Technology in Cancer Research & Treatment
Volume 20: 1-11

DOI: 10.1177/15330338211024551
journals.sagepub.com/home/tct
SAGE

Expression and Clinical Significance of BCL2 Interacting Protein 3 Like in Multiple Myeloma

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Abstract

Multiple myeloma (MM) is one of the main blood disorders threatening human health today. This study aimed to examine the expression of BCL-2/adenovirus E1B 19 kDa-interacting protein 3-like (BNIP3L) in patients with MM and explore its mechanisms in silico. Bone marrow samples (n = 36 from patients with MM and n = 12 from healthy donors) were used to conduct BNIP3L expression analysis using immunohistochemistry. Microarray or RNA sequencing data from the Sequence Read Archive, Gene Expression Omnibus, and ArrayExpress databases were used to appraise BNIP3L expression and its prognostic role in patients with MM. The co-expressed genes of BNIP3L were identified for enrichment and protein-protein interaction (PPI) analyses to determine the associated signaling pathways. Immunohistochemistry indicated that BNIP3L expression in bone marrow of patients with MM was significantly lower than that in bone marrow of healthy donors. BNIP3L mRNA expression was also significantly lower in patients with MM than in healthy donors. The overall standard mean difference (SMD) for downregulation of BNIP3L was −0.62 [−1.17, −0.06], and the area under the curve was 0.81 [0.78, 0.85] based on a total of 694 MM cases. The overall survival analysis demonstrated that BNIP3L levels could act as an independent protective indicator of MM patient survival (HR = 0.79). Moreover, 261 co-expressed genes of BNIP3L were confirmed and found to be mainly involved in the adipocytokine signaling pathway. We preliminarily proved that downregulation of BNIP3L may play an important role in the occurrence and development of MM, and the promoting cancer capacity may be related to the pathway of adipocytokine signaling pathway.

Keywords

multiple myeloma, BNIP3L, immunohistochemistry, suppressor gene, silico

Abbreviations:

MM, Multiple myeloma; BNIP3L, BCL2 interacting protein 3 like; SRA, Sequence Read Archive; GEO, Gene Expression Omnibus; SMD, standard mean difference; ROC, receiver operating curve; sROC, summarized ROC; DEGs, differentially expressed genes; PPI, protein-protein interaction; KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene ontology

Received: August 11, 2020; Revised: March 5, 2021; Accepted: March 31, 2021.

Introduction

Multiple myeloma (MM) is a malignant disease characterized by abnormal proliferation of clonal plasma cells and the secretion of a monoclonal immunoglobulin protein known as M protein or monoclonal protein.[1] MM is the second most common malignant blood cancer globally.[2] The prevalence and incidence of MM in developed countries (North America and Western Europe) are significantly higher than those in developing countries.[3] However, owing to the trend of global population aging, the incidence of MM has been gradually increasing worldwide in recent years.[4]

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There have been many new technological improvements as well as innovations in diagnostic and prognostic assessment of MM, especially resulting from new biomarkers for diagnosis and prognosis.[5,6] Several studies have been conducted to reveal the close association of the development and prognosis of MM with the abnormal expression of vascular epidermal growth factor and hepatic growth factors[7] as well as inappropriate activation of endothelial cell migration and proliferation via ERK1/2 and JNK1/2/3 phosphorylation.[8] Long-chain noncoding RNA, circular RNA, and microRNA have also been found to affect the pathogenesis and prognosis of MM.[9] It has been reported that low expression of miR-30d is associated with the diagnosis and prognosis of MM, and that it has a potential therapeutic effect in MM by inhibiting excessive activation of the PI3K/Akt signaling pathways.[10] The count of circulating myeloma cells reflects tumor load, a finding that has great significance for assessing prognostic and curative effects in MM.[11] The Mayo stratification of myeloma and risk-adapted therapy is of great value in evaluating the depth of treatment responses and tiny residual MM depending on cytogenetic biomarkers and high-risk markers of the gene expression profile.[12]

Significant progress has also been made in targeted therapy, immune therapy, and autologous stem cell transplantation therapy in recent years by including the use of immunomodulatory drugs, protease inhibitors, chimeric monoclonal antibodies, and T-cell antigen receptors. However, owing to the highly heterogeneous and abnormal cytogenetics and molecular biology of MM, most patients with MM experience recurrence or eventually become resistant to treatment, resulting in poor prognosis. Therefore, revealing specific therapeutic targets in the MM microenvironment is necessary to develop effective and novel treatments. Research into MM-related genes is one of the main directions of exploration of the pathogenesis and new therapies of MM.

BCL-2/adenovirus E1B 19 kDa-interacting protein 3-like (BNIP3L) is a member of the genetic family that promotes tumor apoptosis through protein expression. Early research has revealed that BNIP3L is associated with the pathogenesis of many diseases, including cancer and cardiovascular disease,[13] and is a known suppressor gene of lung and breast cancers,[14] but its implications in MM remain unclear.[15]

In this study, the expression levels of BNIP3L in patients with MM patients were determined using different detection methods. This study also investigated the potential molecular mechanism of deregulated BNIP3L in MM via analysis of the associated signaling pathways.

Materials and Methods

BNIP3L Expression in MM and Normal Plasma Cells Using Immunohistochemistry

In terms of morphology, normal plasma cells possess the characteristics of eccentric nuclei. In most cases, the distribution of chromatin in normal plasma cells appears as a clock or wheel with abundant cytoplasm and obvious perinuclear holes. In contrast, MM cells are characterized by pale areas in the cytoplasm close to the nucleus with obvious nuclear abnormalities and dark blue cytoplasm. Sometimes, vacuoles and blue aniline particles can be found. These MM cells are distributed in large patches or clusters. Our immunohistochemical experiments utilized the bone marrow samples of 36 patients with MM (proportion of MM cells: 38%-82%) and 12 healthy donors (proportion of plasma cells: 0.5%-2%). The sections were stained with mouse anti-human BNIP3L antibody (ab155010) at a concentration of 5 μg/mL, according to the immunohistochemistry (IHC) experimental procedure. On each slide, 10 high-magnification fields were randomly selected, and the total number of MM, plasma, and BNIP3L positive cells were counted. The percentage of BNIP3L positive cells was calculated as the average of 10 fields.[16-19] This study protocol was approved by the ethics committee of the First Affiliated Hospital of Guangxi Medical University. All the participants have signed informed consent.

Collection of MM-Related Cohorts

MM-related cohorts were collected from the SRA, GEO, and ArrayExpress databases using the keyword "myeloma." The 2 inclusion criteria were as follows: firstly, the experimental and control groups were human patients with MM and healthy donors, respectively; secondly, the calculated mRNA expression data were to be provided by all included cohorts. The mRNA expression matrix data of each cohort were downloaded, and the mRNA expression data of BNIP3L were extracted. These cohorts were used to identify MM-related genes and evaluate the expression of BNIP3L and its related genes (Supplementary Figure 1). The prognosis-related cohorts were screened using public databases. The inclusion criteria were as follows: firstly, the sample size should be greater than 30; secondly, the survival time and survival status of patients with MM should be presented in the included cohorts. These cohorts were used to evaluate the prognostic value of BNIP3L and its related genes (Supplementary Figure 2). Moreover, cohorts providing clinical parameters were collected separately to explore the relationships between BNIP3L and clinical parameters.

