Identification of biomarkers and signaling pathways during the treatment of multiple myeloma

Hanming Gu (✉ laygmp@gmail.com)
Shanghai University

Research Article

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Abstract

Multiple myeloma (MM) is an incurable hematologic malignancy, which is characterized by increased bone marrow plasma cells, osteolytic lesions, and anemia. Here, our aim is to characterize significant biomarkers and signaling pathways during the treatment of MM by using bioinformatic methods. The GSE180018 dataset was generated by DNBSEQ-G400 (*Homo sapiens*). The KEGG and GO analyses showed the biological processes such as "Protein export", "Protein processing in endoplasmic reticulum", "Vibrio cholerae infection", "Fc gamma R-mediated phagocytosis" are mainly affected in FOXM1 KO MM cells. Moreover, we identified the significant genes including CD44, NOTCH1, SELL, RAC2, HSPA8, VAV1, DDIT3, PLK1, HYOU1, ITGAL in FOXM1 KO MM cells. Therefore, our study may provide further guidance for the drug development of MM.

Introduction

Multiple myeloma (MM) is a hematological malignancy characterized by bone lesions, defective hematopoiesis, and renal failure¹. The assessment of MM is mainly dependent on molecular or flow cytometric analyses, but these tests cannot explain the spatial heterogeneity of MM². Therefore, a great need for developing noninvasive and safe biomarkers will enable higher precision of diseases activity. During the past two decades, advanced therapies for MM have been developed such as immunomodulatory drugs, proteasome inhibitors (PIs), histone deacetylase inhibitors^{3,4}. Although the current therapies provide various opportunities for improving the health quality of MM patients, several challenges such as therapy toxicity and drug resistance remain in existence⁵. Based on these, the establishment of predictive biomarkers may serve the treatment of MM.

Forkhead box transcription factor (FOXM1) plays key roles in several cellular processes including proliferation, differentiation, apoptosis, and redox signaling. Given that FOXM1 is elevated in various cancers and shows in tumor initiation and progression, FOXM1 is considered an important oncogene⁶. FOXM1 is a key transcription factor altered in cancer, which alters KRAS signaling in the development of hepatocellular carcinomas and lung tumorigenesis in RAS-driven tumors⁷. Moreover, FOXM1 is controlled by hepatocyte growth factor (HGF) through the activation of mesenchymal-epithelial transition (MET)-tyrosine kinase receptor⁸. Interestingly, FOXM1 increased the growth, survival and clonogenicity of MM cells, but the knockdown of FOXM1 abolished these features. However, the potential mechanism and function of FOXM1 in MM cells are still unclear⁹.

Here, we evaluated the function and potential mechanism of FOXM1 in MM cells by analyzing the RNA sequence data. We identified several DEGs and the relevant biological process of MM by comparing the WT and FOXM1 KO MM cells. We also performed the functional enrichment and protein-protein interaction (PPI) for finding key protein nodes during the knockout of FOXM1. These critical genes and pathways may shed light on the development of novel MM therapies.

Methods

Data resources, acquisition and preprocessing

Gene expression profiles of FOXM1-deficient (KOXM1 KO) and WT human myeloma cell lines (HMCLs) (GSE180018) include three WT HMCLs and three KOXM1 KO samples. To identify the DEGs between the WT and KOXM1 KO HMCLs, the expression matrix was processed by the multi-array analysis method.

The probe ID was converted to a gene symbol using annotation files obtained from the platform (GPL28038 DNBSEQ-G400). DEGs were identified by the limma algorithm in the R package. A P-value of <0.05 and $\log_2 \text{fold} \geq 1$ were used as the cutoff criteria for this analysis¹⁰.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses

The GO and KEGG analyses were performed by the database for annotation, visualization, and integrated discovery online platform (DAVID; <http://david.ncifcrf.gov/>). The P-value < 0.05 was considered as a statistical significance. The GO contains a biological process (BP), cellular component (CC), and molecular function (MF), which were shown in bubble plots by the R package (<https://cran.rproject.org/web/packages/ggplot2/index.html>).

PPI network

A PPI network was created to define the interactions among proteins. The online Search Tool for the Retrieval of Interacting Genes and proteins (STRING) database (<https://string-db.org/>) was used to construct the PPI network¹¹.

Module selection

To analyze and visualize the clusters of genes in the PPI network, MCODE was used to construct the modules from the PPI network. We set the degree cutoff 2, node score 0.2, and the maximum depth 100 as cutoff criteria.

Reactome analysis

We will select the DEGs ($P < 0.001$) in interesting terms that were used to perform the subsequent REACTOME Pathway enrichment analysis using the online REACTOME Pathway analysis tool (<https://reactome.org/>).

