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HSP70 is the Most Significantly Upregulated Molecule Upon Bortezomib Stimulation: A Study Based on the Multiple Myeloma Database

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Abstract: *Objective*: This study aimed to investigate the changes in gene expression profiles of multiple myeloma (MM) cells after bortezomib treatment by analyzing the GEO database, thereby providing a theoretical foundation for subsequent research on HSP70. *Methods*: The GSE41929 dataset was selected from the GEO database. Screening and analysis were performed to identify differentially expressed genes between bortezomib-treated and non-treated MM cells. *Results*: After bortezomib treatment, 126 genes in MM cells showed the most significant changes in expression ($P < 0.05$, absolute value of logFC ≥ 1.5). Based on the fold change and the most significant gene module, *HSPA1B* exhibited the most notable upregulation after *HMOX1*, followed by *HSPA6* and *DNAJB1*. *HSPA1B* and *HSPA6* are members of the HSP70 protein family, while *DNAJB1* primarily interacts with HSP70 to stimulate its ATPase activity and negatively regulates the transcriptional activity of HSF1 induced by heat shock. *Conclusion*: HSP70 was the most significantly upregulated molecule in MM cells following bortezomib stimulation.

Keywords: Bortezomib; Multiply myeloma; HSP70; GEO database

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1. Introduction

Multiple myeloma (MM) is the second most common hematologic malignancy. It accounts for approximately 1% of all newly diagnosed cancers globally each year [1]. MM predominantly affects middle-aged and elderly individuals, and its exact etiology remains unclear. The pathogenesis involves the clonal proliferation of malignant plasma cells, leading to the excessive production and deposition of monoclonal immunoglobulins in various tissues. This results in clinical manifestations such as anemia, renal failure, hypercalcemia, and bone diseases [2,3].

In recent years, the introduction of novel drugs, including carfilzomib, ixazomib, pomalidomide, panobinostat,

and monoclonal antibodies (e.g., elotuzumab and daratumumab), has significantly improved the response rates and overall survival (OS) in patients with MM [4–6]. Among these, chemotherapy regimens combining proteasome inhibitors (PIs) and immunomodulatory drugs (IMiDs) are considered the first-line treatment for newly diagnosed transplant-eligible MM patients [7,8]. These combination therapies yield higher complete response (CR) rates than the standard high-dose therapy plus stem-cell transplantation (HDT-SCT). However, due to the heterogeneity in initial symptoms, clinical presentations, and survival times, nearly all patients eventually relapse or develop drug resistance [9,10].

Bortezomib is a particular, reversible 26S proteasome inhibitor and the first PI approved by the FDA for treating both newly diagnosed and relapsed/refractory MM. Its introduction has markedly improved MM prognosis, particularly in reversing the poor outcomes of patients with t(4;14) chromosomal abnormalities [11,12]. Nonetheless, primary and secondary resistance leading to relapse or death remains a major challenge in bortezomib therapy. Current research highlights several resistance mechanisms, including the ubiquitin-proteasome system (UPS), endoplasmic reticulum (ER) stress pathways, autophagy, pro-survival signaling, and the bone marrow microenvironment.

Compared to normal cells, malignant cells exhibit heightened proteasome activity. Proteasome inhibition causes the accumulation of ubiquitinated proteins in the ER, triggering ER stress and the unfolded protein response (UPR) [13]. The UPR induces heat shock proteins (HSPs), which serve as chaperones to maintain protein folding and mitigate ER stress—a process implicated in bortezomib resistance [14]. Consequently, HSP inhibitors have been proposed to sensitize tumor cells to bortezomib and enhance its therapeutic efficacy [15]. This study investigates the expression changes of HSP family molecules in MM cells after bortezomib stimulation using GEO database profiles, aiming to provide a theoretical basis for targeted MM therapies.

2. Methods

(1) GEO DataSets were searched for gene expression profiles of MM cells treated with bortezomib.

(2) The GSE41929 dataset (Genome-wide analysis of gene expression in response to bortezomib treatment [human cell lines]) was selected, comprising six samples. The experiment involved stimulating MM.1S and U266 cells with 33 nmol/L bortezomib at different time points and analyzing gene expression changes.

