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Deciphering the prognostic and therapeutic significance of BAG1 and BAG2 for predicting distinct survival outcome and effects on liposarcoma

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Liposarcoma (LPS) is the second most common kind of soft tissue sarcoma, and a heterogeneous malignant tumor derived from adipose tissue. Up to now, the prognostic value of BAG1 or BAG2 in LPS has not been defined yet. Expression profiling data of LPS patients were collected from TCGA and GEO database. Survival curves were plotted to verify the outcome differences of patients based on BAG1 or BAG2 expression. Univariate and multivariate Cox regression models were used to analyze the prognostic ability of BAG1 or BAG2. Chaperone's regulators BAG1 and BAG2 were identified as prognostic biomarkers for LPS patients, which exhibited distinct expression patterns and survival outcome prediction performances. Patients with high BAG2 expression and/or low BAG1 expression had worse prognosis. Enrichment analysis showed that BAG1 was involved in negative regulation of TGF-β signaling. Low expression of BAG1 was associated with high abundance of regulatory T cells (Tregs). The 2-gene signature model further confirmed the improved risk assessment performance of BAG1 and BAG2: high risk patients displayed poor prognosis. BAG1 and BAG2 are supposed to be potential prognostic biomarkers for LPS and have impacts on liposarcomagenesis and immune infiltration in distinctive manners, which may function as potential therapy targets (BAG1 agonists/BAG2 inhibitors) for LPS.

Keywords Liposarcoma, BAG1, BAG2, Prognostic biomarker, Gene signature, Immune infiltration

LPS is the second most common soft tissue sarcoma, accounting for 11.5% of sarcomas[1]. According to the WHO classification of malignant tumors in 2020, LPS was categorized into five histological subtypes with varying degrees of malignancy, including a. Well differentiated liposarcoma (WDLPS); b. Dedifferentiated liposarcoma (DDLPS); c. Myxoid liposarcoma (MLPS); d. Pleomorphic liposarcoma (PLPS); e. Myxoid pleomorphic liposarcoma (MPLPS)[2]. The most malignant subtype is DDLPS, with 41 to 52% local recurrence rate, 15% distant metastasis rate and 30% disease-related mortality rate[3]. Treatment of LPS mainly depends on surgical resection and subsequent anthracycline-based chemotherapy[4,5]. However, due to complex pathological context and molecular heterogeneity of LPS, the therapeutic effect on LPS patients is unsatisfactory with high recurrence and mortality rate[5–7]. In view of this, it has become a current research trend to explore sensitive and specific risk predictors for LPS[8–11], and to facilitate comprehensive molecular targeted therapy of LPS. As a current research hotspot, autophagy plays key roles in tumor progression[12,13]. In the present study, we have focused on autophagy-related genes derived from Human Autophagy Database (HADb), BAG1 and BAG2, which were identified as potential prognostic biomarkers for LPS, especially for DDLPS. BCL2-associated athanogene-1 (BAG1) and -2

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(BAG2) are multifunctional proteins, and function as linker molecules in regulating other proteins (regulators of chaperone), involving in cell proliferation, differentiation, apoptosis, autophagy and motility[14–17].

As previously described, BAG1 or BAG2 expression was associated with various cancers. High expression of BAG1 in B-cell acute lymphoblastic leukemia was associated with poor prognosis of patients[18]. Loss of BAG1 was found to cause oncoprotein MYC-induced apoptosis in osteosarcoma cells. Subsequent studies found that the small isoform of BAG1, BAG1S, cooperated with the HSP70 chaperone complex to selectively mediate cell survival in tumor cells with overexpressed MYC. Therefore, high expression of BAG1 may be related to the progress in osteosarcoma[19]. On the contrary, BAG1 copy numbers are mostly reduced in soft tissue sarcoma[20]. In addition, kidney renal clear cell carcinoma patients with high BAG1 expression presented beneficial prognosis[21]. BAG1 showed the same trend in liposarcoma in this study. BAG2 expression is up-regulated in a variety of malignancies[22–24]. BAG2 promotes the progression and metastasis of hepatocellular carcinoma (HCC) and is associated with adverse prognosis of HCC patients[24]. The expression of BAG2 was decreased in chondrosarcoma under hypoxia, which was the native environment for the growth of chondrosarcoma[25]. So far, the relationship between BAG2 and sarcoma has not been reported in detail. In brief, BAG1 and BAG2 are involved in tumorigenesis and progression. However, up to now, the role of BAG1 or BAG2 in liposarcomagenesis and progression, especially the prognostic ability of BAG1 or BAG2 in LPS, has not been elucidated yet. Therefore, the specific objectives of this study were to investigate the relationship between BAG1 or BAG2 expression and prognosis of LPS patients, and to explore the feasibility of BAG1 or BAG2 as a prognostic biomarker in LPS. Our findings provide new insights into the understanding of the pathogenesis and immune infiltration of LPS, facilitating the development of prognostic biomarkers and targeted treatment for LPS.

