P851 TARGETING GENE DEPENDENCIES IN MYC OVEREXPRESSING MULTIPLE MYELOMA

**Topic:** 13. Myeloma and other monoclonal gammopathies - Biology & Translational Research

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**Background:**

Multiple myeloma (MM) is an incurable hematological malignancy characterized by a proliferation of clonal plasma cells in bone marrow. MM progresses from precursor stages, named monoclonal gammopathy of undetermined significance (MGUS) and smoldering MM (SMM), to the symptomatic myeloma, MM. MYC abnormalities play a critical role in the disease progression. However, MYC is not therapeutically targetable due to its nuclear localization and the short half-life of the protein.

**Aims:**

To overcome this, we hypothesized that the proliferative advantage promoted by MYC overexpression induces differential genomic dependencies on other signaling pathways thus creates vulnerabilities that can be exploited therapeutically.

**Methods:**

We searched for genetic vulnerabilities associated with MYC overexpression by leveraging genome-scale pooled short hairpin RNA screening data for 236 cancer cell lines from Project Achilles. We generated an isogenic model of MYC overexpression (OE) in U266 cell line using EF1A-C-MYC lentiviral vector. Then, we performed RNA-seq, quantitative proteomics by Tandem Mass Tag mass spectrometry (TMT-MS) and a drug screening with ~2000 compounds. For validation, we performed pharmacological inhibition of glutamine catabolism as well as shRNA-mediated GLS1 knockdown. To determine the functional mechanisms, we used capillary electrophoresis-mass spectrometry (CE-MS) and Agilent Seahorse XF analyzer.

**Results:**

Achilles analysis revealed main dependencies associated with MYC overexpression on glutamine metabolism and specifically GLS1 (glutaminase) and SLC1A1 (glutamine transporter). Using a screening of 2000 small molecules, we further observed that inhibitors of NAD synthesis and mTORC1, which rely on the intracellular glutamine pool, had preferential effect on the proliferation of U266/MYC. Both RNA-seq and quantitative proteomics showed no significant upregulation of glutaminolysis related genes, suggesting a non-oncogenic dependency. Our validation tests confirmed this metabolic dependency, MYC OE cell lines failed to proliferate in the context of glutamine starvation and showed higher sensitivity to CB-839 and V-9302 inhibiting GLS1 and SLC1A5, respectively. Using the Seahorse analyzer, we measured the oxygen consumption rate (OCR) induced by glutamine. U266/MYC cells possess the ability to oxidize glutamine at a higher rate compared to U266/Ctrl. Additionally, higher sensitivity of U266/MYC to CB-839 was observed on both baseline and FCCP-induced OCR highlighting the role of glutamine in controlling mitochondrial OXPHOS in MYC OE cells. To understand the differential metabolic rewiring in MYC OE cells, we performed metabolomic analysis and observed higher GLS1 activity in U266/MYC presented by elevated glutamine to glutamate flux. We also identified the enriched metabolic pathways under GLS1 inhibition. Notably, CB-839 in U266/MYC results in more pronounced changes in TCA cycle and energy debt. This effect was blunted by the co-
incubation with a-ketoglutarate, in a rescue experiment. Interestingly GLS1 inhibition was not limited to this effect, but extended to redox balance. CB839 significantly decreased glutathione level in U266/MYC. Furthermore, the intracellular concentrations of the glutamate-dependent amino acids were more depleted under GLS1 inhibition in U266/MYC compared to U266/Ctrl.

**Summary/Conclusion:**

Combining these observations, we were able to identify vulnerabilities to glutamine metabolism in *MYC* overexpressing cells. These results may lead to developing new therapeutic strategies to target MYC in clinical practice.