

3. RELAPSED/REFRACTORY MULTIPLE MYELOMA

MODELING DORMANCY IN MULTIPLE MYELOMA: FUNCTIONAL AND TRANSCRIPTOMIC CHARACTERIZATION OF SERUM STARVATION AND CHEMOTHERAPY-INDUCED STATES

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Introduction. Cancer cell dormancy is a reversible, non-proliferative state that enables long-term survival under therapeutic pressure and is believed to be the cause of late relapse in multiple myeloma (MM), even after a prolonged minimal residual disease (MRD)-negative remission period. Therefore, robust experimental systems that can model and characterize dormancy are essential for identifying the mechanisms that sustain this state and for developing strategies to eliminate dormant cells and ultimately cure MM.

Methods. We established and compared *in vitro* conditions that induce dormancy in MM cell lines (AMOI and L363) using serum starvation and chemotherapy treatment. The cells were cultured in suspension and within three-dimensional (3D) Matrigel models. We assessed the proliferation and cell cycle activity of the cells by flow cytometry using the following markers: EdU incorporation (proliferation), CellTrace Violet dilution (cell division), Ki67 (cell cycle activity), and p27 (quiescence). To confirm the reversibility of dormancy, we cultured the serum-starved cells again in a complete medium and evaluated the cells' ability to re-enter proliferation and form spheroids. Next, we performed bulk RNA sequencing to identify transcriptional programs associated with dormancy-like states. Finally, pathway enrichment analysis was performed using Reactome 2024, WikiPathways 2024, KEGG 2021 and Hallmark 2020.

Results. Serum starvation induced a dormant-like phenotype characterized by high p27 expression and strong CellTrace retention, and markedly decreased EdU incorporation, indicating cell cycle arrest. Re-exposure of serum-starved cells to a complete medium restored proliferation and spheroid formation, demonstrating that the dormant-

-like state is reversible. Chemotherapy treatment significantly decreased viability. Among the surviving cells, minimal EdU incorporation was observed, indicating a predominantly non-proliferative population. This population may exhibit a different type of dormancy than that induced by starvation. Transcriptomic profiling showed downregulation of cell cycle-related program, including E2F targets and DNA replication pathways, across all conditions. The enriched pathways were functionally grouped into stress- and senescence-associated programs (including SASP), immune and cytokine signaling, antigen processing and PD-1 signaling, DNA damage and repair, and epigenetic regulation. Filtering for differentially expressed genes (log2 fold change >1) identified 15 candidate dormancy-associated transcriptional features, primarily protein-coding genes, functionally linked to cell cycle arrest, stress adaptation, and survival signaling. This 15-gene-based signature will be used to study dormancy in patient samples. Additionally, dormant cells will be used in cytotoxicity assays with novel T-cell-based therapies, including bispecific antibodies and CAR T cells.

Conclusions. We established and functionally validated *in vitro* models of dormancy in MM and defined distinct transcriptional programs associated with serum starvation and chemotherapy-induced non-proliferative states. Integrating pathway enrichment from multiple databases revealed stress, immune, and epigenetic programs underlying dormancy. Identifying 15 candidate dormancy-associated transcriptional features and demonstrating their reversibility establishes a framework for the future functional validation and therapeutic targeting of dormant MM cells to prevent relapse.