Drug sensitivity profile of 5TGM1 murine multiple myeloma cell line emphasizes the translational potential of the syngeneic *in vivo* model

AACR 2017
#3838

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Introduction

Multiple myeloma (MM) is the second most common hematologic malignancy that originates from B-cells (plasma cells) and causes 2% of cancer-related deaths. Symptoms of MM include bone pain caused by multiple osteolytic lesions, pathologic fractures, and hypercalcemia. Typically, MM has a low growth fraction and it is highly dependent on the microenvironment. These properties have made MM hard to target by conventional chemotherapy, but could now be exploited by novel stroma-targeting drugs and immunotherapy. These new approaches underline the need for well characterized models with functional immune system and appropriate tumor microenvironment.

Aim of the Study

To gain additional information supporting the use of the syngeneic 5TGM1 murine multiple myeloma model in drug development, we tested drug sensitivity of 5TGM1 cells by screening an extensive panel of drugs.

Materials and Methods

5TGM1 cells have been received from Dr. Oyajobi, Department of Molecular Medicine, University of Texas Health Science Center at San Antonio and were cultured in IMDM, 15% iFBS and 1% penicillin-streptomycin. The drug sensitivity of the cell line was assessed by applying a large panel of drugs covering both cancer chemo-therapeutics and many clinically available and emerging molecularly targeted drugs including conventional chemotherapy, kinase inhibitors, metabolic modifiers, rapalogs, differentiating/epigenetic modifiers, kinesin inhibitors, apoptotic modulators, NSAIDs, hormone therapy, immunomodulators and HSP inhibitors. A panel of approximately 460 compounds was tested in five concentrations covering a 10.000-fold drug-relevant concentration range in 384-well format. Cells were seeded to pre-drugged plates, followed by cell viability measurements (CellTiter-Glo, Promega) after 72 hours. Maximal and minimal responses to drugs were analyzed, the EC50 values were calculated and Drug Sensitivity Score (DSS) was calculated for each drug as a measure of reduced viability (1-3). A selective Drug Sensitivity Score (sDSS) can be calculated to identify the selective drug response pattern of a cancer cell line (1-3).

References

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Results

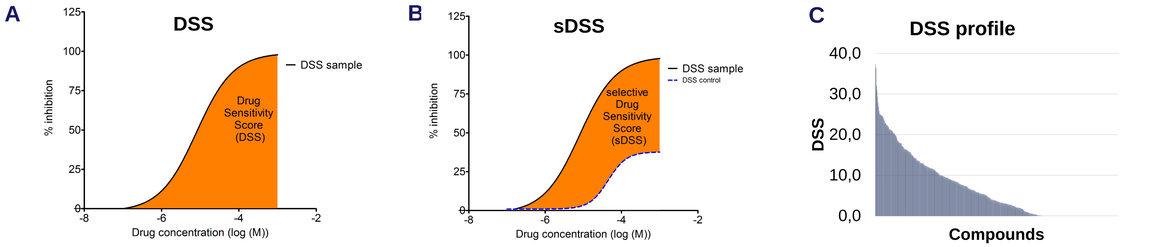

Figure 1. Illustration of A) DSS and B) sDSS determination. C) DSS profile of murine 5TGM1 MM cells. A panel of 460 compounds was tested. DSS = 0 depicts inactivity in cells, DSS = 0-5 depicts low activity, DSS = 5-10 depicts semi-activity, DSS = 10-20 depicts activity and DSS >20 depicts high activity of the compounds in cells.

Table 1. The murine 5TGM1 cells show sensitivity to various MM drugs currently in clinical and research use. Cells were seeded to pre-drugged 384-well-plates followed by cell viability measurements (CellTiter-Glo) after 72 hours.