Identification of MM-Related Genes

Multiple cohorts were used to identify the differentially expressed genes (DEGs) in MM. Different microarrays that shared the same detection platform were integrated and the surrogate variable analysis package was used to remove the batch effect. Based on the mRNA expression profile of each platform, the "limma" package of R was used to calculate the DEGs. Since there were multiple datasets involved, the final DEGs were determined using a robust rank aggregation method reported in previous studies.[20-23]

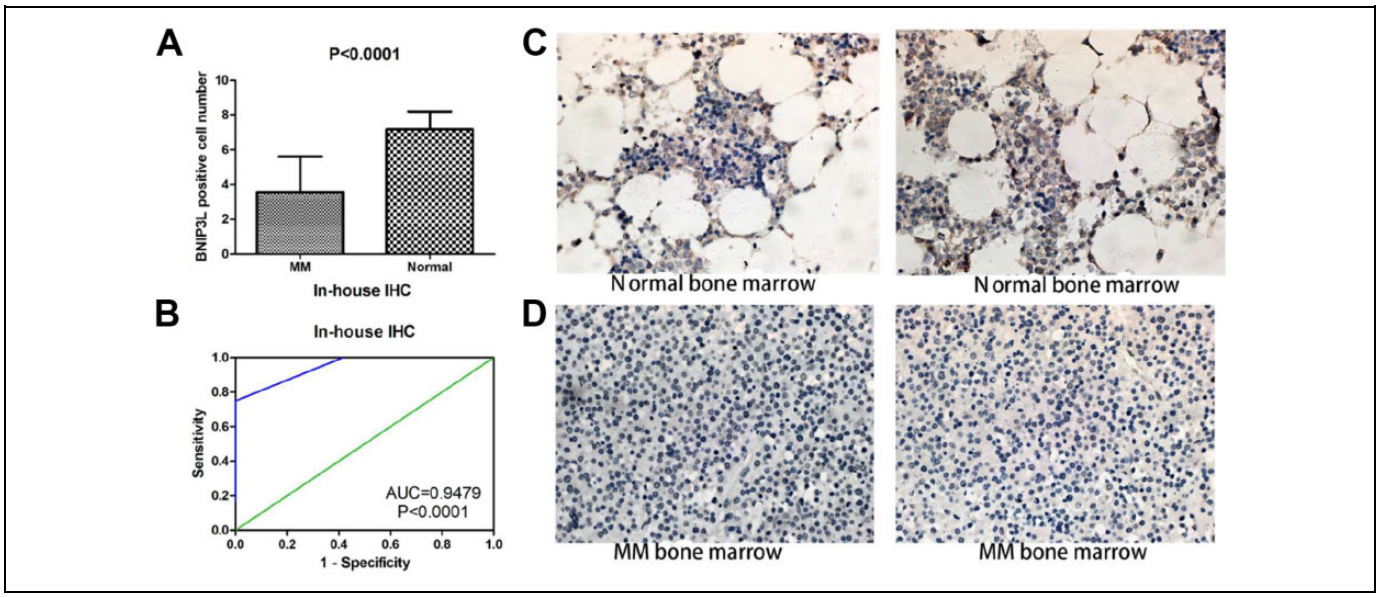

Figure 1. Protein expression of BNIP3L in MM microarray based on IHC. (A) Bar chart, (B) ROC curve, BNIP3L protein expression in (C) normal bone marrow, (D) MM. IHC, ×400. MM indicates multiple myeloma; IHC, immunohistochemistry; AUC, area under the curve.

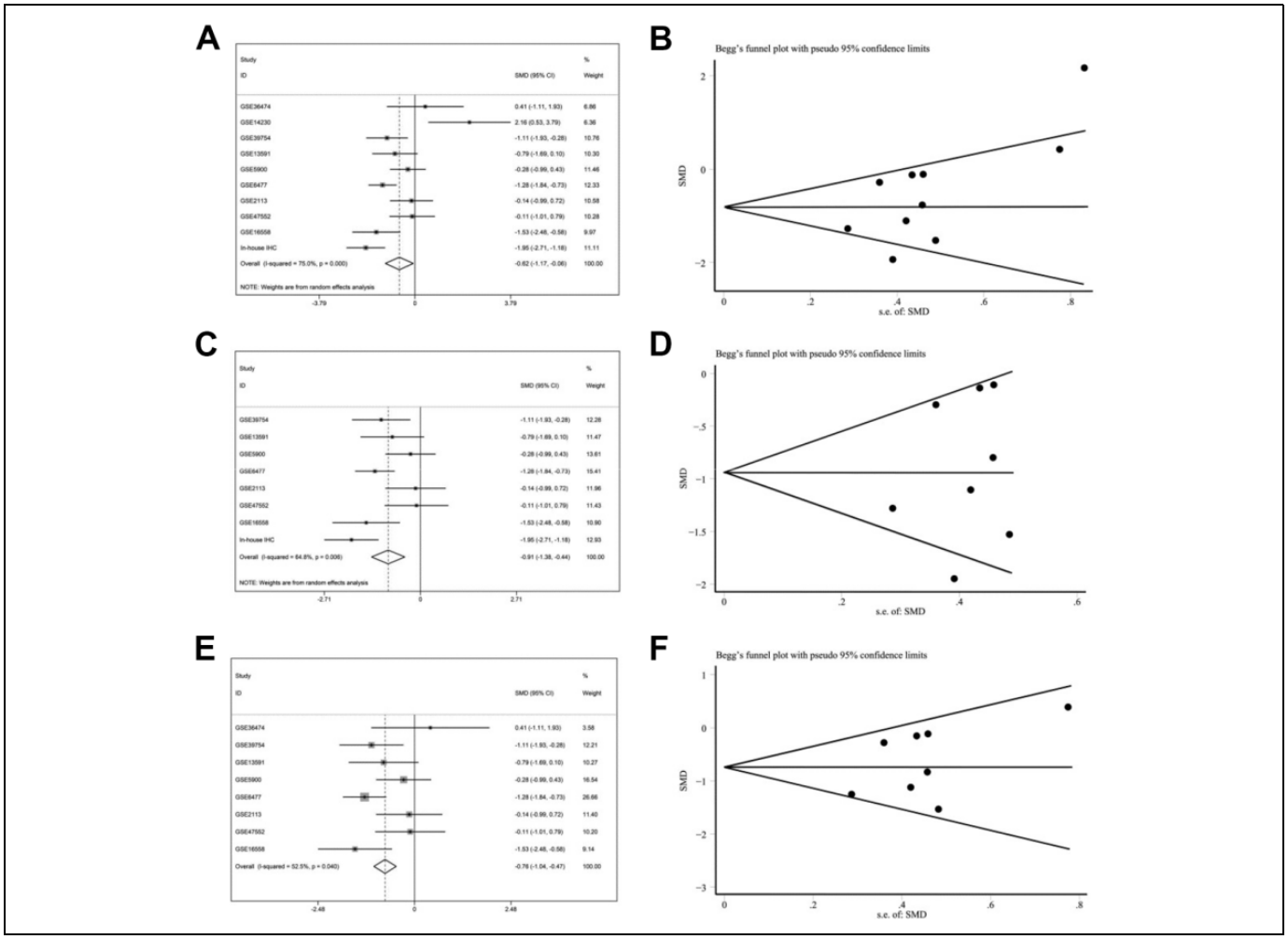

Figure 2. Expression level of BNIP3L based on integrated data. A, Forest plot showing BNIP3L expression in patients with MM based on all available data. B, Funnel plot of A. C, Forest plot showing BNIP3L expression in MM and normal plasma cells. D, Funnel plot of C. E, The mRNA expression of BNIP3L in patients with MM. F, Funnel plot of E.

