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Multiomics Analysis on the Clinical Treatment for Multiple Myeloma (MM)

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Abstract

The innovative speed in Multiple Myeloma (MM) therapy is remarkable in recent years with the arrival of monoclonal antibodies and approval of novel agents with new action mechanisms. Emerging therapies especially immunotherapy Chimeric Antigen Receptor (CAR)-T cells, Bispecific T-cell Engager Antibodies (BiTEs), Antibody-Drug Conjugates (ADCs), newer generations of Monoclonal Antibodies (MoAbs) and small molecule inhibitors/modulators have extended the survival of a patient, advancing with the goal of a cure. In this work, AI driven tools are used for multinomics (GSE156872) studies on the *homosapiens* and *mus musculus*. The transcriptomics data showed 37 downregulated genes (*homosapiens*) and 9 downregulated genes (*mus musculus*), which indicated them to be the potential targets for pathogenesis, diagnosis and treatment. Transcription Factor (*TF*) -gene interactions were seen for ID3, C3, CFBPD, ZNF267, hsr-mir-98-5p and RDGBRA human tumor genes whereas Esr1, Id4, Foxa1, mmu-mir-425-5p and mmu-mir-186-5p *TF* gene interactions were observed for *mus musculus*. The ChIP-seq analysis showed a lower peak of NSD2 in humans and higher in *mus musculus*. The different level of epigenetics in both (human, mice) indicated that the target might not be used for further analysis in mice for knock-in and knock-out or further pharmacokinetics analysis. Integrated KEGG Pathways analysis from (DEGs-ChIP) data predicts carcinogenesis for humans and *mus musculus*. Drug-gene interaction predicted few approved drugs and immunotherapies for the personalized treatment of MM patients. Bioinformatics studies suggested a combination therapy, which is seen in clinical treatment as well.

Keywords: Multiple myeloma; Bone marrow; Transcriptomics; Epigenomics; Malignancy

Introduction

Multiple Myeloma (MM) is a hematological malignancy which occurs due to the unrestrained development of malign plasma cells in the Bone Marrow (BM), over 10% by definition [1]. MM cells adjust BM according to its necessity by changing various BM stromal cell types which result in ontogenesis, bone annihilation, and suppression of immune system. 80% to 90% of patients with MM suffer by these active bone diseases and it affects their quality of life and increases their mortality [2]. In almost all cases, MM is preceded by a premalignant disease such as Monoclonal Gammopathy of Undetermined Significance (MGUS) [3]. Genetic and epigenetic irregularities play significant roles in MM pathogenesis, which dedifferentiate malignant cells to a less mature state and lead to poor disease progression and drug resistance [4]. Chromosomal abnormalities such as Copy-Number Variations (CNVs), translocations, and mutations are critical prime occurrences for the initiation of MM [5]. Additionally, DNA methylation and histone modifications also contribute to MM disease progression, clonal heterogeneity, cellular plasticity, and drug resistance [5,6]. It has been well explained that abnormal histone methylation plays a vital role in the pathological process of MM, as high levels of Histone Methyltransferases (HMTs) and Demethylases (HDMs) are found in MM patients with genetic mutations [7].

Numerous treatment options have entered the landscape of myeloma therapeutics in the last few years, remarkably extending Progression-Free Survival (PFS) and Overall Survival (OS) [8]. Treatment of MM includes IMIDs (Immunomodulatory agents) and PIs (Proteasome Inhibitors), Bi-specific T-cell Engagers Antibodies (BiTEs), Antibodies Drug Conjugates (ADCs), Chimeric Antigen Receptor (CAR)-T cells [9-11], and a series of chemotherapeutic molecules coupled or not with Autologous Stem Cell Transplantation (ASCT) [12,13]. Daratumumab, an antiCD38 antibody initially approved for relapsed/refractory patients is now in the frontline treatment for newly diagnosed multiple myeloma patients. Recently, B-Cell Maturation Antigen (BCMA)-targeted antibody-drug conjugate (belantamab mafodotin) [14], and BCMA-targeted CAR-T cell

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therapy (Idecabtagene vicleucel) [15] are approved for MM patients. The advance therapies using proteosome inhibitors and BCL2 inhibitors or combination of drugs (bortezomib, carfilzomib) and/or (lenalidomide, pomalidomide) with related transplantation of stem cell, alkylating drugs (melphalan) and/or glucocorticoids has increased the life expectancy of MM patients in past 15 years [16,17].

To understand the impact of previously recommended drugs and novel drugs therapies for MM, AI driven tools are used for Multiomics (transcriptomics, epigenetics) studies on the *Homo sapiens* and *Mus musculus* samples (GSE156872) of a published clinical studies [18] in diagnosis of MM.

The clinical studies explore the roles of Steroid Receptor Coactivator-3 (SRC-3) in response to treatment with proteasome inhibitors in myeloma cells. Furthermore, the underlying mechanisms of SRC-3-mediated chromatin remodeling and transcriptomic alterations, and evaluation of the efficacy of a newly developed SRC-3 inhibitor, SI-2, used in overcoming Bortezomib (BTZ) resistance in myeloma cells both *in vitro* and *in vivo* were studied. SI-2 disrupts the SRC-3 (NSD2) interaction, and ease SRC- 3 degradation. SI-2 is considered for clinical use in MM as it affects the drug responsible genes and accordingly influences the survival genes [18]. (SRC-3) is an SRC/p160 coactivator family of three members: SRC-1/NCOA1, SRC-2/TIF2/GRIP1/NCOA2, and SRC- 3/AIB1/NCOA3. SRC-3 is amplified and overexpressed in various human cancers. SRC-3 promotes cancer initiation, progression, and chemoresistance; incorporating nuclear hormone receptors (or other transcription factors) and numerous pathways of cancer-growth [19]. Recently a study suggested that SRC-3 is assumably a driver in chemoresistance as amplification of the *NCOA3* gene is associated with initial chemoresistance in ovarian cancers [20]. Overexpression of SRC-3 is associated with tamoxifen resistance [21] and resistance to cytotoxic agents [22] in breast cancer, to platinum resistance in ovarian epithelial cancer [23], and suppression of SRC-3 protein levels to anticancer reagents for the treatment of prostate cancer and leukemia cells [24,25]. Yet there is no clear mechanism on how SRC-3 is involved with epigenetic regulators (NSD2) in drug resistance in MM.

The integration of different molecular multiomics datasets is used in a standardized way. Combination of epigenetic and RNA-Seq data allows to identify the candidate genes and directly measure gene regulation and gene expression. This multidata approach is used to understand the mechanisms controlling interesting phenotypes and to uncover new regulatory elements for biomarkers and therapeutic targets. Various sequencing platform produce large multiomics data which offer a possibility to answer a lot of complex questions related to specific biological process. Limitations arise when there is a gap in the generated information. It often lead to noisy data with many unwanted divergent results and challenges are still remains to accurately analyse the extraordinary data volumes identifying true signals and understanding the combination and correlation of variable datasets [19].