Compound	DSS	EC50 (nM)	Mechanism	Comments
Bortezomib	32.2	2.5	Proteasome inhibitor (26S subunit)	
Carfilzomib	22.8	10.2	Proteasome inhibitor (20S subunit)	
Carmustine	12.4	1.7	Alkylating agent	
Panobinostat	21	18.8	HDAC inhibitor	
Lenalidomide	0	-	Immunomodulator	Do not bind to murine form of the target cereblon (4)
Melphalan	0	-	Nitrogen mustard alkylating agent	Prodrug, activated in the liver
Pomalidomide	0.6	-	Immunomodulator	Do not bind to murine form of the target cereblon (4)

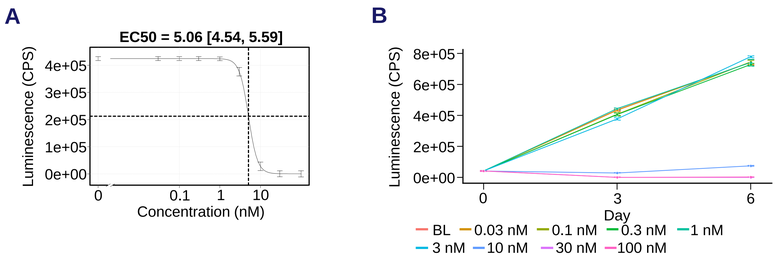

Figure 2. The 5TGM1 cells are sensitive to bortezomib. A) Determination of EC50 value (95% confidence interval) for bortezomib in 5TGM1 cells at day 3. Four-parametric log-logistic (4PL) curve was fitted to the cell viability (luminescence) data. EC50 value was calculated as a half-way value between the minimum and maximum of the fitted curve. B) Effects of bortezomib on 5TGM1 cell proliferation. The results are shown as luminescence (CPS; mean ± SE) measured in the CellTiter-Glo viability assay at days 0, 3 and 6. BL = Baseline (no added compounds). Cells were cultured on a 96-well-plate.

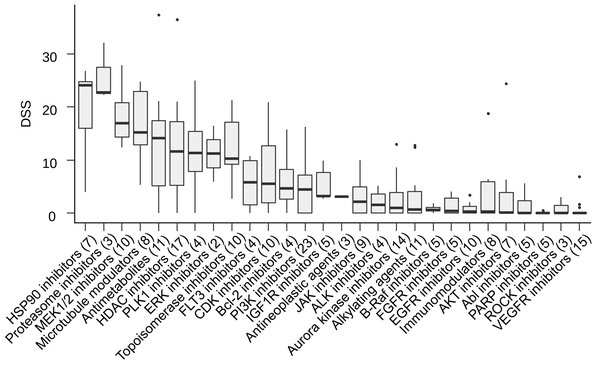

Figure 3. DSS of selected compound groups.

Conclusions

According to DSS analysis, 5TGM1 cells showed sensitivity to conventional chemotherapy, such as antimitotic drugs, and kinase inhibitors, such as MEK1/2 inhibitors. In addition, the cells showed particular sensitivity to several HSP90 inhibitors currently in clinical trials for the treatment of MM. Lenalidomide and pomalidomide, efficient in treating multiple myeloma in humans, both gave low DSS value indicating that 5TGM1 cells are not sensitive to these drugs, which is expected because they do not bind to murine form of the target cereblon. In contrast, 5TGM1 cells were highly sensitive to the proteasome inhibitor bortezomib (DSS 32.2), which is currently in clinical use. In conclusion, the murine 5TGM1 cells show sensitivity to various MM drugs used in the clinic and under development. Evaluating the effects of the microenvironment on the growth and drug sensitivity of 5TGM1 cells *in vitro* and *in vivo* will be essential. Furthermore, the cell-based compound screening combined with DSS analysis provides a possibility to profile cellular responses to an extensive collection of anti-cancer compounds enabling identification of vulnerabilities in cancer cells and functional investigation of cellular pathways behind drug sensitivity or resistance.

Acknowledgements: We thank Dr. Esa Alhoniemi (Avoltus) for skillful technical assistance.