Table 1. The Expression of BNIP3L in Multiple Myeloma Based on Microarrays and In-House IHC.

		Multiple myeloma			Normal bone marrow							
Dataset	Country	Number	Mean	Standard deviation	Number	Mean	Standard deviation	T	*P* value	Sensitivity	Specificity	AUC
GSE36474	Belgium	4	10.7184	0.26084	3	10.6213	0.19767	−0.535	0.616	0.6667	0.75	0.6667
GSE14230	Italy	5	10.4776	0.40357	5	9.7173	0.29153	−3.415	0.009	0.8	1	0.96
GSE39754	USA	170	8.4098	0.69503	6	9.2465	1.89852	1.077	0.330	0.6667	0.9588	0.6637
GSE13591	Italy	142	7.0842	0.83835	5	7.7448	0.63953	1.742	0.084	0.5	0.9371	0.7343
GSE5900	USA	12	10.6462	0.42564	22	10.7879	0.54754	0.776	0.444	0.6364	0.5833	0.5606
GSE6477	USA	125	8.7851	0.8296	15	9.8161	0.52028	4.695	0	1	0.6	0.8624
GSE2113	Italy	46	6.733	0.87927	6	6.85	0.73549	0.311	0.757	0.6667	0.6304	0.5725
GSE47552	Spain	94	8.3086	0.67924	5	8.382	0.44002	0.238	0.812	1	0.3511	0.5319
GSE16558	Spain	60	7.448	0.5319	5	8.2484	0.37953	3.284	0.002	1	0.8	0.8933
In-house IHC	China	36	3.5556	2.04862	12	7.1667	1.02986	5.835	0	1	0.75	0.9479

Abbreviations: IHC, immunohistochemistry; AUC, area under the curve.

Co-Expressed Genes of BNIP3L in MM and Relevant Signaling Pathways

The co-expressed genes of BNIP3L were identified using the Multi Experiment Matrix[24] and COXPRESdb.[25] In the Multi Experiment Matrix, $P < 0.05$ was regarded as statistically significant. In COXPRESdb, the top 2,000 genes were selected based on their correlation coefficients. The co-expressed genes of BNIP3L from the Multi Experiment Matrix and COXPRESdb overlapped, and the results were further intersected with the MM DEGs identified in this study. Finally, the overlapping genes from 3 analyses were considered as co-expressed genes of BNIP3L in MM. The co-expressed genes were submitted to DAVID for enrichment analysis, including gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses. STRING was used to construct a protein-protein interaction network.

Validation of Hub Co-Expressed Genes of BNIP3L

The hub co-expressed genes of BNIP3L were identified based on the connective degree of interaction nodes and further explored. Firstly, 9 microarrays were merged by calculating the standardized mean difference (SMD) to determine the expression levels of the hub genes in MM. Secondly, based on associated cohorts, the ability of hub genes to distinguish MM cells from normal plasma cells was evaluated using receiver operating characteristic (ROC) tests. Thirdly, based on the data of prognostic-related microarrays, a univariate Cox regression analysis was used to evaluate the prognostic value of hub genes, and an integrative result of multiple cohorts was achieved by calculating the summarized hazard ratio (HR).

Statistical Analysis

Independent sample *t*-tests were initially performed with SPSS 19.0 statistical software to evaluate the differences in BNIP3L expression levels between patients with MM and normal donors based on relevant data, and the results were presented as mean $\pm$ SD. The results of the *t*-test were merged in Stata12.0, the SMD was calculated, and the forest plot and funnel plot were exported. The ROC was drawn using GraphPad Prism 8 to evaluate the potential of BNIP3L and BNIP3L co-expressed genes in distinguishing MM samples from normal samples, and the sensitivity and specificity were calculated. Univariate Cox regression analysis was carried out to evaluate the relationship between BNIP3L expression and survival status, and the HR and the lower and upper 95% confidence intervals were exported. The prognostic role of BNIP3L in MM was examined by calculating the summarized HR. Statistical significance was set at $P < 0.05$.

Results

Expression of BNIP3L in Patients With MM Based on In-House Immunohistochemistry

The IHC results of the tissue microarray showed that the positive signal of the BNIP3L protein resulted from mitochondria. In normal plasma cells, moderately positive expression signals were observed with a mean score of 7.17 $\pm$ 1.03, while only a low-level expression signal was observed in MM cells with a mean score of 3.56 $\pm$ 2.05 (Table 1). Through statistical analysis, the protein expression of BNIP3L was clearly lower in MM cells than in normal plasma cells (Figure 1).

Comprehensive Expression of BNIP3L in Patients With MM Based on High-Throughput Data and In-House IHC Data

A total of 9 microarrays from patients with MM and healthy donors were selected to assess BNIP3L expression (Table 1). Among the 9 cohorts, GSE36474 and GSE14230 provided the BNIP3L expression data of bone marrow samples from patients with MM and healthy donors. The other 7 microarrays provided BNIP3L expression data of purified MM plasma and