Computational Details

In these studies, the relevant RNA-seq and ChIP-seq data is taken from the Gene Expression Omnibus database under accession number GSE156872 [26]. Out of total fourteen samples; eight single end samples (Organism: *Homo sapiens*, *Mus musculus*) was taken for RNA-seq analysis. Six paired end samples were taken for ChIP analysis; out of which there is 1 input, 3 treated and two untreated paired end samples. Each sample has (treated or untreated) a set of corresponding separate biological replicated condition.

Reads alignment and differential expression analysis

RNA-Seq reads with FASTQ files are uploaded in Galaxy server [27,28] for quality assessment. The FASTQC [29] tools are used to create a report of sequence quality and MultiQC [30] tools are used to check the quality results. Trimmomatic software [31] is used to delete the adaptors and poor-quality bases and reads (upto 50 bp) length are used for the analyses. An alignment strategy is used to analyse the data of xenograft model. False positives are avoided by discarding the common reads between the two genomes (humans and mouse). Though some information may be lost by this step, but it is essential for analysis. The alignment was performed to filter out mouse-like reads before mapping to the human reference and *vice versa* to differentiate human and mouse expression. STAR software is used to map data against *homo sapiens* (GRCh38/hg38) and *Mus musculus* (GRCm38/mm10) genomes separately. HTSeq software [32] is used for read counts and DESeq2 [33] is used to identify DE genes for each species. The crieteria for these genes were adjusted P-value (adjP) ≤ 0.1 and Fold Change (FC) values ≥ 1.5 and ≤ -1.5 for the up-regulated and down-regulated genes in BTZ class, respectively.

Functional enrichment of GO terms and KEGG analyses

GEne SeT AnaLysis Toolkit (Webgestalt) [34,35] web server is used to perform the GO and KEGG analyses for Human and murine data separately. A list of all DE genes for each species was used separately for the differentially connected genes and gene modules. Hypergeometric analysis is used to obtain the P-values. Gene Ontology (GO) is used to study the nature of genes according to the International Standardized Classification System (ISCS) of gene function. GO is based on the function of molecules, components of cell, and Biological Process (BP) of genes. The calculated hypergeometric p-value is adjusted as q-value to investigate that the genes are from GO (Gene Ontology) term. Significantly enriched GO terms have $q<0.05$. GO enrichment analysis shows the performance of the biological functions of Differentially Expressed Genes (DEGs). The pathway maps represent proteins and genes which are accountable for the reaction networks and molecular interactions. These pathways are manually drawn from KEGG database. Significantly enriched KEGG pathways are also identified, similar to the GO enrichment analysis. The significantly enriched terms have $q<0.05$. The corrected P-values are used for False Discovery Rate (FDR). These terms were considered significant when adjP ≤ 0.1.

Principal Component Analysis (PCA) is carried out with https://gccri.bishop-lab.uthscsa.edu/correlation-analyzer/ [36]. The correlation for clinically expressed *NSD1* gene to the *DE* genes for humans and *mus musculus* is obtained by genome-wide co-expression correlation values for any gene of interest in multiple genes and disease conditions.

Single gene mode to reveal the genome-wide co-expression correlation values for any gene of interest in multiple genes and disease conditions were used to correlate the clinically expressed *NSD1* gene to the *DE* genes for humans and *Mus musculus* data. The tool can provide gene counts from approximately 200,000 sequencing samples. The genome-wide co-expression correlations analysis provides a lot of biological sights for the cancer studies.

ChIP analysis

ChIP-seq analysis is used to identify the genomic locations of transcription factors, histone modifications and many other proteins that bind DNA. Bowtie 2 [37] is used to perform ChIP-seq data alignment and Picard Mark Duplicates remove duplicate reads (bam files). Peak calling was performed using MACS2 [38]. MACS2 is used to detect transcription factor binding sites (narrow peaks) and the larger regions (broad peaks). 1,000 of these high-quality peaks are separated by their positive and negative strand tags and aligned by the midpoint between their centers. Finally top 100 most significant peaks were found which may overlap with any genes. The Transcription Factors (*TF*s) were taken from OmicsNet [39] to study the molecular interactions; Protein-Protein Interaction (PPI), *TF*-gene, *miRNA*-gene and metabolite-protein. OmicsNet also provide system analysis of a single list of molecules; consolidated analysis of multiple lists of various types of molecules; and intuitive visualization of 2D/3D images. *TF*-gene interactions derived from ENCODE CHIP-seq data, with comprehensive PPI containing both known and predicted PPI (set parameters) using STRING software (http://www.string-db.org/) [40]. *TF*-gene interactions were derived from data mining and manually curated data for *Mus musculus*.

Cistrome-GO, a web server http://go.cistrome.org/ [41] is used to conduct functional enrichment analyses of gene regulation by *TF*s in human and mouse with integration of ChIP-seq peaks and *DE* genes dataset.

Drug-gene interaction

DGIdb (Version 4.2.0- sha1 afd9f30b, https://dgidb.genome.wustl.edu/) [42,43] is used to predict the interaction between 64 prognostic core genes and drugs so that predictions should be made regarding the therapeutic targets with the approved drugs and immunotherapies.

Results

Total fourteen samples with eight single end samples (Organism: *Homo sapiens*, *Mus musculus*) and six paired end samples for ChIP analysis are used to check the quality results. The MultiQC reports seem to be good for all single read sequences. Each RNA-Seq sample (GSE) was aligned against two reference genomes; *Homo sapiens* (GRCh38/hg38) and *Mus musculus* (GRCm38/mm10) separately. Results indicated 81.2% of uniquely reads mapped for human and 20.7% of uniquely reads mapped for mouse. 11% of reads mapped to both human and mouse genomes are excluded for further analysis (Supplementary Figure 1).

Detection of Differentially Expressed (DE) genes

A total of 43,388 genes were detected for the reads aligned to the human genome. Variance, sample-sample distance, dispersion estimates and histogram for DE human tumor genes are given in Figure 1. However, 64 DE genes were found for bortezomib-treated group (adjP<0.05). Of these, 27 genes were up-regulated and 37 genes were down-regulated (log2 FC ≤ -0.5) for bortezomib treatment (log2 FC ≥ 0.5) Supplementary Table 1. 25,239 genes were detected for the read alignment in mouse cells. Of these, 12 *DE* genes were found for bortezomib-treated groups (adjP<0.05). Variance analysis for *mus musculus* tumor cells are given in Figure 2. Out of which 3 *DE* genes are up-regulated and 9 *DE* genes are down-regulated (log2 FC ≤ -0.58).

