Dynamic Epigenetic Landscapes are Associated with Multiple Myeloma Progression and Drug Resistance

Rafael Renatino Canevarolo[1], Mark B. Meads[2], Maria L. C. Siqueira Silva[1], Praneeth Reddy Sudalagunta[1], Christopher Cubitt[3], Gabriel De Avila[2], Raghunandan Reddy Alugubelli[4], Amit Kulkarni[5], Qi Zhang[5], Oliver Hampton[5], Kenneth H. Shain[2], and Ariosto Siqueira Silva[1].

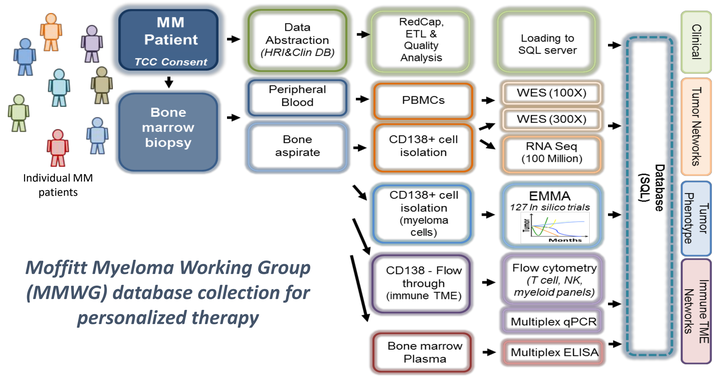
1 – Department of Cancer Physiology; 2 – Department of Malignant Hematology; 3 – Cancer PK/PD Core; 4 – Collaborative Data Services Core; 5 – M2GEN.

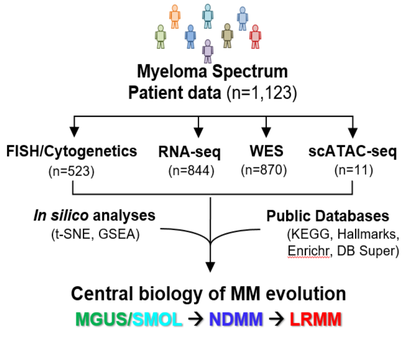
INTRODUCTION

- Multiple myeloma (MM) is an incurable cancer of bone marrow-resident plasma cells, which evolves from a premalignant state, monoclonal gammopathy of undetermined significance (MGUS), to active disease.
- Despite often responding to initial therapy, MM tumors ultimately develop multidrug resistance leading to refractory disease and therapy failure.
- The molecular mechanisms driving MM progression and refractory disease remain poorly understood.
- **Objective: to better understand the mechanisms driving MM progression and evolution of drug resistance by exploring the molecular data of a new cohort of myeloma patients treated at Moffitt Cancer Center.**

APPROACH

We generated a new database comprising 1,123 bone marrow biopsies from MM patients treated at Moffitt Cancer Center, across MM spectrum.





- These samples were characterized clinically, genetically (523 FISH-Cytogenetics, 844 RNA-seq, 870 WES) and epigenetically (11 scATAC-seq).
- We have conducted pathway analysis of this dataset in curated databases (KEGG, Hallmarks, Enrichr, DB Super) to identify biological pathways and driving events associated with MM progression and refractory disease.

RESULTS

1 MM progression from pre-malignant to active disease was associated with under-expression of genes related to *cell adhesion, inflammatory cytokines and hematopoietic cell identity*, while refractory disease presented over-expression of genes linked to *cell cycle, energy metabolism, DNA repair, and protein/RNA synthesis/degradation*.