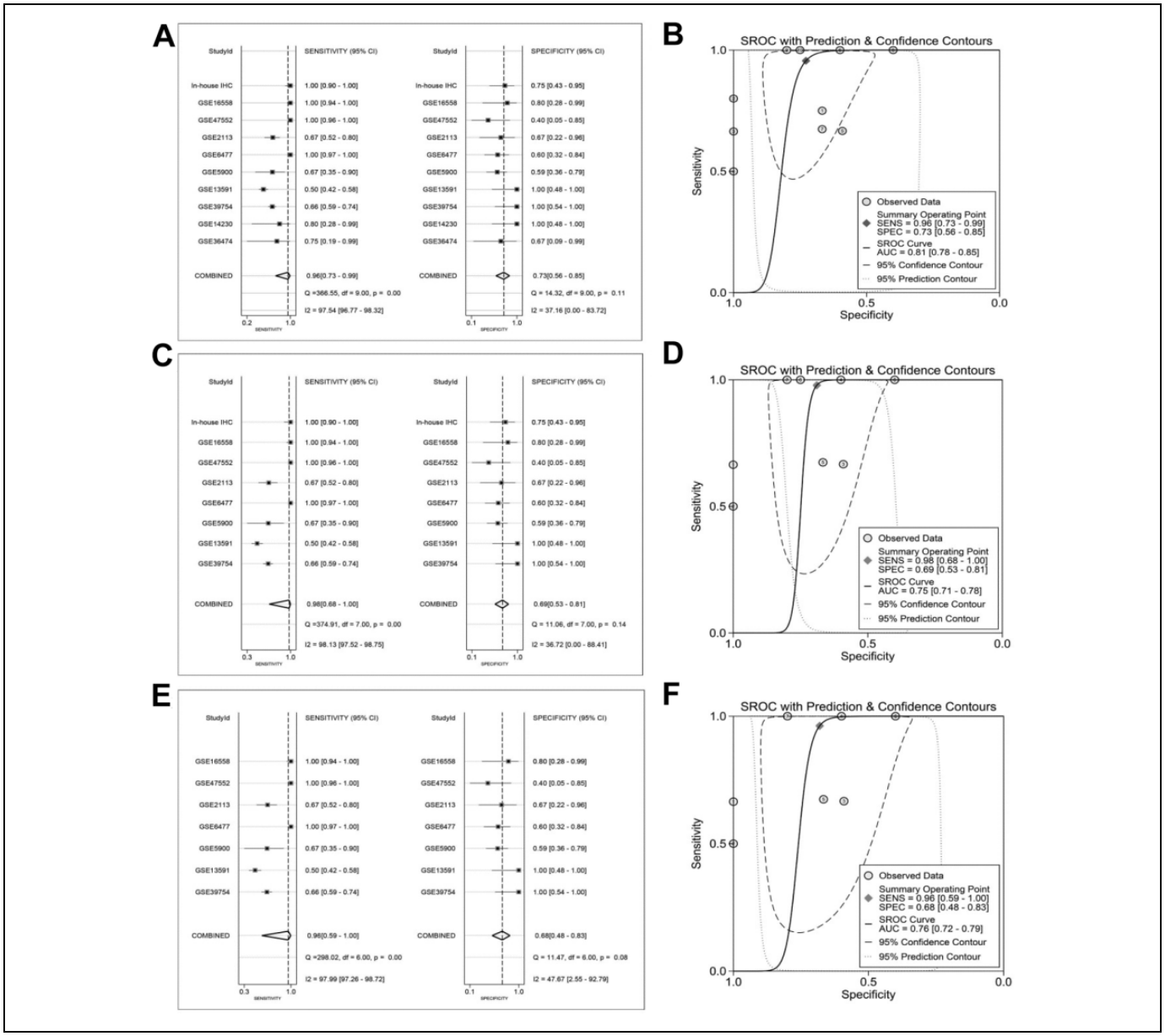

Figure 3. The potential of BNIP3L in identifying MM samples from normal samples. A, Sensitivity and specificity based on all included data. B, The sROC curve of A. C, The sensitivity and specificity of BNIP3L to identify MM and normal plasma cells based on 7 GEO microarrays and in-house IHC data. D, sROC curve of C. E, The sensitivity and specificity of BNIP3 L to identify MM and normal plasma cells based on 7 GEO microarrays. F, sROC curve of E.

normal plasma cells. In the first step, 9 GEO microarrays and the IHC data were integrated to evaluate the expression level of BNIP3L in patients with MM, which revealed that BNIP3L was significantly downregulated in patients with MM, and the corresponding standardized mean difference (SMD) was −0.62 (−1.17, −0.06) (Figure 2A and B). Secondly, the expression of BNIP3L in MM and normal plasma cells was assessed based on the data of 7 cohorts (GSE39754, GSE13591, GSE5900, GSE6477, GSE2113, GSE47552, GSE16558) and in-house IHC (Figure 2C and D), and downregulation of BNIP3L could also be found in MM cells with an SMD of −0.91 (−1.38, −0.44). Thirdly, the mRNA expression level of BNIP3L, as a subgroup, was determined based on the 9 microarrays. The mRNA expression of BNIP3L was consistently downregulated in patients with MM (Figure 2E and F). The ROC curve was drawn based on the relevant data (Supplementary Figure 3), and the summary ROC (sROC) curve was generated by integrating all available data. The area under the curve (AUC) was 0.81 (0.78, 0.85), which reflected a difference in BNIP3L expression between patients with MM and healthy donors. Additionally, sROC based on microarrays and in-house IHC indicated that the downregulation of BNIP3L had favorable sensitivity and specificity for identifying MM and healthy plasma cells. The sensitivity and specificity for MM and

healthy plasma cells were 0.98 (0.68, 1.00) and 0.69 (0.53, 0.81), respectively (Figure 3C and F).

Table 2. The Prognosis Value of BNIP3L Based on Prognosis-Related Microarrays.

Datasets	Sample size	Country	HR	LCI	UCI	*P* value
GSE57317	55	USA	1.058	0.3398	3.292	0.9228
GSE4581	414	USA	0.7232	0.4643	1.126	0.1517
GSE4452	65	USA	1.121	0.4327	2.904	0.8142
GSE4204	538	USA	0.7846	0.5209	1.182	0.2458
GSE24080	559	China	0.7784	0.5767	1.051	0.1016

Abbreviations: HR, hazard ratio; LCI, low confidence interval; UCI, up confidence interval.

Relationship Between BNIP3L Expression and Clinical Parameters

A total of 5 prognosis-related microarrays (GSE57317, GSE4452, GSE4581, GSE4204, and GSE24080) were included in our study (Supplementary Figure 2, Table 2). The HR value was calculated based on an individual microarray (Figure 4A and E). The summarized HR revealed that downregulated BNIP3L was associated with a better prognosis in patients with MM (HR = 0.79, $P < 0.001$) (Figure 4F). The relationship between BNIP3L expression and some clinical parameters was explored based on the clinical parameters of 559 patients with MM in the GSE24080 cohort. Interestingly, BNIP3L expression was negatively correlated with β2 microglobulin and creatinine levels, while it was positively correlated with albumin and hemoglobin levels in patients with MM (Figure 4G and J).

