Abstract topic: Lymphoid malignancies and plasma cell dyscrasias

Title: Targeting of pyrroline-5-carboxylate reductase (PYCR) reduces drug resistance in multiple myeloma *in vitro*.

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Objectives

Multiple myeloma (MM) is a B-cell malignancy and remains incurable due to drug resistance, leading to frequent patient relapse. The hypoxic bone marrow environment induces metabolic changes, making the cancer cells less sensitive to current standard-of-care agents. In this study, we aimed to identify these metabolic changes and further investigate these altered pathways *in vitro*.

Methods

To perform a tracer study, RPMI-8226 cells were supplemented with ^{13}C-glutamine for 48h in both normoxia and hypoxia (<1% O_2, by chamber). Correlation of pyrroline-5-carboxylase reductase 1 and 2 (PYCR1 and PYCR2) with overall survival was investigated in the publically available gene-expression data of MM patients (MMRF and Heidelberg/Montpellier). For further *in vitro* investigation, two human MM cell lines (OPM-2 and RPMI-8226) were used. We used siRNA to establish a knockdown of PYCR1 and/or PYCR2. Levels of apoptosis were measured using AnnexinV and 7-AAD positivity on flow cytometry. Differential protein expression was evaluated with a western blot. Pargyline was used as a PYCR1 inhibitor. All experiments were performed in hypoxic conditions.

Results

We performed a tracer study on RPMI-8226, revealing an increased conversion of ^{13}C-glutamine to proline in hypoxia compared to normoxia. Pyrroline-5-carboxylate reductase 1 and 2 (PYCR1 and PYCR2) are two mitochondrial enzymes that facilitate the last step in the enzymatic conversion of glutamine to proline. Expression of both enzymes correlate with a lower overall survival in the MMRF cohort. Moreover, relapse/refractory patients have significant higher levels of PYCR2. MM cells also express significantly higher levels of PYCR1 compared to bone marrow plasma cells and smouldering myeloma. *In vitro* knockdown of PYCR1 or both PYCR1/2 combined with bortezomib increased apoptotic cell death in OPM-2 and RPMI-8226. In contrast, PYCR2 knockdown combined with bortezomib did not significantly alter apoptosis. Further investigation revealed that knockdown of PYCR1 and PYCR1/2 decrease p-AKT, pMAPK and C-MYC on protein level, suggesting involvement in proliferation and survival. Cleaved PARP and cleaved caspase 3 levels were also increased after knockdown. Inhibition of PYCR1 with pargyline significantly reduced cell viability and increased apoptosis.

Conclusion

Hypoxia increases glutamine to proline conversion in myeloma cell lines. Knockdown of PYCR1 and both PYCR1/2, increases apoptosis markers on protein level and resensitizes the myeloma cells to bortezomib.