P844 CHARACTERIZATION OF MULTIPLE MYELOMA CELL LINES WITH ACQUIRED-RESISTANCE TO PROTEASOME INHIBITORS HIGHLIGHTS A LINK BETWEEN RESISTANCE AND METABOLIC DEREGULATION

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

Hugues De Boussac,1 Amelie Machura,1 Alizée Steer,1 Alboukadel Kassambara,1 Marie Gely,1 Djamilah Chmelal,1 Claire Gourzones,2 Guilhem Requirand,3 Nicolas Robert,3 Laure Vincent,4 Charles Herbaux,4 Angelique Bruyer,1 Jerome Moreaux

1 Diag2Tec, Montpellier, France; 2 UMR 9002 CNRS-UM, Montpellier, France; 3 Laboratory for Monitoring Innovative Therapies, Department of Biological Hematology, CHU Montpellier, Montpellier, France; 4 CHU Montpellier, Montpellier, France; 5 Institute of Human Genetics, UMR 9002 CNRS-UM, Montpellier, France

Background:
Characterized by an abnormal clonal proliferation of malignant plasma cells, Multiple myeloma (MM) is the second most common hematological malignancy. Novel agents have significantly improved clinical outcomes, but most of the MM patients eventually relapse. A better understanding of the drug resistance mechanisms remains of significant interest to improve the treatment of patients.

Aims:
We aimed to investigate the mechanisms involved in the resistance to proteasome inhibitors (PI).

Methods:
For that purpose, we have derived and characterized 6 Bortezomib (Btz)-resistant human myeloma cells lines (BR-HMCLs) from different molecular subgroups and still dependent on the addition of IL-6 including XG1BR t(11;14), XG2BR t(12;14), XG7BR, XG20BR, XG24BR t(4;14) and XG19BR t(14;16). These cell lines were cultured continuously with escalating concentrations of Btz during 12 months.

Results:
Interestingly, BR-HMCLs demonstrated significant resistance to Btz compared to their parental counterparts (CTR-HMCLs) (mean IC50: BR-HMCLs =5nM vs CTR-HMCL=2.3nM, p<0.05). In addition, these BR-HMCLs also showed significant cross-resistance to Carfilzomib (Cfz) and Ixazomib (Ixa) PIs (p<0.05 for Cfz or Ixa). Finally, no significant cross-resistance was observed with other therapeutic agents (melphalan, dexamethasone or IMIDs), indicating that the observed drug resistance mechanisms are especially related to PIs.

We then used genomic approaches (whole genome sequencing and transcriptomic analyses) to understand the PIs resistance mechanisms acquired by MM cells. Remarkably, as observed in relapsed MM patients, among the 40 mutations identified in BR-HMCLs compared to the CTR-HMCLs, we identified a mutation residing in the Btz-binding pocket of the proteasome beta5-subunit (PSMB5), that reduces the PI binding capacity, thus preventing inactivation of the catalytic activity of the 20S proteasome.

Further transcriptomic analyses on BR-HMCLs underlined significant deregulation of genes involved in cell metabolism and drug clearance that could allow the BR-cells to maintain metabolic homeostasis and survival in stringent redox conditions. Thus, in the BR-HMCL we identified an upregulation of enzymes directly involved in glycolytic and energy metabolism (ALDOC, ENO3, HK1, PDK1/3, PFKB3/4, PFKL, SLC2A1 (FC>1.5 p-value <0.05), but also a significant downregulation of 8 solute carrier protein (SLC) intake transporters together with a significant upregulation of xenobiotic receptors (FC> or < 1.5; p-value <0.05).
We then tested the metabolomic of BR-HMCL using the Seahorse assay. This technology allows to analyze the glycolysis via the extracellular acidification rate (ECAR) and cell respiration via the mitochondrial oxidative phosphorylation based on the oxygen consumption rate (OCR). Following the increased gene expression of several glycolytic enzymes found in the BR-HMCLs, we observed that while CTR and BR displayed equivalent respiration and glycolytic activities, their treatment with Btz induces major metabolic modifications, including a significant increase of the glycolytic and mitochondrial activity of the BR-HMCL while it decreases it in the CTR-HMCL, suggesting a higher capacity of the BR-HMCL to respond to cell stress.

**Summary/Conclusion:**

Altogether, we developed acquired PIs resistant HMCLs that exhibit PSMB5 mutation as observed in patients, and we identified pathways linked to metabolism deregulation in these cell lines. These results make our PI-resistant models, an attractive preclinical model to test new therapeutic strategies to overcome PI resistance in MM.