The genes that were found to be up-regulated or down-regulated in drug-treated groups were assigned to pathways using information from several databases. The top ten Gene Ontology (GO) terms for *Homo sapiens* involve extracellular region, external encapsulating structure, extracellular matrix, collagen-containing extracellular matrix, endoplasmic reticulum lumen, external encapsulating structure organization, extracellular matrix organization, extracellular matrix structural constitution, extracellular structure organization and platelet-derived growth factor binding pathways (Table 1, Supplementary Figure 1). The top ten GO terms for *mus musculus* showed that the DE genes were involved in response to acid chemical, development of animal organ and system, animal organ morphogenesis, process for multicellular organism, development of multicellular organism, anatomical structure morphogenesis, retinal

Table 1: Pathways enriched for Differently Expressed (DE) genes for human (GRCh38/hg38) tumor cells.

GO Terms	Category	de_genes
extracellular matrix structural constituent	GO:OOJ5201	SPARC,COL6AI,PXDN,COL4AI,LAMB3,COL5AI,LAMB1,COL14AI,LUM,FBLN2,COL1AI,FBLN1,COL3AI
endoplasmic reticulum lumen	GO:OOJ5788	COL6AI,APOL1,COL4AI,COL5AI,LAMB1,COL14AI,SPP1,COL1AI,IGFBP4,SPARCL1,COL3AI,C3,COL18
extracellular matrix organization	G0:0030198	SPARC,COL6AI,PXDN,COL4AI,LAMB3,PDGFRA,COL5AI,LAMB1,MMP2,CDH1,COL14AI,LUM,FBLN2,
extracellular structure organization	G0:0043062	SPARC,COL6AI,PXDN,COL4AI,LAMB3,PDGFRA,COL5AI,LAMB1,MMP2,CDH1,COL14AI,LUM,FBLN2,
external encapsulating structure organization	G0:0045229	SPARC,COL6AI,PXDN,COL4AI,LAMB3,PDGFRA,COL5AI,LAMB1,MMP2,CDH1,COL14AI,LUM,FBLN2,
Platelet-derived growth factor binding	G0:0048407	COL6AI,COL4AI,PDGFRA,COL5AI,COL1AI,PDGFRB,COL3AI
collagen-containing extracellular matrix	G0:0062023	SPARC,SERPI NG1,COL6AI,PXDN,COL4AI,LAMB3,COL5AI,LAMB1,MMP2,COL14AI,LUM,TI MP3,FBLN
extracellular matrix	G0:0031012	SPARC,SERPING1,COL6AI,PXDN,COL4AI,LAMB3,COL5AI,LAMB1,MMP2,COL14AI,LUM,TI MP3,FBLN
external encapsulating structure	G0:0030312	SPARC,SERPING1,COL6AI,PXDN,COL4AI,LAMB3,COL5AI,LAMB1,MMP2,COL14AI,LUM,TIMP3,FBLN.
extracellular region	GO:OOJ5576	SPARC,PAEP,SERPING1,COL6AI,APOL1,LEFTY1,PXDN,COL4AI,LAMB3,C1R,COL5AI,VNN1,LAMB1,MI
extracellular space	GO:OOJ5615	SPARC,PAEP,SERPING1,COL6AI,APOL1,LEFTY1,PXDN,COL4AI,C1R,COL5AI,LAMB1,MMP2,CDH1,COI
growth factor binding	G0:0019838	COL6AI,PXDN,COL4AI,PDGFRA,COL5AI,COL1AI,IGFBP4,PDGFRB,COL3AI,A2M
structural molecule activity	GO:OOJ5198	SPARC,COL6AI,PXDN,COL4AI,LAMB3,COL5AI,LAMB1,COL14AI,LUM,FBLN2,COL1AI,CLDN4,FBLN1,I
extracellular matrix structural constituent conferring tens	G0:0030020	COL6AI,COL4AI,COL5AI,COL14AI,COL1AI,COL3AI,COL18AI
collagen trimer	GO:OOJ5581	COL6AI,COL4AI,COL5AI,COL14AI,LUM,COL1AI,COL3AI,COL18AI

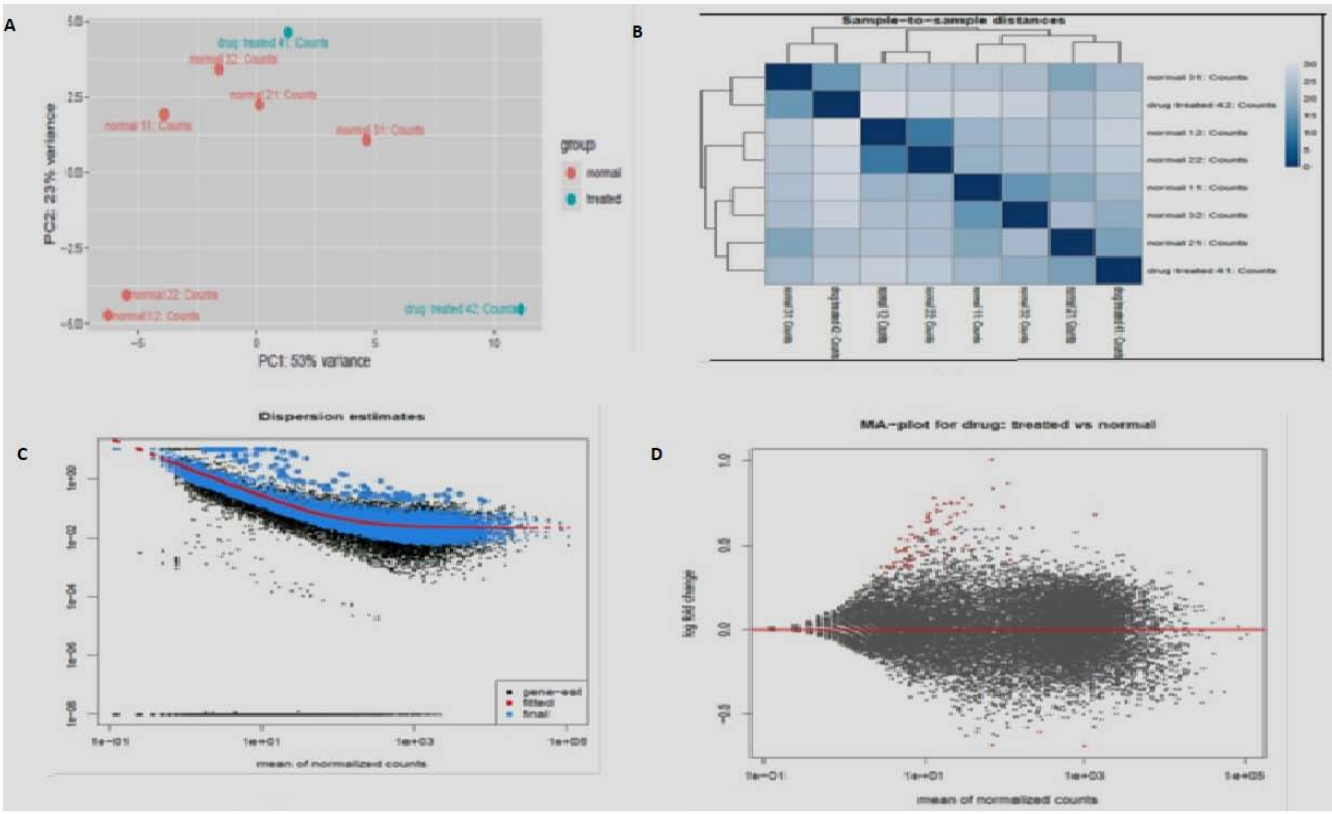

Figure 1: (A) Variance between drugs treated versus normal group. (B) Sample-sample distances for the drugs treated versus normal group counts. (C) Dispersion Estimates for dispersion versus mean of normalized counts. (D) Histogram of p-values for drug treated versus normal group. (For GRCh38/hg38).