Pre-malignant → Active disease

	CANCER HALLMARKS	-log (q value)
UP	MYC TARGETS V2	7.86
UP	UNFOLDED PROTEIN RESPONSE	6.80
DOWN	IL6 JAK STAT3 SIGNALING	10.37
DOWN	KRAS SIGNALING UP	9.52
DOWN	INFLAMMATORY RESPONSE	9.52
DOWN	IL2 STAT5 SIGNALING	9.33
DOWN	ALLOGRAFT REJECTION	8.69
DOWN	COMPLEMENT	8.47
DOWN	HEME METABOLISM	8.02
DOWN	APICAL JUNCTION	7.83

	KEGG PATHWAYS	-log (q value)
UP	AMINOACYL T-RNA BIOSYNTHESIS	6.77
DOWN	HEMATOPOIETIC CELL LINEAGE	13.34
DOWN	PRIMARY IMMUNODEFICIENCY	10.84
DOWN	CHEMOKINE SIGNALING PATHWAY	10.72
DOWN	CYTOKINE CYTOKINE RECEPTOR INTERACTION	8.53
DOWN	ASTHMA	8.50
DOWN	NK MED CYTOTOXICITY	8.47
DOWN	LEISHMANIA INFECTION	8.26
DOWN	CELL ADHESION MOLECULES CAMS	7.87
DOWN	FOCAL ADHESION	6.89

Newly-diagnosed MM → Refractory MM

	CANCER HALLMARKS	-log (q value)
UP	E2F TARGETS	15.00
UP	G2M CHECKPOINT	15.00
UP	MYC TARGETS V1	14.52
UP	OXIDATIVE PHOSPHORYLATION	13.39
UP	DNA REPAIR	11.85
UP	PEROXISOME	11.72
UP	MTORC1 SIGNALING	11.46
UP	FATTY ACID METABOLISM	10.02
UP	PI3K AKT MTOR SIGNALING	7.11
DOWN	KRAS SIGNALING DN	8.56

	KEGG PATHWAYS	-log (q value)
UP	OOCYTE MEIOSIS	15.00
UP	PROTEASOME	15.00
UP	CELL CYCLE	15.00
UP	PEROXISOME	10.98
UP	DNA REPLICATION	10.98
UP	OX PHOS	10.58
UP	ALZHEIMERS DISEASE	10.54
UP	HUNTINGTONS DISEASE	10.52
UP	PARKINSONS DISEASE	10.44
UP	SYSTEMIC LUPUS ERYTHEMATOSUS	9.94

2 These gene sets shared enrichment for specific histone modifications – H3K27me3 and H3K27ac, respectively – which are markers of epigenetic transcriptional regulation through chromatin accessibility modulation.

Pre-malignant → Active disease

Epigenomics Roadmap HM ChIP-seq	Adjusted p-value
H3K27me3 erythroblast mm9	4.93E-15
H3K27me3 GM12878 hg19	5.31E-09
H3K27me3 SK-N-SH hg19	1.64E-08
H3K27me3 H1-hESC hg19	2.83E-08
H3K27me3 spleen mm9	6.89E-07
H3K27me3 MCF-7 hg19	4.91E-06
H3K27me3 A549 hg19	8.57E-07
H3K27me3 mammary epithelial cell hg19	9.41E-07
H3K27me3 keratinocyte hg19	8.82E-06
H3K27me3 fibroblast of lung hg19	2.71E-05

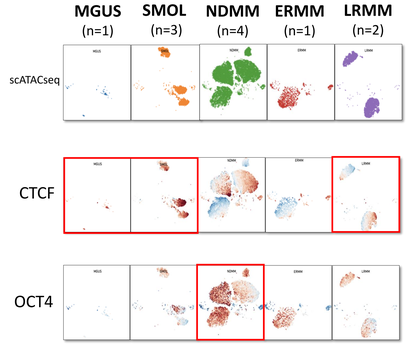
H3K27me3 represses gene expression

Newly-diagnosed MM → Refractory MM

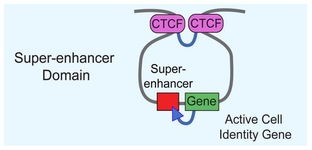
Epigenomics Roadmap HM ChIP-seq	Adj. p-value
H3K27ac CD4 Naive Primary Cells	2.25E-209
H3K56ac IMR90	8.46E-159
H3K27ac Mobilized CD34 Primary Cells	4.27E-165
H3K27ac CD4 Memory Primary Cells	2.41E-132
H3K27ac CD8 Naive Primary Cells	9.23E-126
H3K27ac CD4+ CD25int CD127+ Tmem Primary Cells	4.98E-119
H3K4ac IMR90	1.37E-116
H4K8ac IMR90	1.50E-123
H3K27ac H9	7.68E-103
H3K56ac H1	2.69E-104