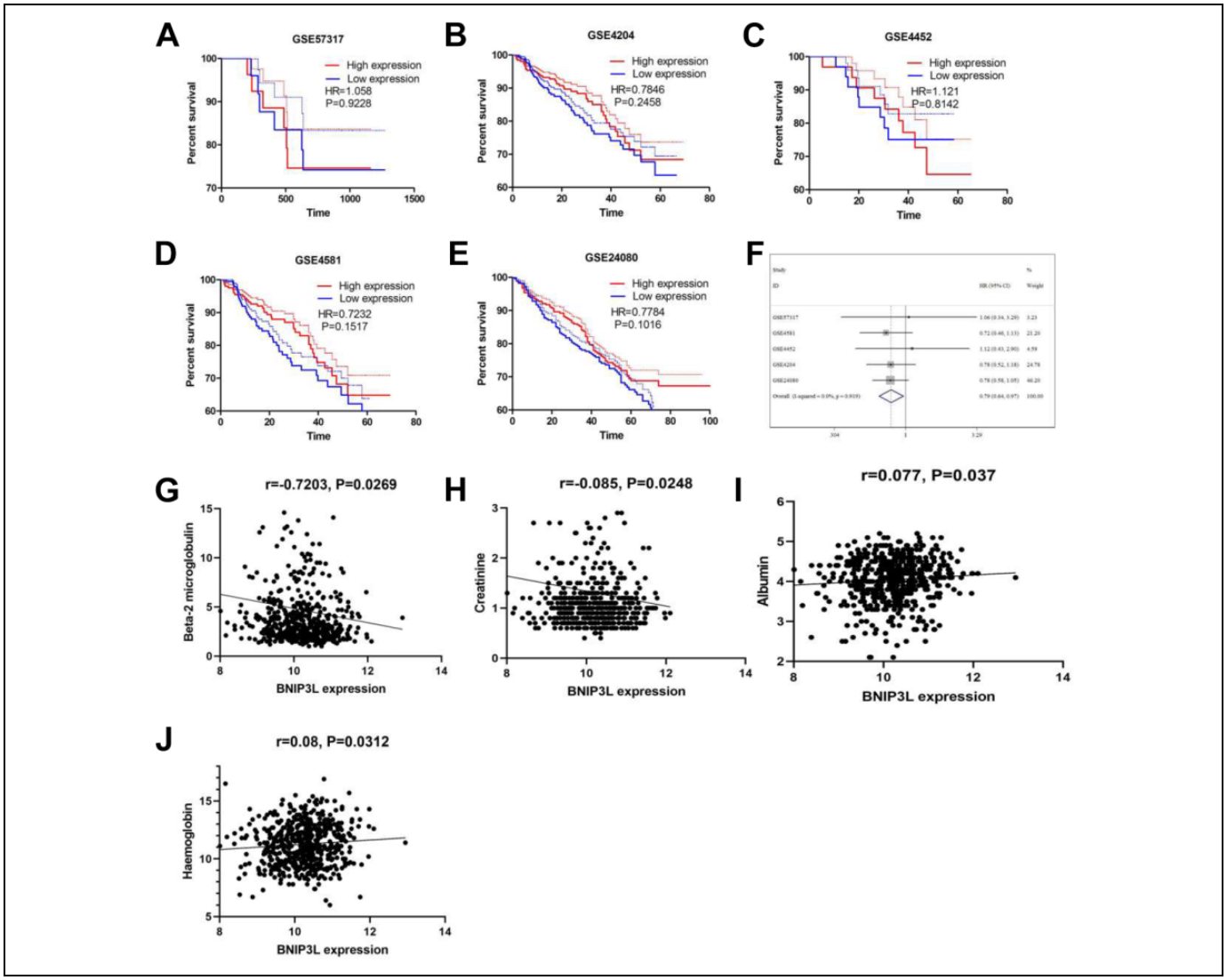

Figure 4. Prognosis value of BNIP3L in multiple myeloma. A, Survival curve based on GSE57317. B, Survival curve based on GSE4204. C, Survival curve based on GSE4452. D, Survival curve based on GSE4581. E, Survival curve based on GSE24080. F, Forest plot combining all datasets. G, The relationship between BNIP3L and β2 microglobulin in patients with MM. H, The correlation between BNIP3L and creatinine in patients with MM. I, The correlation between BNIP3L and albumin in patients with MM. J, The correlation between BNIP3L and hemoglobin in patients with MM.

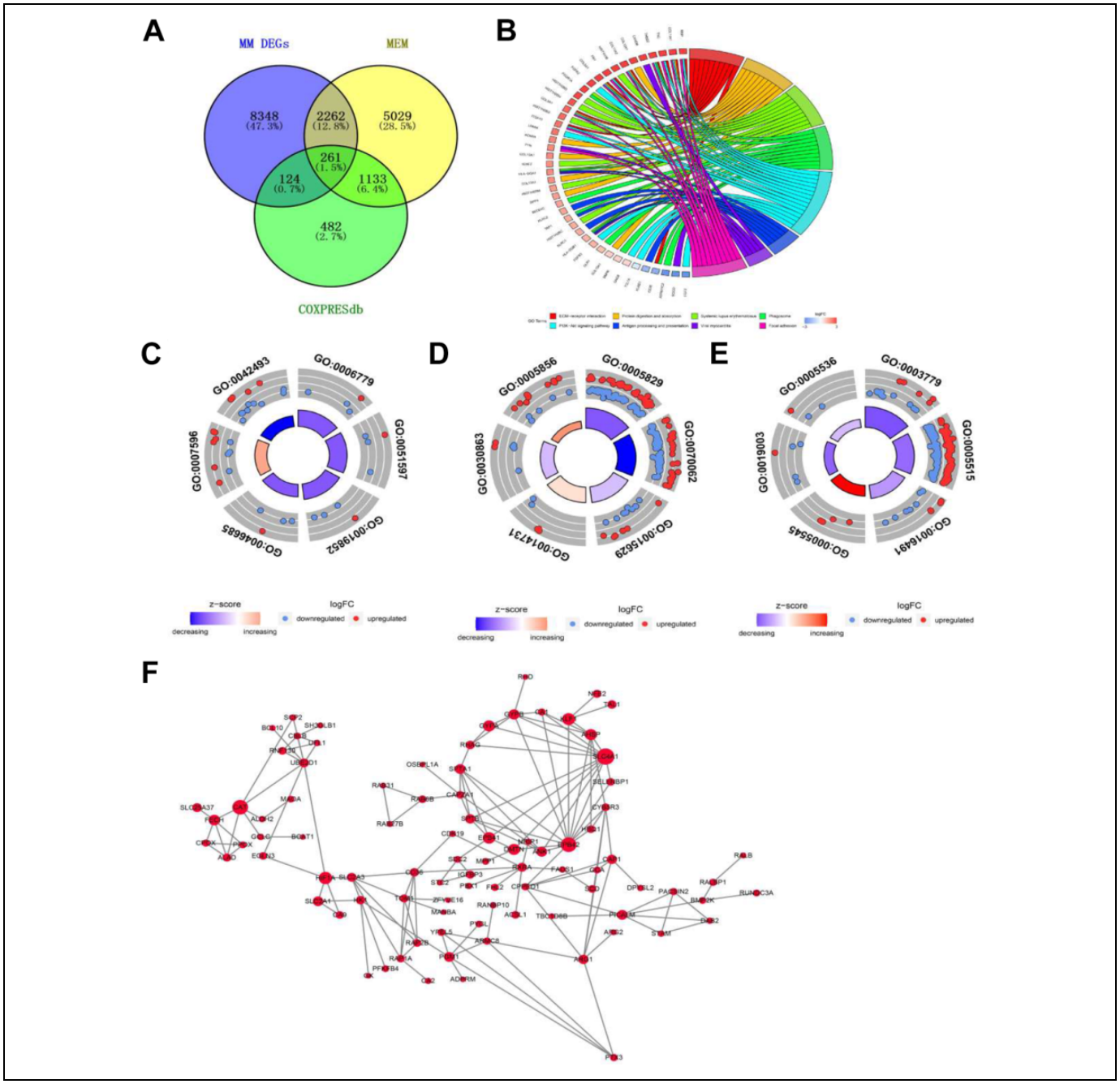

Figure 5. Prospective pathway analysis and protein-protein interaction of BNIP3L co-expressed genes in MM. A, Venn diagram. B, KEGG pathways of BNIP3L co-expressed genes in MM. C, Biological process of GO annotation. D, Cellular component of GO annotation. E, Molecular function of the GO annotation. F, Protein-protein interaction network (combined score > 0.7, the size of the node represents the connective degree).

MM-Related Gene and BNIP3L Co-Expressed Genes

A total of 20 microarrays were obtained from the public database and divided into 5 platforms: GPL570 (GSE36474, GSE29023, GSE28327, GSE4581, GSE4204, GSE4452, GSE24080, GSE16791, GSE58133, and GSE68871); GPL96 (GSE8991, GSE14230, GSE13591, GSE2912, GSE6477, and GSE2113); GPL6244 (GSE47552 and GSE16558); GPL571 (GSE5900); and GPL5175 (GSE39754) (Supplementary Figure 3, Supplementary Table 1). A differential expression analysis was conducted based on the expression matrix of the platform, and the DEGs from the 5 platforms were integrated. A total of 4,459 upregulated and 10,995 downregulated MM-related genes were identified. Furthermore, a total of 8,685 and 2,000 co-expressed genes of BNIP3L were obtained from the Multi Experiment Matrix and COXPRESdb, respectively. After integrating the MM-related genes and BNIP3L

Table 3. The Major GO Items and KEGG Pathways of BNIP3L Co-Expressed Genes in Multiple Myeloma.