Methods

Data collection and processing

Gene expression profiling and clinical data of LPS were collected from the gene expression omnibus (GEO) and The cancer genome atlas (TCGA) database. For TCGA dataset, clinicopathological characters and follow-up data of 59 cases were obtained. For GEO datasets, gene expression profiling or follow-up data derived from GSE159659 (15 normal tissue, 15 WDLPS and 15 DDLPS), GSE21122 (9 normal fat and 47 DDLPS) and GSE30929 (52 WDLPS and 40 DDLPS) was processed as previously described[26].

Identification of potential prognostic biomarkers in LPS

First, gene symbols of 219 autophagy-related genes (ARGs) were obtained from Human Autophagy Database (HADb, http://www.autophagy.lu/index.html). Second, for TCGA dataset, survival curves of OS were plotted for these ARGs based on expression profiling and follow-up data to screen out the potential biomarkers associated with prognosis of LPS ($p < 0.05$). To confirm the prognostic values of these candidate genes, data from GSE30929 dataset was applied to plot survival curves of DRFS (distant recurrence-free survival), and genes with significant p value were included. Then, these potential biomarkers were searched in NCBI PubMed to validate the innovativeness (not reported in LPS). Ultimately, BAG1 was considered as the promising biomarker for LPS. As the same family member, BAG2 was found to have a profound effect on prognosis of LPS as well.

Differential expression analysis of BAG1 or BAG2

GraphPad Prism 8.4.3 was applied to compare the expression level of BAG1 or BAG2 between normal and LPS tissues, and the results were visualized by scatter plots. Moreover, DNA copy number alterations (CNAs) data of BAG1 or BAG2 obtained from TCGA dataset was divided into three categories (copy number amplification, diploid normal copy and single copy deletion), matched with gene expression data, and then imported to Graphpad. $p < 0.05$ was considered to be statistically significant.

Kaplan–Meier survival curve plotting

The correlation between BAG1 or BAG2 expression and the survival outcome in LPS was investigated by plotting survival curve using GraphPad. Patients were separated into two classes according to the expression level (high expression vs. low expression) of BAG1 or BAG2. Kaplan–meier curves of overall survival (OS), disease-specific survival (DSS) and distant relapse-free survival (DRFS) in LPS were plotted and marked with p-value, hazard ratio (HR) and 95% CI.

Correlation matrix analysis

The correlation between BAG1 or BAG2 and previously reported biomarkers or differential expressed genes (DEGs) of LPS was examined using Pearson's rank correlation test in Sangerbox 3.0 (integrated visual drawing toolbox) and visualized by heatmap marked with the corresponding correlation coefficient.

Validation of the prognostic value of BAG1 or BAG2

To investigate the correlation between BAG1 or BAG2 expression and clinicopathological characteristics, Chi-square test was performed by using SPSS Statistics 25.0. Moreover, Univariate and multivariate analysis were performed to verify the prognostic ability of BAG1 or BAG2 in LPS by SPSS. Risk factors assessed by univariate analysis ($p < 0.05$) were included for further multivariate analysis using regression method.

Receiver operating characteristic (ROC) curves

To examine the discrimination ability of BAG1 or BAG2 expression in making a distinction between normal and LPS, or between WDLPS and DDLPS tissues, ROC curves were plotted by using Wilson calculation method, and

presented as the percentage of sensitivity and specificity. In particular, ROC curve with AUC (area under the ROC curve) and *p* value was obtained by plotting the sensitivity (true positive rate) on Y-axis and 1-specificity (false positive rate) on X-axis.