Table 2: Pathways enriched for Differently Expressed (DE) genes in Tumor Microenvironment (TME) (mouse tissue (GRCh38/mm[10])) after bortezomib treatment. C: The number of reference genes in the category; O: The number of genes in the gene set and also in the category; E: The expected number in the category; R: Ratio of enrichment; adjusted by the multiple test adjustment.

DE Genes	Pathway Name	C	O	E	R
Col4a1, Col1a2, Gja1, Id4, Lamb1,Sparc ,Tgm2					
	response to acid chemical	207	7	1.22E-07	0.0017
Col4a1, Col1a2, Gja1, Id4, Lamb1, Sparc, Tgm2					
	animal organ development	1881	13	3.531E-06	0.0248
Col1a2, Gja1, Id4, Lamb1, Tgm2					
	system development	2505	14	9.298E-06	0.0435
Col4a1, Col1a2, Gja1, Id4, Lamb1, Sparc, Tgm2					
	animal organ morphogenesis	632	8	0	0.0657
Col4a1, Col1a2, Gja1, Id4, Lamb1, Sparc, Tgm2	multicellular organismal process	3312	15	0	0.0657
Col4a1, Col1a2, Gja1, Id4, Lamb1, Sparc, Tgm2	multicellular organism development	2758	14	0	0.0794
Col4a1, Col1a2, Gja, Id4, Lamb1, Sparc, Tgm2					
	anatomical structure morphogenesis	1535	11	0	0.0907
Col4a1	retinal blood vessel morphogenesis	6	2	0.0001	0.1212
Col4a1, Col1a2, Gja1, Id4, Lamb1, Sparc, Tgm2					
	anatomical structure development	2950	14	0.0001	0.1303
Col4a1	retina vasculature morphogenesis in camera-type eye	9	2	0.0002	0.2153

blood vessel morphogenesis, anatomical structure development and retina vasculature morphogenesis in camera-type eye Table 2. The results exemplify potential role of drugs for the immune response in the mouse.

The pathway enriched analysis of *DE* genes in MM cancer cells of (*Homo sapiens* and *Mus musculus*) is given in Supplementary Figure 2 and Supplementary Figure 3 respectively. The enriched GO categories are shown in red boxes. Interactive Heatmap was drawn for top variable genes and then it is used to calculate the euclidean distance between samples (Supplementary Figure 4, 5). The functional

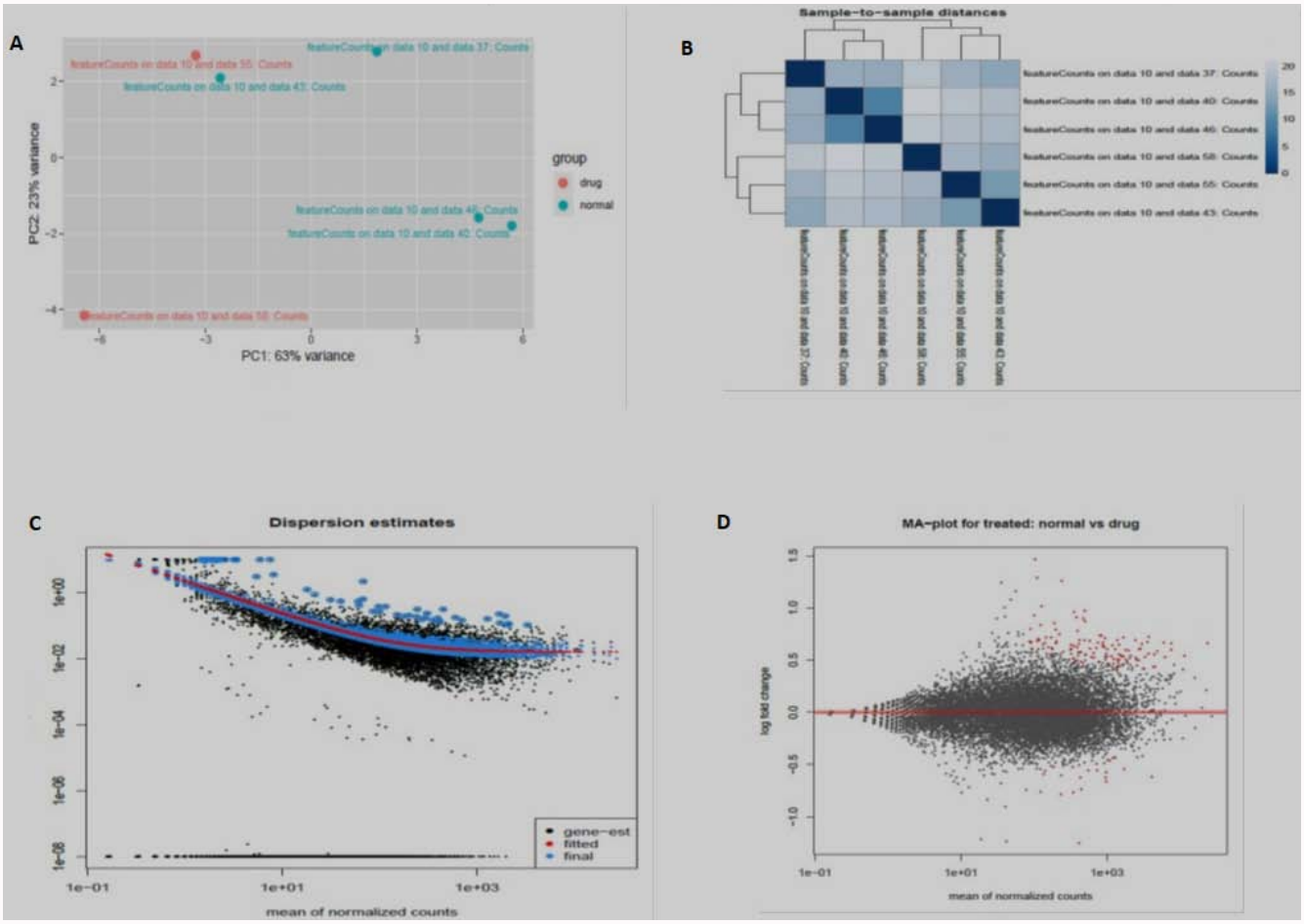

Figure 2: (A) Variance between drugs treated versus normal group. (B) Sample-sample distances for the drugs treated versus normal group counts. (C) Dispersion Estimates for dispersion versus mean of normalized counts. (D) Histogram of p-values for drug treated versus normal group. (For GRCh38/mm10).

