Prolonged Omacetaxine Treatment Alters Fitness and Proteostasis in Multiple Myeloma

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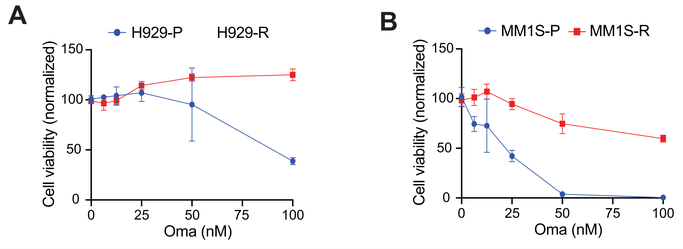
Background and Rationale

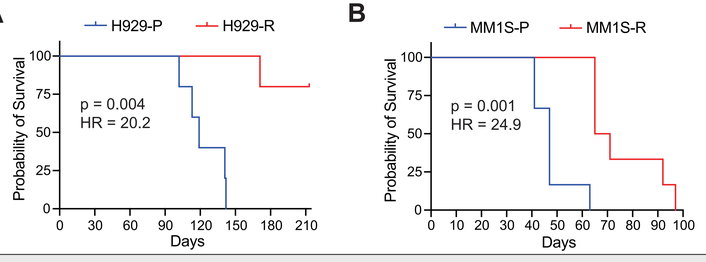
Protein production and homeostasis are critical to life and death decisions of malignant plasma cells in multiple myeloma (MM). While protein homeostasis has been targeted effectively with proteasome inhibitors in MM, inhibition of protein production has not been clinically tested. Protein translation inhibition represents an immediately available avenue with the FDA-approved agent omacetaxine (Oma). Recent in vivo and ex vivo work illustrates the broad sensitivity MM cells have to Oma, irrespective of proteasome inhibitor (PI) or immunomodulatory (IMiD) drug treatment exposure (*Walker et al. Clinical Cancer Research, 2021*). In this study, a high level of baseline protein translation was predictive of high Oma sensitivity in MM patient samples. In clinical trials of protein translation inhibition in MM, knowledge of potential biomarkers of resistance would also be valuable to inform the correlative studies that could be done in parallel. Thus, we sought to study the effect of prolonged Oma exposure in MM by establishing resistance in two human cell lines and comparing them to their isogenic parental lines. Herein, we describe the resulting candidates for Oma resistance.

Establishing Omacetaxine Resistance in MM Cell Lines



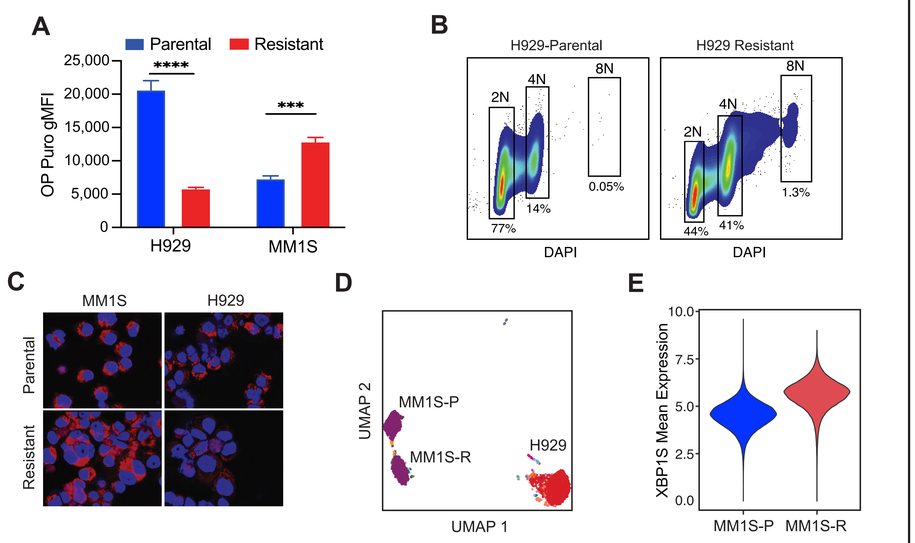
(A) Dose response of 96 h Oma treatment in H929 parental vs H929 resistant cell lines. **(B)** Dose response of 96 h Oma treatment in MM1S parental vs MM1S resistant cell lines.