Exploration of co-expressed genes and protein–protein interaction (PPI)

Co-expressed genes of BAG1 or BAG2 were explored using GeneMANIA database (https://genemania.org/) under the premise that the minimum required interaction score should be no less than 0.4. Furthermore, PPI of BAG1 or BAG2 was explored using STRING database (https://string-db.org/).

Functional and pathway enrichment analysis

Co-expressed genes of BAG1 or BAG2 obtained from GeneMANIA were further mined the biological function annotation of them using DAVID database (https://david.ncifcrf.gov/). Hiplot was employed to perform Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis (https://hiplot.com.cn/).

Gene set enrichment analysis (GSEA)

GSEA was performed to investigate the function enrichment of BAG1 or BAG2. TCGA-DDLPS cases were divided into two groups according to BAG1 or BAG2 expression. The annotated gene sets of 'c2.cp.kegg.v6.2.symbol.gmt' was selected in GSEA 4.2.2. After running, the output results were obtained, including heatmap, peak map and ranked genes. The gene sets with $|NES| > 1$, NOM p-val < 0.05 and FDR q-val < 0.25 were generally considered to be significantly enriched.

Tumor microenvironment and immune infiltration analysis

CIBERSORT was applied to analyze the relative abundance of immune cells based on expression profile data. The relationship between immune score and gene expression was evaluated by using ESTIMATE. In addition, xCell was introduced to obtain the scores of various immune cells. Then the correlation between BAG1 or BAG2 expression and the scores of immune cells in tumor microenvironment (TME) of LPS was illustrated by Sangerbox 3.0.

TIMER2.0 was applied to estimate the abundance of six immune infiltrate types. Thus, the correlation between BAG1 or BAG2 expression and the abundance of immune infiltration in sarcomas was analyzed.

Construction of a BAG1-BAG2-based risk assessment model

To obtain a BAG1-BAG2-based risk assessment model, Lasso regression was applied to establish a 2-gene prognostic signature with regression coefficient (β) of each gene by using the "glmnet" R package. The risk score of individual patients was calculated by the following formula: expression of gene (1) × Lasso regression coefficient β (1) + expression of gene (2) × Lasso regression coefficient β (2) + … + expression of gene (n) × lasso regression coefficient β (n). According to the median of risk scores, patients were categorized into two groups: high risk vs. low risk. The Kaplan–Meier survival curve and ROC curve were plotted to examine the differences in prognosis between high and low risk group and evaluate the accuracy and sensitivity of this prognostic model. Wilcoxon rank-sum test was performed to observe the differences of risk scores between high and low risk group.

Results

BAG1 and BAG2 present the distinct expression features in liposarcoma

To explore the potential biomarkers for LPS, ARGs were screened through analyzing expression profiling and follow-up data of LPS, thus BAG1 and BAG2 were identified as the potential predictors for LPS survival. Then the expression of BAG1 or BAG2 was comparatively assessed in normal adipose, DDLPS and WDLPS tissues. As a result, BAG1 was significantly down-regulated in DDLPS or WDLPS compared to normal tissues (Fig. 1A and B), on the contrary, BAG2 was significantly overexpressed in DDLPS compared to normal tissues (Fig. 1D and E). Compared to WDLPS tissues, BAG1 was significantly down-regulated in DDLPS, in contrast, BAG2 was significantly up-regulated in DDLPS (Fig. 1B, C,E and F). These results showed that the obvious discrepancy of the expression features between BAG1 and BAG2 in LPS, indicating the distinct roles of BAG1 and BAG2 in the tumorigenesis and development of LPS.

Then, ROC curve of BAG1 or BAG2 was plotted based on their expression in DDLPS and WDLPS tissues (AUC > 0.8 and $p < 0.0001$) (Fig. 1G), suggesting an outstanding discrimination ability of BAG1 or BAG2 between two types of LPS (DDLPS VS. WDLPS). Moreover, BAG1 or BAG2 was able to discriminate normal fat from DDLPS or WDLPS tissues (Fig. 1H and I), revealing that the expression level of BAG1 or BAG2 is of diagnostic significance for LPS.

