SINGLE-CELL TRANSCRIPTOMICS IDENTIFIES INTRA-TUMOR HETEROGENEITY IN HUMAN MYELOMA CELL LINES

Amit Kumar Mitra[1], Holly Stessman[2], Michael A. Linden[3] and Brian G. Van Ness[1,4]

1. Department of Genetics, Cell Biology & Development, University of Minnesota, Minneapolis, MN;
2. Department of Genome Sciences, University of Washington, Seattle, WA;
3. Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN;
4. PUMA-Institute of Personalized Medicine, College of Pharmacy, University of Minnesota, Minneapolis, MN.

BRIEF OVERVIEW

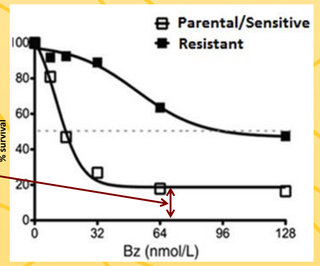
Multiple myeloma (MM) is a difficult disease to cure with median survival rate of around 7 years. In a recently published study, we confirmed the presence of residual proteasome inhibitor (PI)-resistant sub-population in drug-naïve MM tumors. We hypothesize that this pre-existing resistant subclone may give rise to emerging resistance in course of PI treatment.

In the current study, we used single cell transcriptomics to identify tumor subclones within human myeloma cell lines (HMCLs) based on a 48-gene model of predictive genetic signature for baseline PI response.

Our results demonstrated the presence of pre-existing subclones of cells within untreated myeloma cells with a characteristic genetic signature profile distinct from the pre-treatment overall profile.

This may help identify the presence and extent of intra-tumor heterogeneity in MM and may define residual pre-existing subclones resistant to PI therapies.

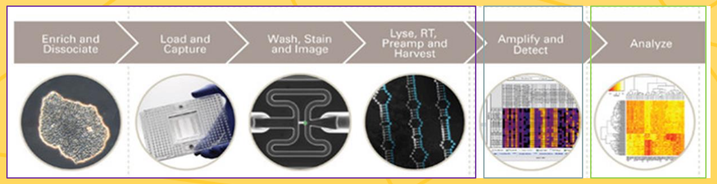
OBJECTIVE



Does GEP profile help in identifying pre-existing drug resistant clonal subpopulations within sensitive population?

To perform automated single-cell capture & cDNA synthesis from cellular RNA in individual cells and single-Cell qRT-PCR-based gene expression analysis of human myeloma tumor prior to exposure to PI treatment to identify resistant pre-existing sub-clones using a GEP-based model (48-gene panel of baseline responsiveness to PIs).

METHODS



- Automated single-cell capture and cDNA synthesis from cellular RNA were performed using Fluidigm's C1™ Single-Cell Auto Prep System.
- The cDNA was harvested and transferred to BioMark HD System for single-cell targeted high-throughput qPCR-based gene expression analysis of a gene-panel using Fluidigm DELTAgene assays.

- Stessman *et al* identified a 23-gene expression signature of baseline responsiveness to PI that was successful in stratifying good versus poor outcomes in human MM subjects from MM TT3 trial and APEX MM clinical trials (both using PI therapy).
- Shaughnessy *et al* identified a 17-gene model from newly diagnosed MM patients on TT3 trial that could discriminate between low and high-risk myeloma with accuracies of over 95%.
- We used a GEP signature-based model derived from combination of these gene panels of baseline PI response (and a set of internal control genes) to identify pre-existing PI-resistant subclones within untreated bulk myeloma tumor cell populations.

SSBP4	HIST1H1C	CXCR4	KIF20B	CTBS	E2F1	CDK4
SNAI3	DDX3Y	TSPAN32	TMPO	CLCC1	TP53	CDKN1A
SMOC1	PMPCB	SCD5	NADK	LTBP1	FOXO4	GAPDH
CBS	TAP2	SCD	LARS2	JUN	CCNA1	ACTB
FZD6	CD53	KIF14	TBRG4	FOS	CCNB1	
IFITM1	SLC35B1	SLC19A1	AIM2	MYC	CCND1	
RGS16	LGALS1	CKS1B	ASPM	MYB	CCNE1	
ENO1	SRR	YWHAZ	AHCYL1	RB1	CDK2	

- Statistical analysis was performed using a combination of Fluidigm's Singular Analysis Toolset and the R Statistical analysis package.

RESULTS

U266 Parental vs U266 PI-resistant cells

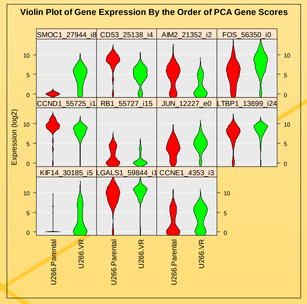


Figure: Violin plot representing the top significant genes (p<0.05) that distinguish U266 parental cells from clonally-derived PI-resistant U266 cells.