(3) Online grouping was performed in the database, followed by GEO2R analysis.

(4) All data were saved, and differentially expressed genes were screened using $P < 0.05$ and logFC absolute value ≥ 1.5.

(5) Protein-protein interaction (PPI) networks were constructed using STRING and visualized with Cytoscape (v3.7.2).

3. Results

3.1. Gene expression profile changes in MM cells after bortezomib stimulation

Using the GEO public gene chip database in the NCBI database, data retrieval of gene expression profile changes after bortezomib stimulation of MM was conducted. And the GSE41929 data was ultimately selected as the analysis object. The GSE41929 gene expression profile was developed and established by Illumina based on the GPL13369 (catalog number: DA–801–1003) platform. When downloading GSE41929 data online, it was found that there were a total of 6 chip samples. The samples were defined by “Define groups” and divided into a

control group and a bortezomib treatment group. **Figure 1** shows the changes in expression levels of all samples in a box plot. Then, the GEO2R analysis was performed, and the data were saved. Differential gene expression screening was performed based on $P < 0.05$ and the absolute value of logFC ≥ 1.5 times. A total of 126 differential genes were screened out. The top 10 genes with significantly upregulated expression and the top 10 genes with significantly downregulated expression were shown in **Tables 1** and **2**, respectively.

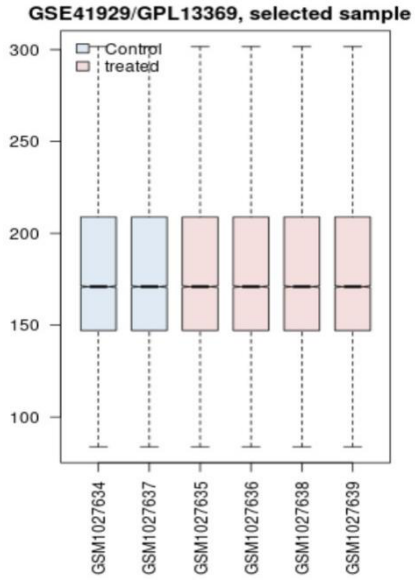

Figure 1. Box plot showed expression changes across all samples in the GSE41929 series

Table 1. Significantly upregulated genes

GB_ACC	Gene	*P* value	logFC value
NM_002133.1	HMOX1	6.63E-08	5.55846593
NM_005346.3	HSPA1B	6.76E-05	4.58144941
NM_002155.3	HSPA6	1.36E-02	4.43704114
NM_006145.1	DNAJB1	1.40E-04	3.89714309
NM_002228.3	JUN	3.01E-04	3.71489188
NM_004281.3	BAG3	2.35E-04	3.65778646
NM_182491.1	ZFAND2A	4.61E-04	3.62264090
NM_014330.2	PPP1R15A	8.92E-05	3.10443174
NM_001040619.1	ATF3	4.87E-04	3.06525544
NR_004400.1	RNU1-5	1.02E-04	3.02232600

Note: GB_ACC stands for gene bank accession number

Table 2. Top 10 significantly downregulated genes

GB_ACC	Gene	*P* value	logFC value
NM_024665.3	TBL1XR1	3.17E-02	-1.00003988
NM_144772.1	APOA1BP	6.99E-03	-1.00117889
NM_001110.2	ADAM10	3.18E-03	-1.00742746
NM_003200.1	TCF3	7.70E-04	-1.00825865
NM_001031684.1	SFRS7	1.32E-03	-1.01020377
NM_018079.3	SRBD1	4.70E-02	-1.01112345
NM_005916.3	MCM7	7.62E-03	-1.01404815
NM_006290.2	TNFAIP3	1.28E-03	-1.01417679
NM_004219.2	PTTG1	1.60E-03	-1.01583864
NM_024758.3	AGMAT	4.84E-02	-1.01685617