H3K27ac promotes gene expression

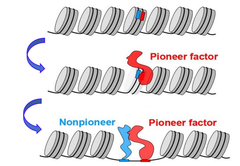
3 scATAC-seq data on 11 MM samples ranging from MGUS to late relapse (LRMM) confirmed that the binding sites of epigenetic regulators involved in the formation of super-enhancers – like CTCF and YY1 – were more accessible in pre-malignant and LRMM stages, while newly-diagnosed MM (NDMM) showed increased chromatin accessibility for transcriptional factors related to pluripotency and cell stemness – such as OCT4 and SOX2.



4 This epigenetic "memory" state is kept by Super enhancer-regulated core regulatory circuitries, which are super-enhancer controlled, self-regulated master TFs that can act as pioneer TFs and confer identity to cells and tissues. Many of them were differentially expressed across disease transitions.



Modified from Dowen *et al.*, Cell 2014, 159(2):374-387



Modified from Mayran *et al., Nat Comm* 2019, 10: 3807

Pre-malignant → Active disease

UP	DOWN	
PRDM1	EBF1	FOXP1
IRF4	ZEB2	ELF1
MZF1	RB1	KLF6
	SPI1	IKZF1

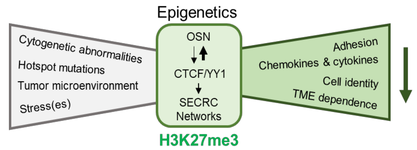
Newly-diagnosed MM → Refractory MM

UP			DOWN
RFX5	ZNF189	NFIL3	JUN
YY1	ZNF217	KLF13	MZF1
NBN	FOXP1	NFE2L2	ZHX2
CTCF	SMARCA5	ARID2	CHD2
ELF1	BCOR	PBX2	KLF6
IRF2	RELA	RARA	
SP3	ERF	TSHZ1	
ZNF184	CREB1	IKZF1	

5 Our data lead us to propose a new bow-tie model for myelomatogenesis and disease progression in which tumor microenvironment, stress, cytogenetic abnormalities and mutation signals are inputs that converge to epigenetic reprograming, leading to changes in chromatin accessibility of SE-regulated master TFs of B cell identity.

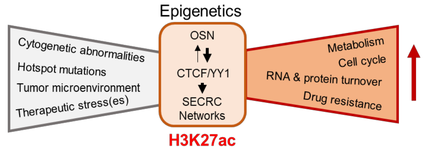
- From pre-malignant to active MM, initial hits would increase Histone 3 methylation, leading to a genome-wide decreased gene expression, lose of B cell identity with simultaneous activation of pluripotency-associated TFs like OCT4, SOX2 and NANOG.

Pre-malignant → Active disease



- From NDMM to LRMM, new mutations and stress caused by therapy regimens would increase Histone 3 acetylation, leading to a genome-wide increased gene expression, partial reacquisition of B cell identity with simultaneous activation of pathways related to accelerated metabolism and proliferation, independence of the TME and multidrug resistance.

Newly-diagnosed MM → Refractory MM



CONCLUSION

MM evolutionary dynamics in response to TME and therapeutic stress is triggered by diverse genetic and cytogenetic events, but ultimately implemented through epigenetic master regulators of the transcriptome.

ACKNOWLEDGEMENT

This work was sponsored in part by the Pentecost Family Foundation and Miles For Moffitt Milestone Award. We also acknowledge the Molecular Genomics and the PK/PD Core Facilities at Moffitt Cancer Center.