Category	Term	Count	*P* value
Biological process			
GO:0006779	porphyrin-containing compound biosynthetic process	4	1.46E-04
GO:0051597	response to methylmercury	4	1.46E-04
GO:0019852	L-ascorbic acid metabolic process	4	2.16E-04
Cellular component			
GO:0005829	cytosol	80	1.52E-07
GO:0070062	extracellular exosome	69	9.58E-07
GO:0015629	actin cytoskeleton	15	1.90E-06
Molecular function			
GO:0003779	actin binding	16	1.04E-05
GO:0005515	protein binding	154	1.10E-04
GO:0016491	oxidoreductase activity	11	5.81E-04
KEGG pathway			
hsa04920	Adipocytokine signaling pathway	7	0.001150328
hsa00860	Porphyrin and chlorophyll metabolism	5	0.005406182
hsa03320	PPAR signaling pathway	6	0.005450632
hsa00520	Amino sugar and nucleotide sugar metabolism	5	0.00869394
hsa04152	AMPK signaling pathway	7	0.018063576
hsa01130	Biosynthesis of antibiotics	9	0.027608801
hsa05231	Choline metabolism in cancer	6	0.02842234
hsa04910	Insulin signaling pathway	7	0.029810666
hsa00910	Nitrogen metabolism	3	0.033024794
hsa04931	Insulin resistance	6	0.036465802

Abbreviations: GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

co-expressed genes from the 2 websites, a total of 261 genes were obtained and considered as co-expressed genes of BNIP3L in MM (Figure 5A).

BNIP3L-Related Signaling Pathways and PPI Network Analysis

Pathway analysis was performed using 261 co-expressed genes of BNIP3L. GO analysis indicated that these genes were mainly related to the gene annotations of porphyrin-containing compound biosynthetic processes, cytosol, and actin binding, while the KEGG analysis showed that these genes were mainly associated with the adipocytokine signaling pathway (Table 3, Figure 5B and E). The PPI network showed that the top 10 hub co-expressed genes of BNIP3L in MM were SLC4A1, EPB42, CAT, HIF1A, KLF1, EPB41, AHSP, DMTN, GYPA, and FECH (Figure 5F).

Further Verification of Hub Correlated Genes

Further expression validation of the top 10 hub co-expressed genes was performed. Firstly, 9 cohorts (GSE36474, GSE14230, GSE39754, GSE13591, GSE5900, GSE6477, GSE2113, GSE47552, and GSE16558) were used to access the expression of hub genes in patients with MM, and it was found that 6 genes (SLC4A1, EPB42, CAT, HIF1A, KLF1, and EPB41) were significantly downregulated in the MM patients (Figure 6). Secondly, the hub genes had a certain sensitivity and specificity in distinguishing MM cells from normal plasma cells (Supplementary Figure 4). Thirdly, positive correlations were noted between the expression of BNIP3L and the 6 co-expressed genes (Supplementary Figure 5). Univariate Cox analysis showed that 6 hub genes were associated with the prognosis of patients with MM. Among them, 3 hub genes (HIF1A, KLF1, and EPB41) were associated with a poorer prognosis, while SLC4A1 was associated with a better prognosis in patients with MM (Supplementary Figure 6).

Discussion

In this study, multiple microarrays were integrated to evaluate the expression levels of BNIP3L in 694 patients with MM and 84 healthy donors. Notably, BNIP3L expression was significantly downregulated in patients with MM. Furthermore, decreased BNIP3L expression was negatively correlated with the expressions of β2 microglobulin and creatinine in patients with MM, while it was positively correlated with the albumin and hemoglobin levels of patients with MM. Survival analysis showed that BNIP3L was associated with a good prognosis in patients with MM. Functional analysis revealed that BNIP3L is associated with the adipocytokine signaling pathway.

Studies have yet to report the clinical significance of the expression level of BNIP3L in patients with MM. However, its clinicopathological implications in other malignancies have been reported. For instance, decreased expression of the BNIP3 L protein was reported in lung cancer, and the downregulation of BNIP3L protein was partially rationalized by inhibition at the transcriptional level.[26] Besides the solid tumor, BNIP3L expression in tumors of the lymphatic hematopoietic system provide a basis to the clinical role of BNIP3L in MM. Lower expression of BNIP3L has been reported in mantle cell lymphoma cells.[27] BNIP3L was also downregulated in cases of myelodysplastic syndromes (MDS), acute myeloid leukemia (AML) with myelodysplasia-related changes (AML-MRC), and denovo AML compared to that in healthy donors.[28]

In this study, both the protein and mRNA expression levels of BNIP3L were significantly decreased in patients with MM compared to those in normal donors. This downregulation of BNIP3L was supported by an in-house tissue microarray, several public microarrays, and an integrated analysis of 694 cases of MM. These results support the hypothesis that BNIP3L may play the role of a suppressor gene in MM. This raises the possibility that the detection of BNIP3L may be used as a biomarker for MM; however, methods to determine the

