reveals a Proliferative Subset with Glycolytic Gene Expression

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INTRODUCTION

Tumor cells are characterized by high reactive oxygen species (ROS) levels, low mitochondrial content and increased glycolytic capacity. Whereas cancer stem cells are reported to utilize oxidative phosphorylation (OxPhos) to produce ATP, a metabolic phenotype associated with stemness that constitutes a platform to understand the biology of residual and drug resistant cancer cells.

Previous data:

- Residual multiple myeloma plasma cells (MMPCs)
  - more mitochondria than normal PCs and MMPCs from active disease
  - lower ROS levels than MMPCs from active disease

To recapitulate the metabolic characteristics of residual MMPCs, myeloma cell lines (MMCLs) were cultured in medium with galactose substituted for glucose to force cells to use oxidative phosphorylation.

- Galactose-conditioned MMCLs:
  - reduced ROS levels
  - increased mitochondrial content
  - Increased sensitivity to drugs that target OxPhos
  - decreased sensitivity to drugs that target glycolysis

Hypothesis: The transition from dormancy to proliferative disease can be detected by determining the metabolic state of residual MMPCs.

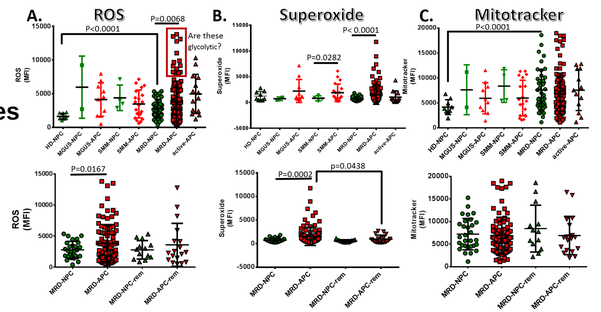
METHODS

- Detected by FACS:
  - In WBM of primary samples:
    - ROS (H_2DCFDA),
    - superoxide (DHE)
    - mitochondrial content (Mitotracker)
  - In MMCLs:
    - CD147
    - Glucose uptake (NBDG)
- Serum LDH was used as a marker of glycolytic activity and proliferation.
- Gene expression
  - gene expression profiling (GEP) by microarray
  - qRT-PCR in MMCLs and CD138-selected MMPCs from active disease

The metabolic score (MS) is derived from quartiles for mitochondrial content, superoxide, ROS and LDH level from measurements of residual MM cases. High superoxide and mitochondrial content (lower score) were considered to indicate dormancy and high ROS and LDH (higher score) indicate glycolytic and proliferative activity.

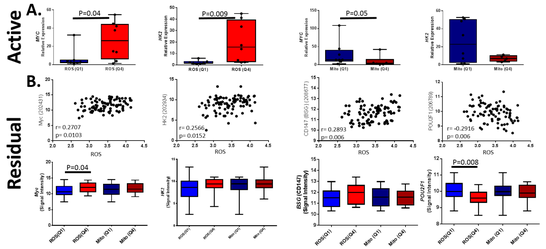
RESULTS

Figure 1: A subset of cases with residual MMPCs have elevated ROS levels.



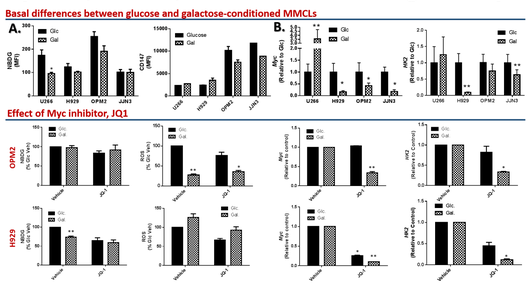
Basal metabolic parameters of PCs in whole bone marrow (WBM). Cells in RBC lysed WBM were stained to identify viable (DAPI$^-$), PCs (CD38, CD45). ROS level (*left*), superoxide (*middle*) or active mitochondria (*right*) was detected in normal (NPC) and abnormal PCs (APC) in healthy donor (HD, n=9), MGUS (n=10), SMM (n=20), MRD (n=113) and active MM (n=14) by FACS. A subset of residual MMPCs have **A**) higher ROS and **B**) superoxide than normal PCs; in most cases this was mutually exclusive. **C**) Mitochondrial content was variable in all groups but HD NPCs. Comparison of residual PCs from MM cases in remission to those that were not (*lower*) demonstrated that superoxide levels were lower in residual MMPCs from cases in remission (n=18) than those not in remission (n=95).