Results

Identification of DEGs between the WT and FOXM1 KO MM cells

To determine whether FOXM1 KO MM cells play a role in regulating the development of MM, we compared the wild type of MM cells with the FOXM1 KO MM cells. A total of 2,332 genes were identified to be differentially expressed in the FOXM1 KO MM cells with the threshold of $P < 0.01$. The heatmap and volcano plot show the DEGs were up-regulated and down-regulated in FOXM1 KO MM cells in comparison with the WT MM cells (Figure 1). The selected top 10 up- and down-regulated genes are listed in Table 1.

Enrichment analysis of DEGs between the WT and FOXM1 KO MM cells

To analyze the potential effects of FOXM1 on the development of MM, we performed the KEGG and GO analyses by using DAVID and R package (Figure 2). Our study showed the top ten significant KEGG pathways, including "Protein export", "Protein processing in endoplasmic reticulum", "Vibrio cholerae infection", "Fc gamma R-mediated phagocytosis", "B cell receptor signaling pathway", "Choline metabolism in cancer", "FoxO signaling pathway", "MAPK signaling pathway", "Regulation of actin cytoskeleton", and "Pathways in cancer".

We then analyzed the GO enrichments. We identified ten significant items in BP including "Regulation of apoptotic signaling pathway", "Proteasome-mediated ubiquitin-dependent protein catabolic process", "Response to endoplasmic reticulum stress", "Response to topologically incorrect protein", "Protein folding", "Response to unfolded protein", "Regulation of mitochondrion organization", "Cellular response to topologically incorrect protein", "Cellular response to unfolded protein", and "Endoplasmic reticulum unfolded protein response". We further identified the top ten CC items: "Cell-substrate junction", "Focal adhesion", "Cell leading edge", "Intrinsic component of organelle membrane", "Integral component of organelle membrane", "Spindle", "Nuclear membrane", "Ruffle", "Melanosome", and "Pigment granule". We also identified the top ten MF items: "Protein serine/threonine kinase activity", "Ubiquitin-like protein ligase binding", "Nucleoside-triphosphatase regulator activity", "Ubiquitin protein ligase binding", "Cadherin binding", "GTPase regulator activity", "GTPase activator activity", "Ribonucleoprotein complex binding", "Chaperone binding", and "SnoRNA".

PPI network and Module analyses

The PPI network was created to further study the relationships of DEGs in MM cells. We constructed the PPI network by using the 277 nodes and 312 interactions between WT and FOXM1 KO MM cells. The top two significant modules were shown to depict the functional annotation of genes (Figure 3 and Table 2).

To further study the relationship of these proteins, we also performed the Reactome analysis (Figure 4 and Table S1). The results show seven significant biological processes including "Unfolded Protein Response (UPR)", "IRE1alpha activates chaperones", "XBP1(S) activates chaperone genes", "Response of EIF2AK1 (HRI) to heme deficiency", "Insulin-like Growth Factor-2 mRNA Binding Proteins

(IGF2BPs/IMPs/VICKZs) bind RNA”, “Regulation of PLK1 Activity at G2/M Transition”, and “RUNX3 regulates RUNX1-mediated transcription”.

Discussion

MM is related to genomic instability that affects numerous oncogenic pathways^{12, 13}. Despite the medical advances, MM remains incurable in the majority of patients¹⁴. The tumor microenvironment plays a key role in the development and progression of a number of tumor types¹⁵. The effect of the microenvironment between MM and bone marrow cells is critical for disease progression and drug development¹⁶. As one of the key factors, FOXM1 is a promising molecular target for high-risk MM and microenvironment⁹. Thus, identifying whether targeting FOXM1 as a novel therapeutic strategy for MM is of great importance. Our KEGG analysis showed MM is involved in protein quality control and immune activation. Tagoug I et al reported that the enhancement of ER stress can sensitize the MM cells to proteasomal inhibitors¹⁷. Tang Ch et al found the blocking of ER stress associated with IRE-1/XBP-1 can inhibit the leukemia cell survival¹⁸. ER stress has been found to play a crucial role in maintaining the cell homeostasis and physiological function^{19, 20}. Here, we found the FOXM1 treatment mainly affects the protein export and protein processing in endoplasmic reticulum, which suggests that protein control is a critical process of MM. Besides the ER stress, the immune-related pathways also play an important role^{21–24}. We found the Fc gamma R-mediated phagocytosis is activated during the treatment and the MAPK signaling pathway is also triggered. Similarly, J Yang et al reported the activated p38 MAPK in tumor cells results in bone lesions in MM²⁵. Teru Hideshima et al found the inhibition of p38 MAPK increases the cytotoxicity against the MM cells²⁶. Interestingly, the GO analysis showed the apoptosis and ubiquitination are involved in the treatment of MM. The previous report showed a number of MM therapeutics are involved in the proapoptotic and antiapoptotic Bcl-2 family of proteins and of inhibitors of apoptosis²⁷. As a chemical tool for exploring proteasomal function, the proteasome inhibitors are now widely used at all stages of MM²⁸.