Then the expression profile of BAG1 in different clinicopathological features was estimated. As shown, patients suffered from DDLPS with higher BAG1 expression significantly more responded to treatment than patients with lower BAG1 expression (complete response vs. progressive disease) (Fig. 1J). Moreover, BAG1 expression was significantly lower in the deceased cases than that in the living cases (Fig. 1K). Tumors with larger volume were correlated with decreased BAG1 expression while smaller tumors with increased expression of BAG1 (Fig. 1L).

To sum up, these results revealed that BAG1 and BAG2 may be involved in the tumorigenesis and development of LPS, and exert distinct influences on LPS survival.

Then, the relationship between BAG1 or BAG2 mRNA expression and DNA CNVs was examined. BAG1 or BAG2 expression in patients with copy number amplification increased significantly ($p = 0.003$; $p = 0.0232$),

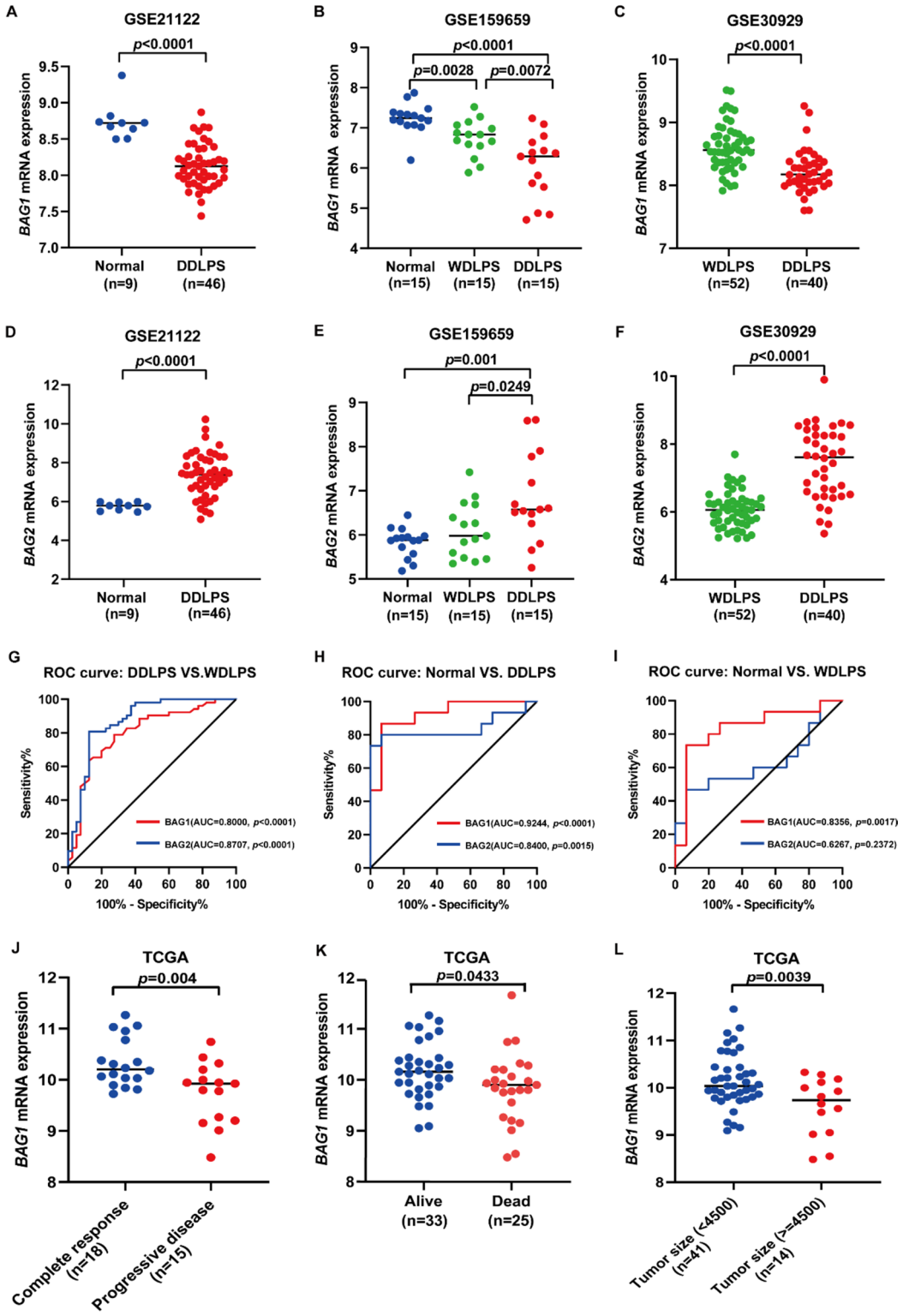


Figure 1. BAG1 and BAG2 displayed distinct gene expression patterns. (**A**–**F**) BAG1 expression was down-regulated while BAG2 was up-regulated in DDLPS. Scatter plots of BAG1 (**A**–**C**) or BAG2 (**D**–**F**) expression in normal adipose tissue, DDLPS and WDLPS. ROC curve of BAG1 or BAG2 expression compared between DDLPS and WDLPS (**G**); between normal adipose tissue and DDLPS (**H**); between normal adipose tissue and WDLPS (**I**). (**J**–**L**) Comparison of BAG1 expression in DDLPS with different clinicopathological features. BAG1 expression difference: (**J**) in distinct responses to treatment of patients; (**K**) compared with the survival status of patients; (**L**) in different tumor volume sizes. Patients were subdivided into two groups according to the tumor volume (≥4500 vs. <4500).

compared to the single copy deletion (n = 15) cases (Fig. S1), revealing that DNA copy number of BAG1 or BAG2 is consistent with its expression level.