Note: GB_ACC stands for gene bank accession number

3.2. Protein-protein interaction network and hub genes

STRING is an online tool for retrieving gene-protein interaction relationships, which predicts potential interactions between proteins or genes. As shown in **Figure 2**, the protein-protein interaction (PPI) network of differentially expressed genes from the GSE dataset analyzed in this study was presented. The PPI network was imported into Cytoscape (v3.7.2) for visualization enhancement, where upregulated genes were highlighted in red and downregulated genes in blue. To further elucidate the core module among the differentially expressed genes, the MCODE plugin in Cytoscape was employed. The algorithm parameters were set as follows: Degree cutoff = 2, K-score = 2, Node Score Cutoff = 0.2. Then select Cluster Finding Haircut to calculate and analyze the weighted reconnection between network structures and nodes, and screen out the most significant modules, namely the regions where the Hub genes are located (**Figure 3**). The hub genes network includes 9 nodes and 28 pairs of edges. The functional annotations of each Hub gene are shown in **Table 3.**

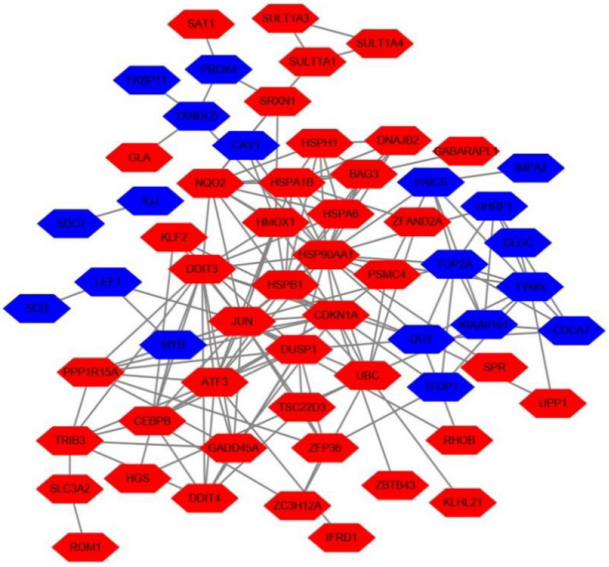

Figure 2. PPI network of differentially expressed genes (red: upregulated genes; blue: downregulated genes)

Following differential expression analysis of the GSE41929 mRNA expression dataset, genes meeting the criteria of absolute value of logFC ≥ 1.5 and *P* value < 0.05 were selected for protein-protein interaction (PPI) network construction. Red markers indicate upregulated genes, while blue markers indicate downregulated genes.

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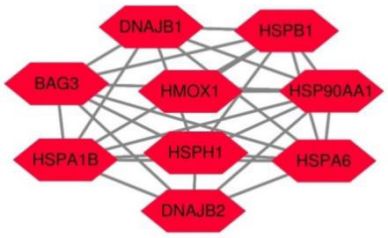

Figure 3. The most significant module

This module is obtained from a PPI network with 9 nodes and 28 pairs of edges.

Table 3. Functional annotations of hub genes

Gene	Full name	Function
HMOX1	Heme oxygenase 1	Heme oxygenase is an essential enzyme in heme catabolism, and excessive free heme can cause cell apoptosis.
HSPA1B	Heat shock protein family A member 1B	HSPA1B is a member of the HSP70 family. After binding with other heat shock proteins, this protein stabilizes existing proteins to prevent aggregation and participates in the ubiquitin proteasome pathway.
HSPA6	Heat shock 70 kDa protein 6	It involves multiple cellular processes, including protecting proteins from stress, folding and transporting newly synthesized peptides, hydrolysis and activation of misfolded proteins, and formation and dissociation of protein complexes. Plays a crucial role in ensuring correct protein folding, refolding misfolded proteins, and controlling protein targeting for subsequent degradation.
DNAJB1	DnaJ homolog subfamily B member 1	Interacts with HSP70 and can stimulate its ATPase activity. Negative regulation of heat shock induced HSF1 transcriptional activity during the attenuation and recovery stages of heat shock response。
BAG3	BAG family molecular chaperone regulator 3	HSP70 and HSC70 chaperone protein molecules. Acting as a nucleotide exchange factor (NEF), it promotes the release of ADP from HSP70 and HSC70 proteins, thereby triggering the release of client/substrate proteins.
DNAJB2	DnaJ homolog subfamily B member 2	Acting as a partner molecule, regulating substrate binding and activating ATPase activity of HSP70/heat shock protein 70 family partners. Meanwhile, it also contributes to the ubiquitin-dependent proteasome degradation of misfolded proteins.
HSPB1	Heat shock protein beta-1	Small-molecule heat shock proteins act as molecular chaperones and may keep denatured proteins in a folded state. Through its molecular chaperone activity, many biological processes can be regulated, including phosphorylation of neurofilament proteins and axonal transport.
HSP90AA1	Heat shock protein HSP 90-alpha	It can dynamically interact with various auxiliary partners to promote the maturation and structural maintenance of specific target proteins. Participate in cell cycle control and signal transduction.
HSPH1	Heat shock protein 105 kDa	NEF acts as a partner protein for HSPA1A and HSPA1B, promoting the release of ADP from HSPA1A/B and triggering the release of client/substrate proteins.