Table 3: Integrated KEGG Pathways analysis from 100 peaks ChIP data and DE Genes (*Homo Sapiens*) from Cistrome-GO.

KEGG Pathways	Enrichment	P-Value	FDR
Viral carcinogenesis	12.083	6.30E-21	6.64E-19
Transcriptional misregulation in cancer	7.396	6.64E-09	4.20E-07
TNF signaling pathway	28.193	6.26E-03	1.52E-01
Systemic lupus erythematosus	29.94	4.10E-55	1.30E-52
RNA degradation	28.04	6.09E-03	1.52E-01
Relaxing signaling pathway	23.855	9.34E-03	1.97E-01
Neurotrophin signaling pathway	26.061	7.65E-03	1.73E-01
Necroptosis	8.755	6.75E-10	5.33E-08
Mitophagy - animal	48.456	2.12E-03	8.25E-02
GnRH signaling pathway	33.346	4.33E-03	1.37E-01
Colorectal cancer	5.634	5.61E-03	1.52E-01

Table 4: Integrated KEGG Pathways analysis from 100 peaks ChIP data and DE Genes (*Mus musculus*) from Cistrome-GO.

KEGG Pathways	Enrichment	P-Value	FDR
Systemic lupus erythematosus	33.377	2.38E-34	7.43E-32
Alcoholism	24.632	3.82E-32	5.97E-30
Viral carcinogenesis	12.668	1.35E-13	1.41E-11
Necroptosis	10.726	7.04E-08	5.49E-06
Transcriptional misregulation in cancer	8.683	9.18E-06	5.73E-04

Table 5: Approved drugs/Immunotherapies from drug-gene interactions (*Humans*) with DGIdb.

Differential Expressed (DE) Genes	Approved Drugs/ Immunotherapies
GJA1	BLEOMYCIN, PROPYLTHIOURACIL
PDGFRA	IMATINIB, SUNITINIB, PONATINIB, NILOTINIB, DASATINIB, PACLITAXEL, PAZOPANIB, SORAFENIB, CARBOPLATIN
MMP2	CYCLOSPORINE, BEVACIZUMAB, VINBLASTINE, PACLITAXEL, STREPTOZOCIN
CDH1	CAPECITABINE
GSTP1	DAUNORUBICIN, CYTARABINE, BUSULFAN, PREDNISONE, PACLITAXEL, BLEOMYCIN, MELPHALAN, THIOTEPA, EPIRUBICIN, ETOPOSIDE, CYCLOPHOSPHAMIDE, DECITABINE, IFOSFAMIDE, CISPLATIN, DEXAMETHASONE, OXALIPLATIN, AZACITIDINE, FLUOROURACIL, CARBOPLATIN, DOXORUBICIN, DOCETAXEL,
SPP1	TACROLIMUS
PDGFRB	SUNITINIB, PAZOPANIB, NILOTINIB, PONATINIB, DASATINIB, IMATINIB, SORAFENIB
GDA	THIOGUANINE

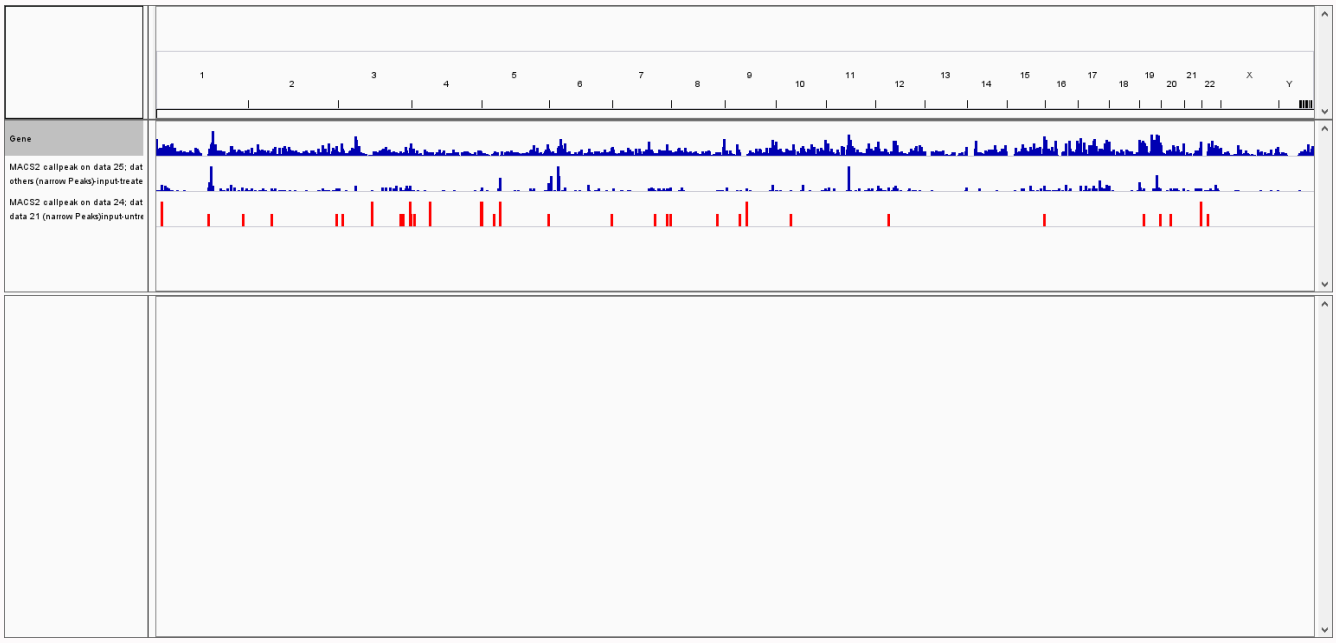

Figure 3: 100 peak calling for DE tumor cells genes in *homosapiens*(GRCh38/hg38).

significance of gene list with pathways analysis for *Homo sapiens* and *Mus musculus* data was given in the Supplementary Figure 6, 7.

Principal component analysis

An unbiased PCA for *DE* genes were performed using highly variable genes to explore the global transcriptomic forms in RNA-seq data. It is anticipated that any two genes are likely to display some similarities or dissimilarities regarding to their correlation value distributions. In a gene list, gene groups can be identified as the members of the list who share correlations in common that are not shared with other members. Principal Component Analysis (PCA) derives the principal components within the gene correlation matrix and the multidimensional dataset is shown graphically as a 2-dimensional scatter plot. The 64 statistically significant principal components in the PCA were reduced to 2D values using t-SNE and these *DE* genes were divided into 7 discrete clusters (*Homo sapiens*). (Supplementary Figure 8) For *mus musculus*, the *DE* genes are divided in 3 cluster groups Supplementary Figure 9.