Figure 2: Residual cases with higher ROS levels have increased expression of glycolytic genes.



Expression of metabolic genes was detected by **A**) qRTPCR in CD138-selected cells from active MM and by **B**) GEP in archived samples from residual cases (n=89) with variable metabolic phenotypes. ROS levels were correlated with metabolic gene expression in residual cases (*middle*) and expression in extreme ROS and mitochondrial levels were compared (Q1 vs Q4, *lower*).

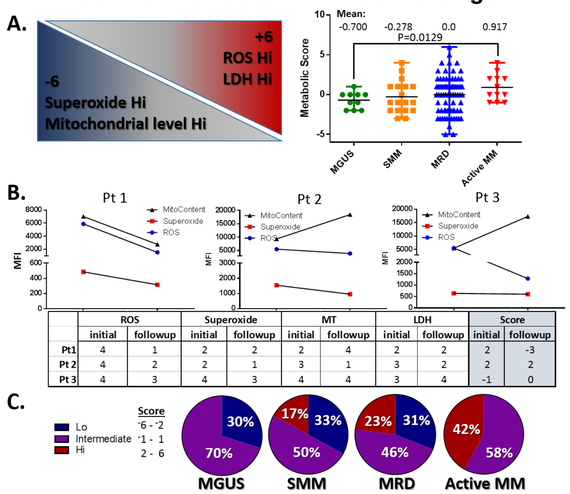
Figure 3: MM cell lines forced to use OxPhos have lower glucose uptake, CD147, and *HK2* and *MYC* gene expression



Differences in glycolytic parameters in MMCLs cultured in galactose compared to glucose **A**) MMCLS cultured in galactose had lower glucose uptake and CD147 expression than cells cultured in glucose. **B**) Expression of glycolytic genes *Myc* and *HK2* was lower in MMCLs cultured in galactose. The effect of Myc inhibitor, JQ1 (48h), was examined in these same cells (*lower*). JQ1 reduced **A**) glucose uptake and ROS levels and **B**) *Myc* and *HK2* expression. No significant growth inhibition was observed at 48h.

RESULTS

Figure 4: The metabolic score defines the metabolic state of abnormal PCs and increases with disease stage.



	ROS		Superoxide		MT		LDH		Score	
	initial	followup	initial	followup	initial	followup	initial	followup	initial	followup
Pt1	4	1	2	2	2	4	2	2	2	-3
Pt 2	4	2	2	1	3	1	3	2	2	2
Pt 3	4	3	4	3	4	4	3	4	-1	0

A) The metabolic score (MS) is derived from measurements of mitochondrial content and superoxide (-, OxPhos/dormancy) and ROS and LDH (+, glycolytic/proliferative) in residual MM cases divided into quartiles (*left*). **The metabolic score is significantly higher in abnormal PCs from active MM than those from MGUS cases** (*right*). **B**) **The metabolic state of MMPCs is dynamic.** Quartiles for the four parameters included in the MS in initial sample and followup for each case are shown. Repeated measurements after 3 months in 3 cases showed that the MS for Pt 1 improved from 2 to -3, Pt 2 remained at 2, and Pt 3 had a slight change from -1 to 0, suggesting that in the absence of external stimuli the metabolic state of MMPCs is dynamic and most likely responds during dormancy to cues from the microenvironment. **C**) **The proportion of the patient population with a high metabolic score increases with disease stage.** Each group was divided into Lo (-6 - -2), Intermediate (-1-1), or Hi (2-6) based on their metabolic score and expressed as a percentage.

CONCLUSIONS

- Most residual MMPCs have lower ROS and higher superoxide and mitochondrial content.
- A subset of residual cases had higher ROS levels along with elevated *Myc, HK2*, *CD147* and reduced *POU2F1* expression, possibly indicating loss of dormancy and transition to active disease
- Metabolic characteristics of residual disease were recapitulated in galactose-conditioned MMCLs and had reduced *Myc*, *HK2* and CD147 expression and reduced glucose uptake. After Myc inhibition, these parameters were also reduced in MMCLs cultured in standard glucose-containing medium.
- We developed a metabolic score to detect increased glycolytic and proliferative activity and found that it increases with disease stage.
- The metabolic score will be used for precision targeting of residual MMPCs with anti-metabolic drugs and to examine efficacy of predicting progression of residual MM cases based on metabolic changes in prospective studies.

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The authors have no conflict of interest to disclose.

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