4. Discussion

Multiple myeloma (MM) is a malignant plasma cell tumor characterized by the clonal proliferation of plasma

cells in the bone marrow. In the past decade, the introduction of new drugs such as proteasome inhibitors and immunomodulators has extended the survival of MM patients. Proteasome inhibition is an important therapeutic target for MM. The US FDA approved the first-generation proteasome inhibitor bortezomib as a first-line treatment for MM in June 2008. Although newly diagnosed MM patients have a very high overall response rate to bortezomib [16], most patients eventually relapse because MM cells develop resistance to treatment [9,10].

Heat shock proteins (HSPs) are a class of proteins that protect cells from stress. In mammalian cells, HSPs can be classified into HSP100, HSP90, HSP70, HSP60, and HSP27 based on their molecular weight. As tumors progress, tumor cells rely on HSPs for "adaptive survival." It has been reported that HSPs contribute to the drug resistance of MM. After stimulation with bortezomib, the expression of *HSP90* and *HSP27* in MM cells was significantly upregulated [17,18]. However, the clinical application of HSP90 inhibitors has been hindered due to their drug toxicity and other reasons. There are also a few preclinical research experiments using HSP27 inhibitors for MM. Therefore, scholars have gradually paid attention to the important changes of *HSP70* in the development of tumors.

The HSP70 family is the most conserved, predominant, and abundant type of protein in HSP. The HSP70 family consists of five important members, including cytosolic HSP70 (HSPA1A or HSP72) and HSC70 (HSPA8), cell surface HSP70-2 (HSPA1B), mitochondrial GRP78/mortalin (HSPA9), and GRP78 (HSPA5), among which GRP78 is mainly localized in the endoplasmic reticulum (ER). Research has found that *HSP70* is highly expressed in various cancers and is associated with tumor grading, metastasis, prognosis, and drug resistance [19,20].

This study investigated the expression profile of GSE41929 gene chip in GEO database, screened the differential gene expression changes between bortezomib treatment group and non-bortezomib treatment group in MM cells, and found that after bortezomib treatment, the expression changes of 126 genes in MM cells were the most significant (P value < 0.05, absolute value of logFC ≥ 1.5 times). According to the expression fold and combined with the most important gene module, it can be seen that besides *HMOX1*, *HSPA1B* is upregulated the most significantly, followed by *HSPA6*, *DNAJB1*. *HSPA1B* and *HSPA6* are both members of the HSP70 protein family. The main function of *DNAJB1* is to interact with HSP70 molecules and stimulate their ATPase activity, negatively regulating heat shock-induced HSF1 transcriptional activity.

By analyzing the expression profile of the GSE41929 gene chip, it can be seen that after bortezomib stimulation of MM cells, the upregulation of *HSP70* is most significant. *HSP90*, *HSPB1*, and *HSPH1* are also upregulated to varying degrees, but none of them is as significantly upregulated as *HSP70*. This study suggests that HSP70 may be an important HSP that promotes MM resistance, and further attention and development of HSP70 inhibitors are needed to enhance the anti-MM effect of bortezomib.

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Disclosure statement

The authors declare no conflict of interest.

References

[1] Pirzaman AT, Ebrahimi P, Hasanpour AH, et al., 2023, miRNAs and Multiple Myeloma: Focus on the Pathogenesis, Prognosis, and Drug Resistance. Technol Cancer Res Treat, 22: 1–13.

[2] Dhodapkar MV, 2024, Immune Pathogenesis of Myeloma. Hematol Oncol Clin North Am, 38(2): 281–291.