Resistant Cells Exhibit Decreased Fitness In Vivo



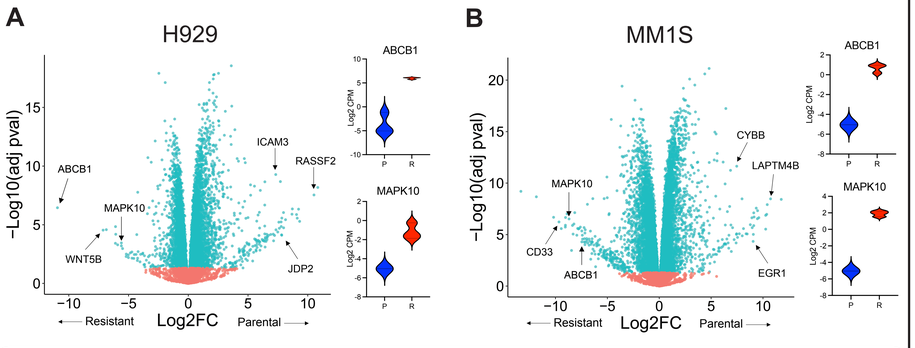
(A-B) Kaplan-Meier survival curves of H929 and MM1S parental vs resistant untreated xenograft model in NOD *scid* gamma mice (n = 5/group).

Oma Resistant Cell Lines Have Altered Phenotypes



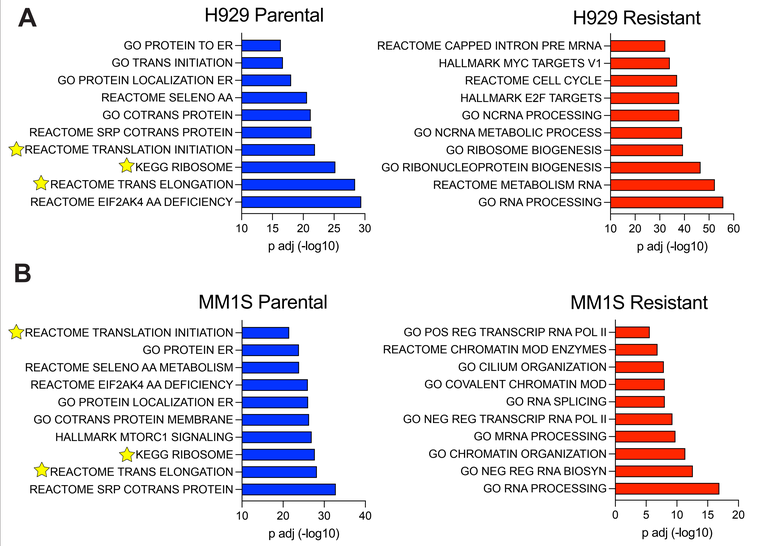
(A) Protein translation rates of H929 and MM1S cells measured by OP-Puromycin. **(B)** Flow cytometry plots of DAPI cell cycle staining of H929 cells. **(C)** Confocal miscroscopy of H929 and MM1S cells stained with DAPI (blue) and MitoTracker (red). **(D)** Uniform Manifold Approximation and Projection (UMAP) clustering of parental and resistant cells generated from CyTOF results. **(E)** Mean expression of XBP1s in MM1S cells determined via CyTOF.

ABCB1 and MAPK10 are Upregulated in Resistant Cells



(A) Differential mean gene expression analysis in H929 parental and resistant cells with violin plots of ABCB1 and MAPK10 ($p = 3.2x10^{-8}$, $p = 3.5x10^{-4}$ respectively). **(B)** Differential mean gene expression analysis in MM1S parental and resistant cells with violin plots of ABCB1 and MAPK10 ($p = 1.7x10^{-5}$, $p = 3.0x10^{-7}$ respectively).

Oma Resistance Alters MM Transcriptional Profiles



(A) Over Representation Analysis (ORA) of Hallmark, KEGG, REACTOME, and Gene Ontology (GO) BIO pathways in H929 parental and resistant cells (adj p cut off = 0.01). **(B)** ORA of Hallmark, KEGG, REACTOME, and GO BIO pathways in MM1S parental and resistant cells (adj p cut off = 0.01). Stars represent translational signatures lost with Oma resistance.

Conclusion and Future Directions

- Oma resistant cell lines exhibited a stressed cell state evident through unfolded-protein response, decline of in vivo fitness, and multi nucleation.
- Bulk RNAseq uncovered drug efflux pump ABCB1 and the MAPK pathway as highly expressed in resistant cells.
- Pathway analysis affirms a shift away from translation related gene sets.
- Future work includes validating targets XBP1s, ABCB1 and MAPK10 for their role in Oma resistance via shRNA and pharmacological knockdown.
- Ribosome profiling is currently underway to further understand the changes in the translational state of Oma resistant MM cells.
- Once validated, including markers of Oma resistance may be useful as correlative biomarkers to measure in clinical trials.

Acknowledgements

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