Identification of BAG1 and BAG2 as potential prognostic biomarkers for LPS patients

By plotting Kaplan–Meier (KM) survival curves, we identified that both BAG1 and BAG2 had prognostic values in LPS but indicated the opposite clinical significance. In detail, DDLPS patients with low BAG1 expression were significantly associated with poor OS ($p = 0.0041$) and DSS ($p = 0.0111$), while high BAG1 expression predicted better OS and DSS (Fig. 2A and B). On the contrary, elevated BAG2 expression corresponded with unfavorable OS ($p = 0.0312$) and DSS ($p = 0.0361$) of DDLPS patients (Fig. 2C and D), indicating the opposite prediction effect of BAG1 and BAG2 on survival. To validate this, we depicted the survival curves of DRFS. Low expression of BAG1 forecasted worse DRFS of LPS ($p = 0.0095$) (Fig. 2E). In contrast, high expression of BAG2 was significantly associated with adverse DRFS of LPS ($p = 0.0133$) or DDLPS patients ($p = 0.0171$) (Fig. 2F and G). According to Fig. 2F and G, in validation set (GSE30929), BAG2 has significant prognostic value at cutoff 50%. While in training set (TCGA), BAG2 has statistically significant prognostic value at cutoff 30% vs. 70%, unfortunately, not 50%. In addition, a comprehensive survival curve was plotted to elaborate the relevance between BAG1/BAG2 expression and prognosis of LPS patients (BAG1 + or BAG2 + denotes high expression of BAG1 or BAG2; BAG1- or BAG2- denotes low expression of BAG1 or BAG2) (Fig. 2H and I). As a result, patients with BAG1 + and BAG2- exhibited the best OS or DRFS, while BAG1- and BAG2 + group presented the worst OS or DRFS (Fig. 2H and I), further verifying the opposite prediction effect of BAG1 and BAG2 on LPS survival (BAG1 acts as a protective factor; BAG2 as a risk factor for LPS), and speculating that BAG1- and/or BAG2 + may have a significant driving role in LPS progression.