Single gene *vs.* gene set

Further the location of *DE* genes in our studies is correlated with the primary gene's (NSD) family for correlation distribution. The correlation is less significant for human and murine tumor cells Supplementary Figure 10, 11.

ChIP analysis

ChIP analysis provides an insight towards gene regulatory process when the transcriptomic profiles from expression microarrays are combined with ChIP Sequencing data. Transcription Factor (*TF*) genes play a major role in transcription by regulating gene expression. Next Generation Sequencing (NGS) technologies are cost effective, have genome coverage over wide range of data, and recover weaker binding events. ChIP-seq analysis depends on the variety of DNA-binding factor, sensitivity and specificity of antibody towards the research area. It can be estimated by the total sub-sampling sequencing reads, and computing the recovery rate of ChIP-seq peaks [44]. The peak calling is used to detect regions with significant enrichment of ChIP signals with respect to the irrespective background. Gene regulation can also be understood by the ChIP-seq data of *TF*s, chromatin factors, and histone marks. It requires the prediction of *TF* regulated target genes, and its binding sites and binding patterns in Figure 3 (*Homo sapiens*) and Figure 4 (*Mus musculus*) for the 100-

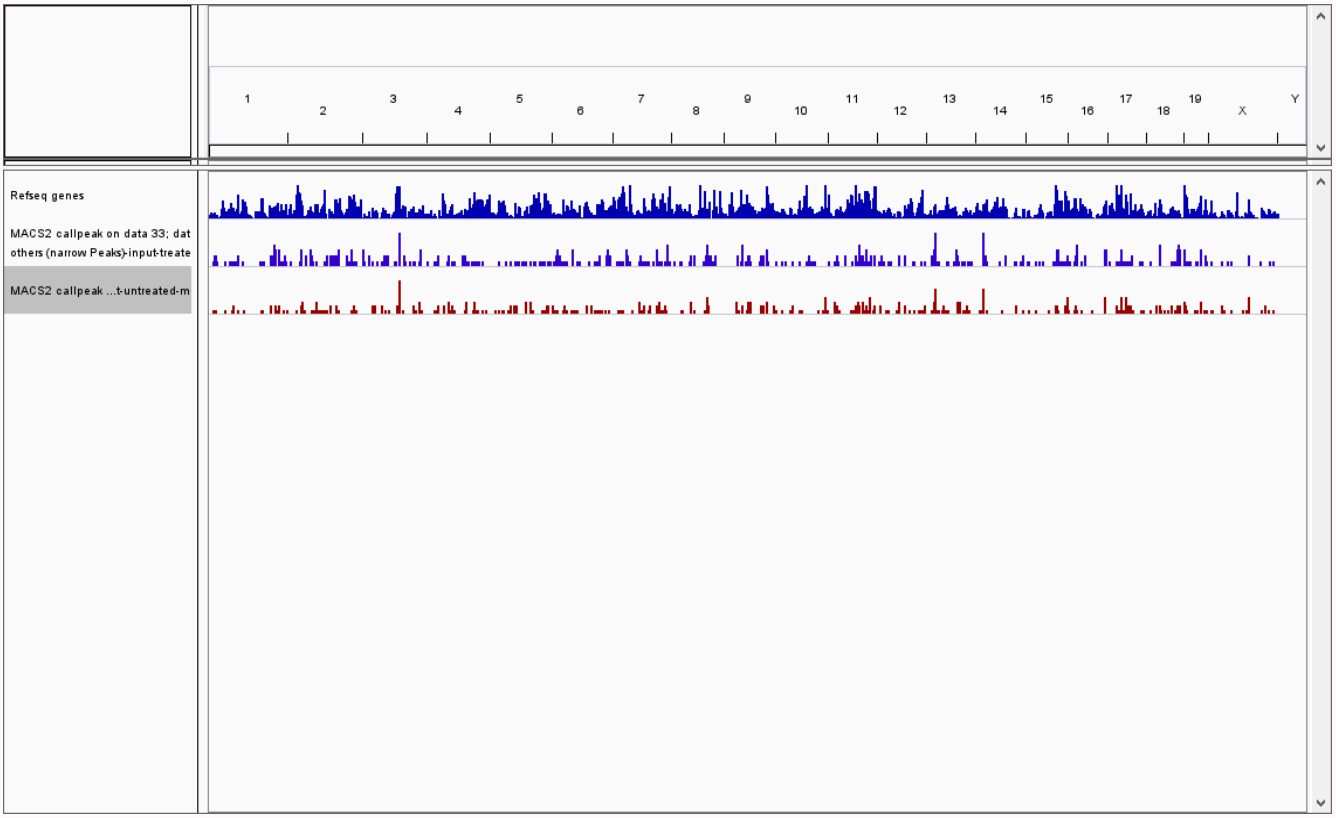

Figure 4: 100 peak calling for DE tumor genes in *mus musculus*. (GRCh38/mm10).

Table 6: Approved drugs/Immunotherapies from drug-gene interactions (*Mus musculus*) with DGIdb.

Differential Expressed (DE) Genes	Approved Drugs/ Immunotherapies
Vcan	CYCLOSPORINE
Gja1	BLEOMYCIN, PROPYLTHIOURACIL
Tgm2	SIROLIMUS, GEMCITABINE, DOXORUBICIN, CISPLATIN, CORTICOTROPIN

peak calling.

The ten KEGG pathways from integrated (ChIP-DEGs) analysis predicted viral carcinogenesis, transcription misregulation in cancer, TNF signalling pathway, systemic lupus erythematosus, RNA degradation, relaxin signalling pathways, neurotrophin signalling pathway, necroptosis, mitophagy, GnRH signalling pathway and colorectal cancer for the homo sapiens data (Table 3) and systemic lupus erythematosus, alchohalism, viral carcinogenesis, necroptosis and transcription misregulation in cancer for *mus musculus*. All these pathways are associated with the genetic disorders (Table 4).

Drug-gene interaction

Approved drugs and immunotherapies have been predicted for *GJA1*, *PDGFRA*, *MMP2*, *CDH1*, *GSTP1*, *SPP1*, *PDGFRB* and *GDA* gene in Humans. Few of these approved inhibitors have already been used for the treatment of MM patients (Table 5). The other approved drugs/immunotherapies have been predicted for *VCAN*, *GJA1* and *TGM2* differentially expressed genes for *mus musculus* (Table 6). For example: PACLITAXEL IMATINIB, SUNITINIB, BLEOMYCIN, PONATINIB, VINBLASTINE [45,46], among many others are already used as combination therapies for MM patients. The possible major drug targets for PACLITAXEL (Cytochrome P450 2C8), IMATINIB (BCR-ABL tyrosine kinase), SUNITINIB (receptor tyrosine kinase), BLEOMYCIN (DNA), PONATINIB (BCR-ABL tyrosine kinase), VINBLASTINE (Tubulin alpha-1A chain) is mentioned in DrugBank [47]. In a previous study [48], it was observed that increased TXNDC5 expression in plasma cells and serum is related to a poor response for bortezomib-based therapy in patients with newly diagnosed MM and in those with relapsed MM. So, it is vital to understand the biology of MM and to identify the drug-resistance biomarkers for personalized treatments. Our studies suggested a combination therapy, which is seen in clinical treatment as well.