[3] Gau Y, Yeh T, Hsu C, et al., 2022, Pathogenesis and Treatment of Myeloma-related Bone Disease. Int J Mol Sci, 23(6): 3112.

[4] Hatic H, Inselman S, Inselman J, et al., 2022, Time to First Treatment is an Independent Prognostic Factor For Multiple Myeloma (MM). Leuk Res, 123: 106966.

[5] Mimura N, Hideshima T, Anderson KC, 2015, Novel Therapeutic Strategies for Multiple Myeloma. Exp Hematol, 43(8): 732–741.

[6] Yang Y, Li Y, Gu H, et al., 2020, Emerging Agents and Regimens for Multiple Myeloma. J Hematol Oncol, 13(1): 150.

[7] Bazarbachi AH, Al Hamed R, Malard F, et al., 2022, Induction Therapy Before Autologous Stem Cell Transplantation (ASCT) in Newly Diagnosed Multiple Myeloma: An Update. Blood Cancer J, 12(3): 47.

[8] Facon T, San-Miguel J, Dimopoulos MA, et al., 2022, Treatment Regimens for Transplant Ineligible Patients with Newly Diagnosed Multiple Myeloma: A Systematic Literature Review and Network Meta-analysis. Adv Ther, 39(8): 3868–3869.

[9] Robak P, Drozdz I, Szemraj J, et al., 2018, Drug Resistance in Multiple Myeloma. Cancer Treat Rev, 70: 199–208.

[10] Vo JN, Wu Y, Mishler J, et al., 2022, The genetic heterogeneity and Drug Resistance Mechanisms of Relapsed Refractory Multiple Myeloma. Nat Commun, 13(1): 3750.

[11] Mikhael JR, Dingli D, Roy V, et al., 2013, Management of Newly Diagnosed Symptomatic Multiple Myeloma: Updated Mayo Stratification of Myeloma and Risk-Adapted Therapy (mSMART) Consensus Guidelines 2013. Mayo Clinic Proceedings, 88(4): 360–376.

[12] Jiang H, Wang Y, Wang J, et al., 2022, Posttranslational Modification of Aurora A-NSD2 Loop Contributes to Drug Resistance in t(4;14) Multiple Myeloma. Clin Transl Med, 12(4): e744.

[13] Wang G, Fan F, Sun C, et al., 2022, Looking into Endoplasmic Reticulum Stress: The Key to Drug-Resistance of Multiple Myeloma? Cancers (Basel), 14(21): 5340.

[14] Kubicki T, Bednarek K, Kostrzewska-Poczekaj M, et al., 2022, Bortezomib and Carfilzomib Resistant Myeloma Cells Show Increased Activity of All Three Arms of the Unfolded Protein Response. Am J Cancer Res, 12(7): 3280–3293.

[15] Ferguson ID, Lin Y, Lam C, et al., 2022, Allosteric HSP70 Inhibitors Perturb Mitochondrial Proteostasis and Overcome Proteasome Inhibitor Resistance in Multiple Myeloma. Cell Chem Biol., 29(8): 1288–1302.

[16] Richardson PG, Weller E, Lonial S, et al., 2010, Lenalidomide, Bortezomib, and Dexamethasone Combination Therapy in Patients with Newly Diagnosed Multiple Myeloma. Blood, 116(5): 679–686.

[17] Cavenagh J, Oakervee H, Baetiong-Caguioa P, et al., 2017, A Phase I/II Study of KW-2478, an HSP90 Inhibitor, in Combination with Bortezomib in Patients with Relapsed/Refractory Multiple Myeloma. British Journal of Cancer, 117(9): 1295–1302.

[18] Heinrich JC, Donakonda S, Haupt VJ, et al., 2016, New HSP27 Inhibitors Efficiently Suppress Drug Resistance Development in Cancer Cells. Oncotarget, 7(42): 68156–68169.

[19] Albakova Z, Armeev GA, Kanevskiy LM, et al., 2020, HSP70 Multi-Functionality in Cancer. Cells, 9(3): 587.

[20] Zhao K, Zhou G, Liu Y, et al., 2023, HSP70 Family in Cancer: Signaling Mechanisms and Therapeutic Advances. Biomolecules, 13(4): 601.