In this study, we characterized the PPI network during the FOXM1 treatment of MM. Wenxing Gu et al found the CD44-specific A6 short peptide (KPSSPPEE) can enhance targetability and anticancer efficacy toward MM²⁹. Anita Skrtić found that the MM expressed 92.31% (72/78) NOTCH1 cases and NOTCH1 staining was strong in the majority of MM cases³⁰. The previous report also showed that the MicroRNA-27 represses the autophagy but enhances the MM cell proliferation through the NEDD4/NOTCH1 Axis³¹. Circadian gene clocks play a critical role in regulating the cell cycles, metabolism, progression, differentiation, and cell functions^{32–36}. Also, circadian rhythm controls the majority of genes activity that involves various diseases including bone diseases, arthritis, aging, and metabolic diseases^{32, 37, 38}. As one of the circadian controlled genes, NOTCH1 can be regulated by PER3 during adipogenesis³⁹. H Angharad Watson found the L-selectin (CD62L) can increase the T cells to eliminate cancer cells⁴⁰. GPCR and RGS family proteins are closely related to the cell metabolism and functions^{41–43}, loss of which will cause various diseases including cancer, bone diseases, inflammatory diseases, and nerve diseases^{44, 45}.

As a kind of prognostic biomarker, Rac family small GTPase 2 can enhance the progression of kidney cancer⁴⁶. As a candidate marker for endometrial carcinoma, HSPA8 is also involved in the acute MM^{47, 48}. As a tumor suppressor, VAV1 combines with Bbl-b and ICN1 to promote the degradation of ICN1⁴⁹. Lin H found the DDIT3 can regulate the cancer stemness in gastric cancer by mediating CEBPbeta⁵⁰. Robert P Evans et al reported that PLK1 is highly expressed in MM patients' bone marrow and is an independent adverse prognostic factor⁵¹. HYOU1 was reported to promote tumor cell proliferation and metastasis through PI3K/AKT signaling in epithelial ovarian cancer⁵². Integrin subunit alpha L was reported to be relevant to thyroid cancer by bioinformatic study⁵³.

In summary, our study provides evidence for the treatment of MM by FOXM1. The ER stress and Immunol signaling are the main biological processes during the treatment of MM. Our future studies will further explore FOXM1's mechanism of treatment for MM. Thus, this study may guide the drug development and clinical therapy of MM.

Declarations

Author Contributions

Hanming Gu: Conceptualization, Methodology, Writing- Reviewing and Editing.

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Declarations of interest

The author declares that there is no conflict of interest.

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Tables

Table 1

Entrez gene	Gene Symble	Fold-change	Regulation
Top 10 down-regulated DEGs			
27019	DNAI1	-2.915444524	Down
11045	UPK1A	-2.905284028	Down
79977	GRHL2	-2.703445298	Down
102724219	LOC102724219	-2.608568444	Down
2994	GYPB	-2.444313829	Down
284525	SLC9C2	-2.320280303	Down
347168	OR1J1	-2.283834562	Down
6540	SLC6A13	-2.211703973	Down
290	ANPEP	-2.196158711	Down
434	ASIP	-2.185374364	Down
Top 10 up-regulated DEGs			
100169851	PATE3	2.97403579	up
100129520	TEX13C	2.29248125	up
538654	TMEM110	2.241526861	up
440087	SMCO3	2.201838731	up
150356	CHADL	2.03630614	up
105371191	LOC105371191	1.979453368	up
112267874	LOC112267874	1.923565413	up
346	APOC4	1.894025701	up
3681	ITGAD	1.871475957	up
79585	CORO7	1.842316162	up

Table 2. Top ten genes demonstrated by connectivity degree in the PPI network

Gene Symbol	Gene title	Degree
CD44	CD44 molecule	19
NOTCH1	Notch receptor 1	18
SELL	Selectin L	15
RAC2	Rac family small GTPase 2	14
HSPA8	Heat shock protein family A (Hsp70) member 8	14
VAV1	Vav guanine nucleotide exchange factor 1	12
DDIT3	DNA damage inducible transcript 3	11
PLK1	Polo like kinase 1	11
HYOU1	Hypoxia up-regulated 1	11
ITGAL	Integrin subunit alpha L	11

Figures

Figure 1

Heatmap and volcano plots of differential expression. (A) Heatmap shows DEGs between WT and FOXM1 KO groups. Each row of the heatmap represents the z-score transformed log2 values of one DEG across all samples. (B) Volcano plots of DEGs.

Figure 2

KEGG and GO analyses. (A) KEGG: the chart of enrichment results grouped by clusters (labeled on the left-hand side of each panel). (B) GO analyses were created by the R package. BP, Biological process; CC, cellular component; MF, Molecular function.

Figure 3

The protein-protein interaction (PPI) network analysis. The 227 DEGs were input into the STRING database. The main cluster 1 (A) and cluster 2 (B) were analyzed and constructed by MCODE in Cytoscape.

Figure 4

Reactome map indication of the distribution of biological processes.