Discussion

MM is a multifactorial disease, which includes wide variety of risk factors that effects various aspects of life. Different studies showed different causes for MM. In one such study among 22 meta-analyses for MM patients, the risk factors for 9 were due to occupational factors, 4 were assessed due to lifestyle (smoking, alcohol, body mass index) changes, 5 were due to presence of other diseases, and 4 due to genetic factors [48]. In our studies, with 64 *DE* genes, 50 genes were matched with the 1937 genes/proteins of *MM* genes from the dataset of curated CTD Gene-Disease Associations [49]. The studied upregulated and downregulated genes play a role in various molecular functions, biological process, cellular component and various pathways. The differentially expressed up-regulated SPARC glycoprotein gene in the bone binds calcium. In MM, calmodulins mediates Ca2+, and cell

cycle progresses with increase of calmodulin in a cell. So, calmodulin inhibitors are used for treatment of MM as these inhibitors induce apoptosis in cancerous cells [50]. In a previous study, it was reported that mutated TAP gene and *calreticulin* gene are used to produce calcium binding protein, a major part of endoplasmic reticulum; which is involved in regulating calcium homeostasis [51]. *Calreticulin* produces a calcium-binding protein that is a major component of the endoplasmic reticulum and has been shown to be involved in various cell types for regulating calcium homeostasis, as a ligand for integrins, and as a component of phagocytic synapses [51]. The up-regulation of *calreticulin* gene is an adverse prognostic factor. Also, it acts as a dominating pro-phagocytic signal in diverse tumors and is correlated with increased *CD47* expression in cancer cells.

Surface membrane antigens as CD38, CD138, CD56, CD117, and CD33 identify the accumulated plasma cells in the bone marrow of patients with MM. In one such study, the whole transcriptome data revealed different expression levels of several genes, which showed myeloma pathogenesis. When these 50 genes were subjected to the GSEA using MSigDB, 11 of these genes had increased expression in plasma cells from patients with MM which were reported as up-regulated genes in the Munshi multiple myeloma data set [52]. In another study the expression of *FGFR3*, *NSD2*, *MAF*, *CCND1*, *CCND2*, *CCND3* and *MYC* were obtained for MM [53]. Zhan et al. has divided the gene expression in 7 MM subgroups based on GEP [54] in which the gene expression in four groups (CD-1/CD-2, MF, MS) were genetically defined by recurrent translocations and one by hyperdiploidy (HY) [55]. *EEF1G*, *ITM2C*, *FTL*, *CLPTM1L*, and *CYBA* were identified as possible genes associated with MM [56], but no correlation between the gene expressions was observed for other studies [57].

Cisplatin resistance-related protein *CRR9p* (CLPTM1L) gene, *EEF1G*, *ITM2C*, *FTL*, *CLPTM1L*, *CYBA* and many other differentially associated genes associated with myelomagenesis were also reported [57]. As MM is a heterogeneous disease, the phenotype appears same across patients, while the genotype varies significantly among individual patients [58]. Due to the heterogeneity, it is very difficult to determine causal drivers of MM [47]. Results from previous study predicted that in mutations of these expressed driver genes (*KRAS*, *NRAS*, and *IRF4*), treatment or mutations had no influence on clustering, rather it is influenced by karyotypic events [59].

In a latest study, the RNA-seq-based risk score is correlated to specific MM somatic mutation profiles and their responses to targeted treatment including EZH2, TOPK/PBK, MELK, and aurora kinase inhibitors is analysed. These were characterized with more than 20 mutations compared to the unmutated MM patients. The high-risk patients were identified with the RNA-Seq-based risk score with the following pathways; enrichment of genes related to cell proliferation, growth factor signaling, MYC pathway and epigenetic deregulation [59]. RNA-seq data of newly diagnosed MM patients treated with high-dose melphalan and autologous stem cell transplantation showed gene risk score for 267 genes in two independent cohorts (n=674 and n=76). The prognosis was predicted based on several Gene Expression Profiling (GEP)-based signatures including UAMS (70 genes) [60], *HOVON-65/GMMG-HD4* (92 genes), [61] and *IFM* (15 genes) [62]. The public datasets of RNAi [63,64], and CRISPR-Cas9 [65] (Dependency Map data, Broad Institute, www.depmap.org) was used to identify the essential genes in myeloma cell lines compared to other cancer cell lines. One hundred forty-two associated genes with poor survival compose the RNA-seq based risk score. Seven genes (*ATP8B1*, *FGFR4*, *FOXD4*, *MX1*, *NPTXR*, *TMEM171*, and *TNFRSF10B*) with a significantly lower DEMETER2 score were found in the myeloma cell lines (n=16) as compared to other cancer cell lines (n=695). GEP is a useful parameter to predict prognosis and screening for drug resistance biomarkers in MM with potential benefits for clinical management. High-risk patients were identified by genes expression involved in several major pathways implicated in MM pathophysiology, including cell proliferation, MYC pathways, and epigenetic regulation [52]. The studies further predicted higher RNA-seq based risk score values in the MM cells of patients characterized by *ASXL1*, *ATM*, *BRAF*, *DIS3*, *EP300*, *FGFR3*, *KMT2B*, *LRP1B*, *MAP3K1*, *MAX*, *NOTCH2*, *NUP214*, *PRDM1*, *PTPRD*, *RB1*, *ROS1*, *SETD2*, *TP53*, *TRRAP*, and *ZFHX3* mutations compared to patients with unmutated MM cells. Indeed, the great majority of MM patients relapses and eventually become resistant to all treatments. Compared to the *DE* genes, pathways and the risk factors involved in the above studies, the *DE* genes of our data predicted that the MM patients are not in the high-risk zone. Combination therapy is suggested to be a better option, which is already used in the clinical treatment for the MM patients [18]. In another studies on MM, 424 DEGs with 350 up-regulated genes and 74 down regulated genes showed that the spread and progression of MM is due to enrichment of transcription regulation, cell adhesion, cell differentiation of RNA polymerase II promoter, significant enrichment in protein binding rate, cell adhesion [66].