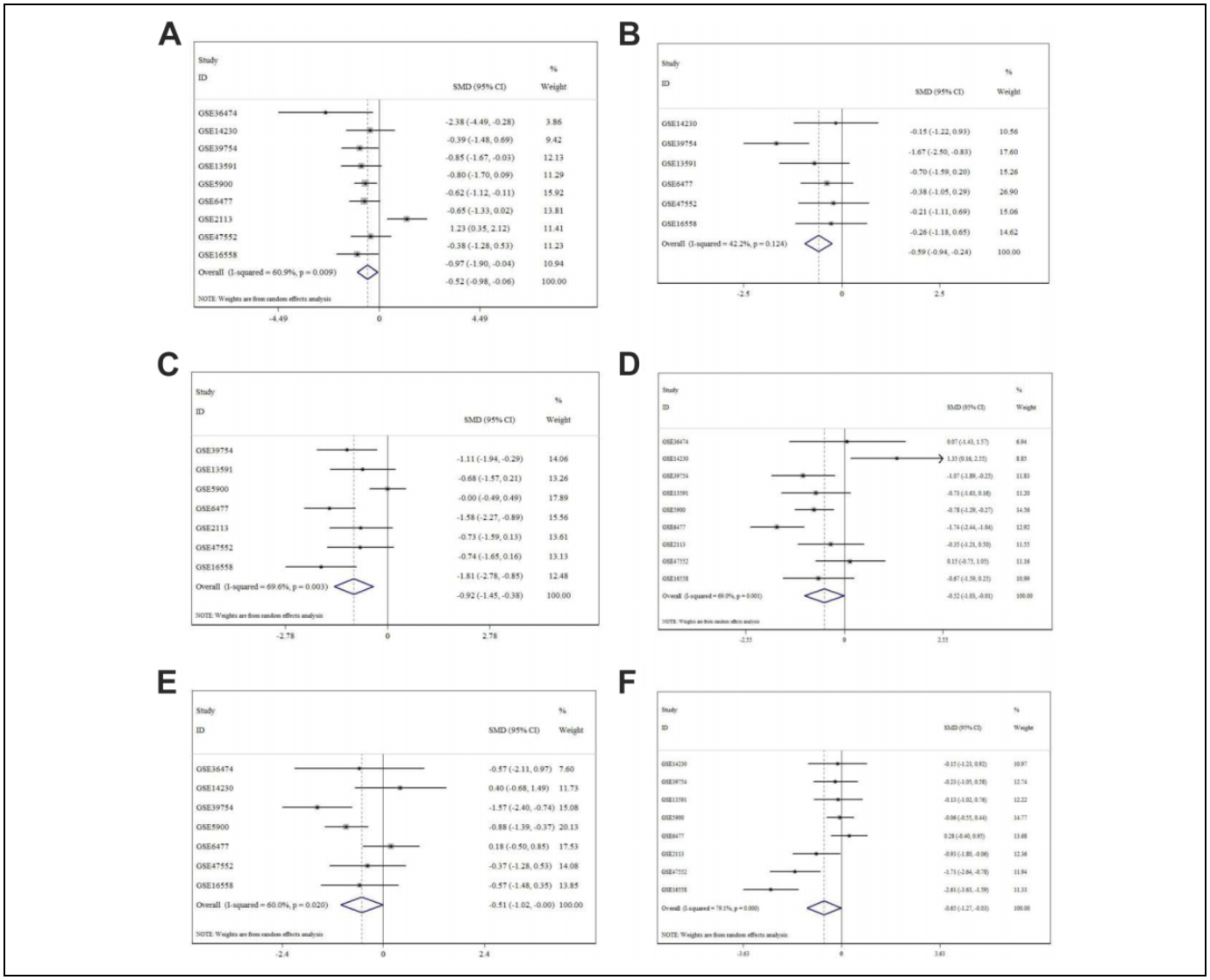

Figure 6. Expression of hub co-expressed genes of BNIP3L in MM. (A) SLC4A1; (B) EPB42; (C) CAT; (D) HIF1A; (E) KLF1; (F) EPB41.

expression level of BNIP3L in bodily fluids should be developed as a non-invasive clinical test.

Only 2 studies have been conducted concerning the prognostic role of BNIP3L in various cancers. One study reported that decreased BNIP3L expression level could serve as an independent prognostic marker for worsened overall survival and event-free survival of patients with MDS.[27] The other study considered a prognostic model to predict the survival of MM with autophagy-related genes based on the data from GSE24080.[15] A risk model with 16 genes was constructed based on multivariate Cox regression analysis, and BNIP3L was one of the 16 candidates. However, the previous study did not investigate the prognostic value of BNIP3L as an individual marker. We found the summarized HR of BNIP3L in MM to be 0.79 (<1), which not only reflected the findings of a previous study,[15] but also furnished evidence for the protective role of BNIP3L in the survival of patients with MM. Moreover, in this study, the relationship between BNIP3L and some clinical parameters (such as β2-microglobulin, creatinine, albumin, and hemoglobin) was evaluated based on clinical data from 559 patients with MM. According to existing clinical studies, these indicators are closely related to the clinical stage and prognosis of patients with MM. For example, one study found that β2-microglobulin was a serum producer of tumor burden in hematological malignancies.[29] In most patients with stage I MM, β2-microglobulin levels are normal, while elevated β2-microglobulin levels were reported in patients with advanced disease stage.[29] A comprehensive analysis based on 10,750 newly diagnosed patients with MM from multiple clinics and laboratories found that serum β2-microglobulin, serum albumin, and serum creatinine levels were powerful predictors of patient survival.[30] Another study reported that hemoglobin level is a protective factor, while creatinine level is a risk factor in patients with MM.[31] Therefore, we speculate that BNIP3L may directly or indirectly affect the survival and disease progression of patients with MM by affecting the

production of these factors. These findings point to BNIP3L as a potential marker for predicting survival in patients with MM.

In the current study, to explore the role of BNIP3L in the occurrence and development of MM, we conducted a functional analysis based on BNIP3L co-expressed genes. It was noted that the most enriched pathways were not classic apoptosis or autophagy pathways. The adipocytokine signaling pathway was the top result from KEGG analysis, providing new insight into possible BNIP3L mechanisms in MM. We also investigated the expression levels of several hub co-expressed BNIP3L genes and found that 6 of them were significantly downregulated in patients with MM. Association of 5 of the 6 genes with MM has never been reported. Whether direct or indirect correlations exist among them remains to be explored. However, hypoxia-inducible factor-1 alpha (HIF1A) inhibitors have proven valuable as anti-multidrug resistance agents for the treatment of melphalan-resistant MM, since upregulation of HIF1A contributes to melphalan resistance in MM cells by activating ERK1/2, Akt, and NF-κB.[32] There may be a synergistic effect between HIF1A and BNIP3L, but the specific details and potential mechanisms require further research.

It has been found that BNIP3L has connections with clinical treatment. Treatment of breast cancer cells with cetuximab (an antibody targeting epidermal growth factor receptors) or trastuzumab (an antibody targeting human epidermal growth factor receptor-2) can enhance the induction of BNIP3L and chemosensitization. After BNIP3L transfection into breast cancer cells, the sensitization to chemotherapy-induced apoptosis was greatly promoted. In contrast, knockdown of BNIP3 L remarkably reduced the chemosensitizing ability of cetuximab. Hence, blocking epidermal growth factor receptors or human epidermal growth factor receptor-2 was able to increase the level of BNIP3L, which is needed for breast cancer chemosensitization.[33] Another example showed that AML cells with BNIP3L deficiency were reported to be more sensitive to mitochondria-targeting drugs.[34] Furthermore, BNIP3L expression was also upregulated by decitabine in myeloid cells.[28] These studies will help incorporate BNIP3L into new therapeutic strategies for MM.

There are several limitations to this study. This study requires more *in vivo* and *in vitro* experiments to verify the effects of BNIP3L on the occurrence and development of MM. In addition, the relationships between BNIP3L and its co-expressed genes were assessed based on a public database, but this co-expression requires further experimental verification, such as through co-immunoprecipitation in cell lines and mass spectrometry. Furthermore, there still exists the challenge of widespread acceptance of translational medicine; nonetheless, the conclusions of this study warrant further consideration.

Conclusions

The most clinically relevant finding of this study is that the downregulation of BNIP3L may play a role in both the initiation and progression of MM. A strong relationship between BNIP3L and some cancer treatments has been reported in the literature providing a novel rationale for therapeutic strategies aimed at activating BNIP3L expression. However, small molecular enhancers of BNIP3L are currently unavailable. Our future work will focus on the therapeutic effects and molecular mechanisms of BNIP3L in MM.

Authors' Note

Ruolin Li, Zhian Ling and Gang Chen drafted the overall design of this paper. Yiwu Dang and Rongquan He conducted experiments. Ruolin Li, Angui Liu and Gang Chen conducted data curation and analyzed the data by using Software. Ruolin Li wrote the original draft article. Zhian Ling and Jie Ma reviewed and edited the original draft. This research program was approved by the Ethics Committee of First Affiliated Hospital of Guangxi Medical University (NO.2019(KY-E-145)). All participants signed informed consent forms.

Acknowledgments

The authors would like to thank GEO, ArrayExpress, SRA and other public data.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by grants from Guangxi Natural Science Foundation (2020GXNSFAA297052), Guangxi Province Health Technology Development and Application Project (No. S2018076), the Research Foundation of Guangxi Education Department (No. 2019KY0107) and Science and technology plan project of Qingxiu region of Guangxi, Nanning (NO.2019027).

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Supplemental Material

Supplemental material for this article is available online.