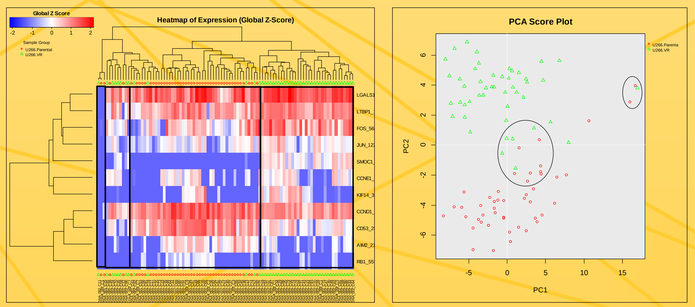


Figure: Heat map generated using unsupervised hierarchical clustering (HC) analysis and Principal Component Analysis (PCA) score plot based on significantly associated genes (p<0.05) displaying pre-existing subclones of parental U266 cells corresponding to the expression patterns of PI-resistant U266 cells, distinct from the pre-treatment overall profile of U266.

MMM1

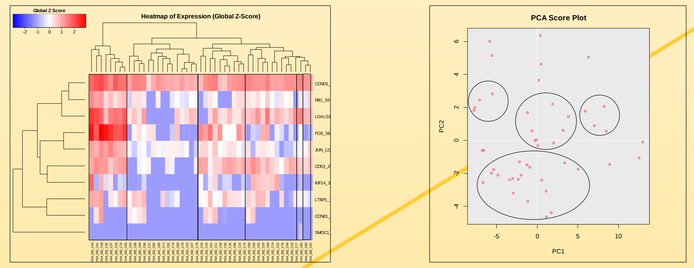


Figure: Heat map generated using unsupervised hierarchical clustering (HC) analysis and Principal Component Analysis (PCA) score plot for MMM1 cells based on top GEP signatures of PI resistance.

Primary Myeloma cells (from Patient)

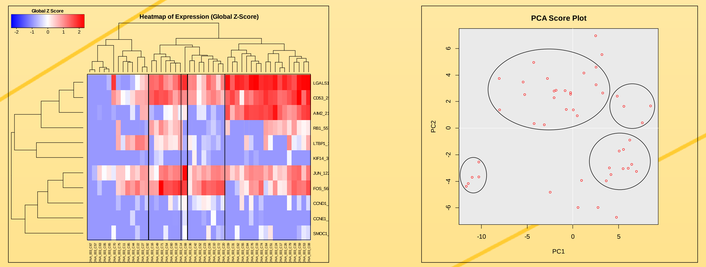


Figure: Heat map generated using unsupervised hierarchical clustering (HC) analysis and Principal Component Analysis (PCA) score plot for primary myeloma cells based on top GEP signatures of PI resistance.

CONCLUSIONS

- Multiple subclones of cells are present within untreated myeloma cells with a characteristic genetic signature profile distinct from the pre-treatment overall profile.
- PI-resistant sub-populations represent a pre-existing subset of PI-sensitive cell lines based on GEP profile that may give rise to emerging resistance in course of treatment with PIs.
- Similar findings have also been observed using multicolor flow cytometry. Immunophenotypic profiling of human myeloma cell lines (HMCLs) demonstrated unique signatures representing sub-clonal populations.

FUTURE PERSPECTIVES

- Our work will help identify the presence and extent of intra-tumor heterogeneity in MM and may define residual pre-existing subclones resistant to PI therapies.
- This will establish the use of microfluidics technology-based single-cell approaches as novel strategy to detect therapy-resistant subclones within bulk tumor populations from patient samples.

REFERENCES

- Rajkumar SV. Multiple myeloma: 2013 update on diagnosis, risk-stratification, and management. Am J Hematol. 2013;88(3):226-235.
- Shaughnessy JD,Jr, Zhan F, Burington BE, et al. A validated gene expression model of high-risk multiple myeloma is defined by deregulated expression of genes mapping to chromosome 1. Blood. 2007;109(6):2276-2284.
- Stessman HA, Baughn LB, Sarver A, et al. Profiling bortezomib resistance identifies secondary therapies in a mouse myeloma model. Mol Cancer Ther. 2013;12(6):1140-1150.

ACKNOWLEDGEMENTS

This research was supported in part by University of Minnesota Genomics Center (UMGC) Fluidigm C1 Single-cell Genomics Grant.
There are no relevant conflicts of interest to disclose.