The top 10 GO pathways of human samples in the studies included extracellular region, external encapsulating structure, extracellular matrix, collagen-containing extracellular matrix, endoplasmic reticulum lumen, external encapsulating structure organization, extracellular matrix organization, extracellular matrix structural constitution, extracellular structure organization and platelet-derived growth factor binding, which do not show any high risk for the patients. Various groups have reported activated pathways such as cMYC, E2F activation, and chromosomal instability-defined GEP signature for higher risk of progression to MM with MGUS or SMM stage, [52] enhanced programmed cell death, NF-κB, DNA repair, and cytokines signaling pathway-related genes in MM cells in comparison with MGUS cells [53]. The impact of microenvironment on gene expression of MM cells revealed activation of crucial pathways, such as NF-κB, Notch, and Ras, and genes affecting cell amplification, cell endurance, and the regulatory activity of cell-cycle [67]. In the expression data of 229 MM patients and 20 healthy adults (GSE6477 and GSE13591) datasets, the top 5 Biological Processes (BPs) included the "immune response," "phagocytosis, engulfment," "positive regulation of B cell activation," "B cell receptor signaling pathway," and "phagocytosis, recognition" for MM patients. Potentially important target genes for 114 DEGs have been expressed in the membrane-bounded organelle (GO:0043227), organelle (GO:0043226), and intracellular organelle (GO:0043229), which have a number of annotated genes of 97/114; 100/114; 96/114 and FDR values of 2.80e-06; 1.45e-05; and 1.45e-05, respectively [68].

The correlation between the single gene (*NSD1*) versus *DE* genes indicated low correlation values (-0.25-0.25) for the human (Supplementary Figure 10) and *mus musculus* samples (Supplementary Figure 11).

The ChIP-seq analysis showed 100 lower peaks in humans and higher peaks in *mus musculus*. Since the level of these peaks are not similar in both Humans and *mus musculus*, it indicates that the target

cannot be used for further analysis in mice for knock-in and knock-out or further pharmacokinetics analysis (Figure 3, 4) respectively.

The KEGG pathways from the integrated (ChIP-DEGs) analysis also predicted carcinogenesis [67] for the Human (Table 3) and *Mus musculus* (Table 4) The ten KEGG pathways from integrated (ChIP-DEGs) analysis predicted viral carcinogenesis, transcription misregulation in cancer, TNF signalling pathway, systemic lupus erythematosus, RNA degradation, relaxin signalling pathways, neurotrophin signalling pathway, necroptosis, mitophagy, GnRH signalling pathway and colorectal cancer for the *Homo sapiens* data (Table 3) and systemic lupus erythematosus, alchohalism, viral carcinogenesis, necroptosis and transcription misregulation in cancer for *mus musculus*. All these pathways are associated with the genetic disorders.

Several new classes of small molecules can also be used to specifically target epigenetic regulators for preclinical and clinical trials. These are *ID3*, *C3*, *CFBPD*, *ZNF267*, *hsr-mir-98-5p* and *RDGBRA* for human tumor genes while *Esr1*, *Id4*, *Foxa1*, *mmu-mir-425-5p* and *mmu-mir-186-5p TF* gene interactions for *mus musculus*, which has been identified in Transcription Factors (*TF*), Protein-Protein interactions and *miRNA* interactions [69] (Supplementary Figure 12, 13) respectively.

In tRNA-derived fragments as a key regulatory factor in MM patients, the targets of tRF-60:77-Tr-TGT-1 from bioinformatics studies showed that Ras signaling pathway, cGMP-PKG signaling pathway, thyroid hormone signaling pathway and FoxO signaling pathways participated in MM [70]. The prognosis associated long noncoding RNA (mRNA) network for MM on bioinformatics analysis on microarray based 559 patients with MM from the GSE24080 included signal transduction by a p53 class mediator, cell cycle G2/M transition and mitotic cell cycle G2/M transition which is closely involved in proliferation of tumors [71]. KEGG pathway enrichment of 424 DEGs showed enrichment in PI3K Akt signaling pathway, actin cytoskeleton regulation, AGE-RAGE signaling pathway in diabetes complications, HIF-1 signaling pathway and TGF-β Signaling pathway, Rap1 signaling pathway, and tumor necrosis factor signaling pathway [66]. Bioinformatics studies for (GSE6477 and GSE13591) showed significantly enriched in "systemic lupus erythematosus," "influenza A," "antigen processing and presentation," "The cell differentiation," "hematopoietic cell lineage," "intestinal immune network for IgA production," and "graft-*vs.*-host disease". The significantly enriched pathways for 114 genes were Epstein-Barr Virus (EBV) infection (hsa05169), MicroRNAs in cancer (hsa05206), PI3K-Akt signaling pathway (hsa04151), and p53 signaling pathway (hsa04115) during development of MM [68].

The Drug-Gene interaction studies recommended combination therapies for Humans (Table 5) and *Mus musculus* data (Table 6). The combinations of Carfilzomib, lenalidomide, dexamethasone, cyclophosphamide, dexamethasone, Elotuzumab (or daratumumab), lenalidomide, and dexamethasone are already used for treatment of other MM patients [72,73]. Previous studies indicated the combination therapies from daratumumab, selinexor (XOP1 nuclear export inhibitor) and isatuximab (another anti-CD38 monoclonal antibody), antibody-drug conjugate (belantamab mafodotin) and BCMA-targeted CAR-T cell therapy (Idecabtagene vicleucel) are recently approved inhibitors to treat MM, while few others such as (Bispecifc T Cell Engager Antibodies (BiTEs), Antibody-Drug Conjugates (ADCs), Monoclonal Antibodies (MoAbs), Immunomodulatory Drugs (IMiDs), Proteasome Inhibitors (PIs) and small molecule inhibitors and modulators are at phase 1 and phase 2 clinical trials [8]. In 37 open clinical trials for treatment resistant MM combination of Bortezomib and other chemotherapeutic agents were recommended [68].

Conclusion

The identification of DEGs is now widely available via high throughput analysis of transcriptomes. However, the quality of data generated is highly dependent on the experimental design, quality of RNA, and sequencing depth. The ChIP-seq technique can provide valuable information about transcriptional regulation based on transcription factor binding to target DNA promoter motifs for coordinating transcriptional regulation in response to environmental cues, while RNA-seq alone does not provide complete information. However, a combination of these technologies opens up new prospects to better elucidate more comprehensive gene regulatory networks. In this study, Artificial Intelligence (AI) driven bioinformatics tools are used to predict the treatment for the MM patients using RNA-Seq and epigenetics data [18]. *TF*-gene interactions were seen for ID3, C3, CFBPD, ZNF267, *hsr-mir-98-5p* and *RDGBRA* human tumor genes while *Esr1*, *Id4*, *Foxa1*, *mmu-mir-425-5p* and *mmu-mir-186-5p TF* gene interactions were observed for *mus musculus*. The different level of epigenetics peaks found in both Humans and *Mus musculus* indicated that further analysis with mice is not required for knock-in and knock-out or pharmacokinetics analysis. The integrated (ChIP-DEGs) KEGG pathway analysis showed genetic disorders. Any conflicting transcriptomics and ChIP studies between human and rodent could be because of the small sample size. Drug-gene interaction predicted few approved drugs and immunotherapies for MM patients. Bioinformatics studies suggested a combination therapy, which is seen in clinical treatment as well. Overall, the bioinformatics studies showed positive correlation to the clinical results upto some extent, yet it is advisable that the treatment recommended to the patients will only be implemented after expert advice of Oncologists along with *in vivo* and *in vitro* studies.

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