References

1. Kumar SK, Rajkumar V, Kyle RA, et al. Multiple myeloma. *Nat Rev Dis Primers*. 2017;3:17046.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin*. 2020;70(1):7-30.
3. Wang S, Xu L, Feng J, et al. Prevalence and incidence of multiple myeloma in urban area in China: a national population-based analysis. *Front Oncol*. 2019;9:1513.
4. Liu W, Liu J, Song Y, et al; Union for China Leukemia Investigators of the Chinese Society of Clinical Oncology, Union for China Lymphoma Investigators of the Chinese Society of Clinical Oncology. Mortality of lymphoma and myeloma in China, 2004-2017: an observational study. *J Hematol Oncol*. 2019;12(1):22.
5. Rajkumar SV, Kumar S. Multiple myeloma: diagnosis and treatment. *Mayo Clin Proc*. 2016;91(1):101-119.

6. Landgren O, Rajkumar SV. New developments in diagnosis, prognosis, and assessment of response in multiple myeloma. *Clin Cancer Res*. 2016;22(22):5428-5433.
7. Saltarella I, Morabito F, Giuliani N, et al. Prognostic or predictive value of circulating cytokines and angiogenic factors for initial treatment of multiple myeloma in the GIMEMA MM0305 randomized controlled trial. *J Hematol Oncol*. 2019;12(1):4.
8. Zarfati M, Avivi I, Brenner B, Katz T, Aharon A. Extracellular vesicles of multiple myeloma cells utilize the proteasome inhibitor mechanism to moderate endothelial angiogenesis. *Angiogenesis*. 2019;22(1):185-196.
9. Sun XQ, Bi FQ, Cui X. Reaserch progress on non-coding RNA in multiple myeloma—review [in Chinese]. *Zhongguo Shi Yan Xue Ye Xue Za Zhi*. 2020;28(2):713-716.
10. Manier S, Liu CJ, Avet-Loiseau H, et al. Prognostic role of circulating exosomal miRNAs in multiple myeloma. *Blood*. 2017; 129(17):2429-2436.
11. An G, Qin X, Acharya C, et al. Multiple myeloma patients with low proportion of circulating plasma cells had similar survival with primary plasma cell leukemia patients. *Ann Hematol*. 2015;94(2):257-264.
12. Kapoor P, Ansell SM, Fonseca R, et al. Diagnosis and management of Waldenstrom macroglobulinemia: Mayo stratification of macroglobulinemia and risk-adapted therapy (mSMART) guidelines 2016. *JAMA Oncol*. 2017;3(9):1257-1265.
13. Vasagiri N, Kutala VK. Structure, function, and epigenetic regulation of BNIP3: a pathophysiological relevance. *Mol Biol Rep*. 2014;41(11):7705-7714.
14. Manka D, Spicer Z, Millhorn DE. Bcl-2/adenovirus E1B 19 kDa interacting protein-3 knockdown enables growth of breast cancer metastases in the lung, liver, and bone. *Cancer Res*. 2005;65(24): 11689-11693.
15. Zhu FX, Wang XT, Zeng HQ, Yin ZH, Ye ZZ. A predicted risk score based on the expression of 16 autophagy-related genes for multiple myeloma survival. *Oncol Lett*. 2019;18(5):5310-5324.
16. Zheng HP, Huang ZG, He RQ, et al. Integrated assessment of CDK1 upregulation in thyroid cancer. *Am J Transl Res*. 2019; 11(12):7233-7254.
17. Zhong F, Lu HP, Chen G, et al. The clinical significance and potential molecular mechanism of integrin subunit beta 4 in laryngeal squamous cell carcinoma. *Pathol Res Pract*. 2020;216(2): 152785.
18. Huang WT, Yang X, He RQ, et al. Overexpressed BSG related to the progression of lung adenocarcinoma with high-throughput data-mining, immunohistochemistry, in vitro validation and in silico investigation. *Am J Transl Res*. 2019;11(8):4835-4850.
19. Liu AG, Zhong JC, Chen G, et al. Upregulated expression of SAC3D1 is associated with progression in gastric cancer. *Int J Oncol*. 2020;57(1):122-138.
20. Liu Y, Chen TY, Yang ZY, Fang W, Wu Q, Zhang C. Identification of hub genes in papillary thyroid carcinoma: robust rank aggregation and weighted gene co-expression network analysis. *J Transl Med*. 2020;18(1):170.
21. Qin YY, Huang SN, Chen G, et al. Clinicopathological value and underlying molecular mechanism of annexin A2 in 992 cases of thyroid carcinoma. *Comput Biol Chem*. 2020;86:107258.
22. Zhu J, Wang Z, Chen F, Liu C. Identification of genes and functional coexpression modules closely related to ulcerative colitis by gene datasets analysis. *Peer J*. 2019;7:e8061.
23. Yan J, Wu L, Jia C, et al. Development of a four-gene prognostic model for pancreatic cancer based on transcriptome dysregulation. *Aging (Albany NY)*. 2020;12(4):3747-3770.
24. Adler P, Kolde R, Kull M, et al. Mining for coexpression across hundreds of datasets using novel rank aggregation and visualization methods. *Genome Biol*. 2009;10(12):R139.
25. Obayashi T, Kagaya Y, Aoki Y, Tadaka S, Kinoshita K. COXPRESdb v7: a gene coexpression database for 11 animal species supported by 23 coexpression platforms for technical evaluation and evolutionary inference. *Nucleic Acids Res*. 2019;47(D1): D55-D62.
26. Sun JL, He XS, Yu YH, Chen ZC. Expression and structure of BNIP3L in lung cancer [in Chinese]. *Ai Zheng*. 2004;23(1): 8-14.
27. Ripperger T, von Neuhoff N, Kamphues K, et al. Promoter methylation of PARG1, a novel candidate tumor suppressor gene in mantle-cell lymphomas. *Haematologica*. 2007;92(4):460-468.
28. Lazarini M, Machado-Neto JA, Duarte AD, et al. BNIP3L in myelodysplastic syndromes and acute myeloid leukemia: impact on disease outcome and cellular response to decitabine. *Haematologica*. 2016;101(11):e445-e448.
29. D'Anastasi M, Notohamiprodjo M, Schmidt GP, Dürr HR, Reiser MF, Baur-Melnyk A. Tumor load in patients with multiple myeloma: β2-microglobulin levels versus whole-body MRI. *AJR Am J Roentgenol*. 2014;203(4):854-862.
30. Greipp PR, San Miguel J, Durie BG, et al. International staging system for multiple myeloma. *J Clin Oncol*. 2005;23(15): 3412-3420.
31. Chen J, Liu H, Li L, et al. Clinical features and treatment outcome of elderly multiple myeloma patients with impaired renal function. *J Clin Lab Anal*. 2019;33(5):e22888.
32. Tsubaki M, Takeda T, Tomonari Y, et al. Overexpression of HIF-1alpha contributes to melphalan resistance in multiple myeloma cells by activation of ERK1/2, Akt, and NF-kappaB. *Lab Invest*. 2019;99(1):72-84.
33. Chen YY, Wang WH, Che L, et al. BNIP3L-dependent mitophagy promotes HBx-induced cancer stemness of hepatocellular carcinoma cells via glycolysis metabolism reprogramming. *Cancers (Basel)*. 2020;12(3):655.
34. Hao BB, Li XJ, Jia XL, et al. The novel cereblon modulator CC-885 inhibits mitophagy via selective degradation of BNIP3L. *Acta Pharmacol Sin*. 2020;41(9):1246-1254.