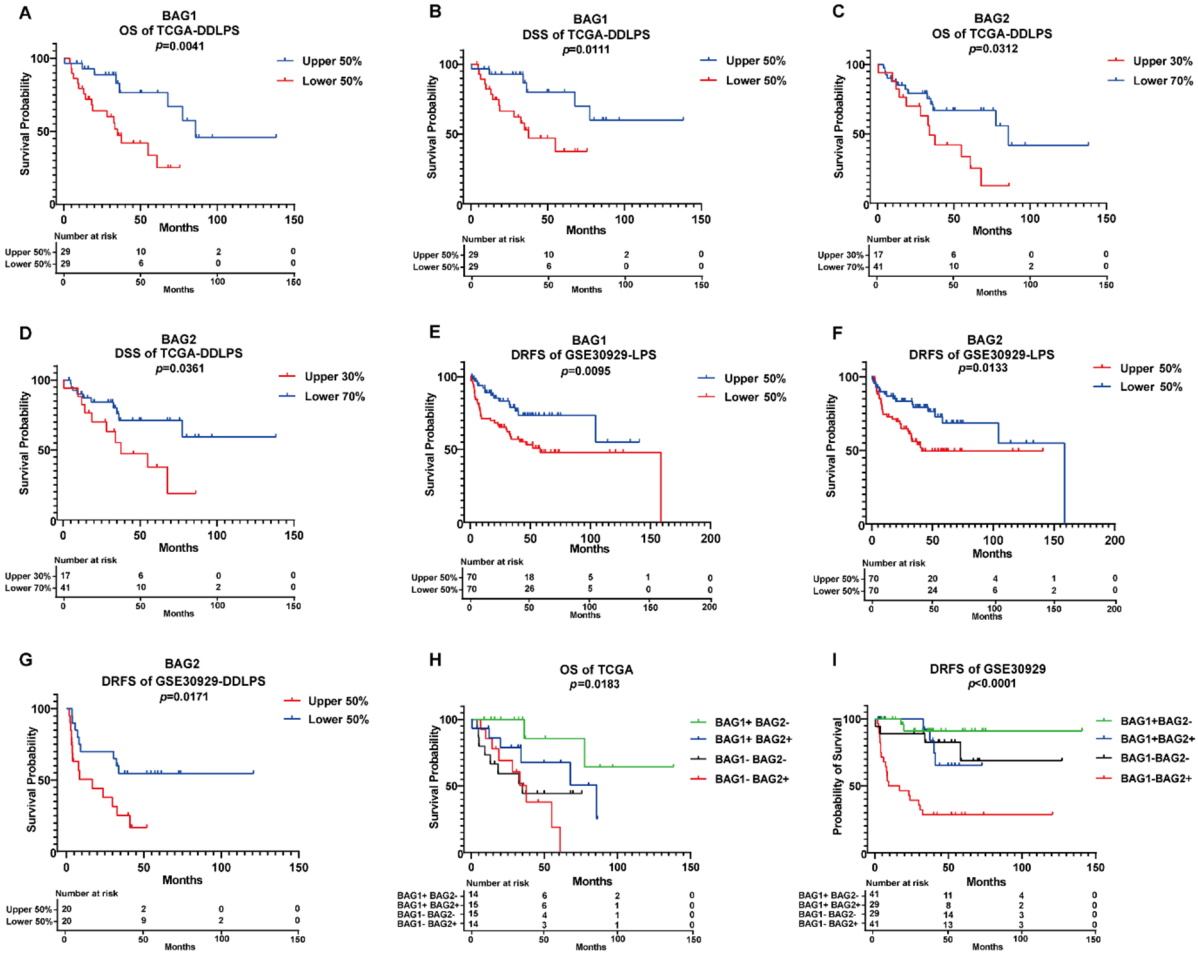


Figure 2. BAG1 and BAG2 have distinct predictive performances for LPS prognosis. (**A**–**D**) Kaplan–Meier plots of OS and DSS for DDLPS patients classified by BAG1 (**A**,**B**) or BAG2 (**C**,**D**) expression. Survival curves based on BAG1 (**E**) or BAG2 (**F**,**G**) expression of patients suffered from LPS and DDLPS, respectively. (**H**,**I**) Survival curves were plotted to compare OS or DRFS of patients with differential expression of BAG1 and BAG2: patients were subdivided into four groups. BAG1 + BAG2–: the group with high BAG1 but low BAG2 expression; BAG1 + BAG2 + : the group with high BAG1 and BAG2 expression; BAG1- BAG2–: the group with low BAG1 and BAG2 expression; BAG1– BAG2 + : the group with low BAG1 but high BAG2 expression; cut-off: 50%.

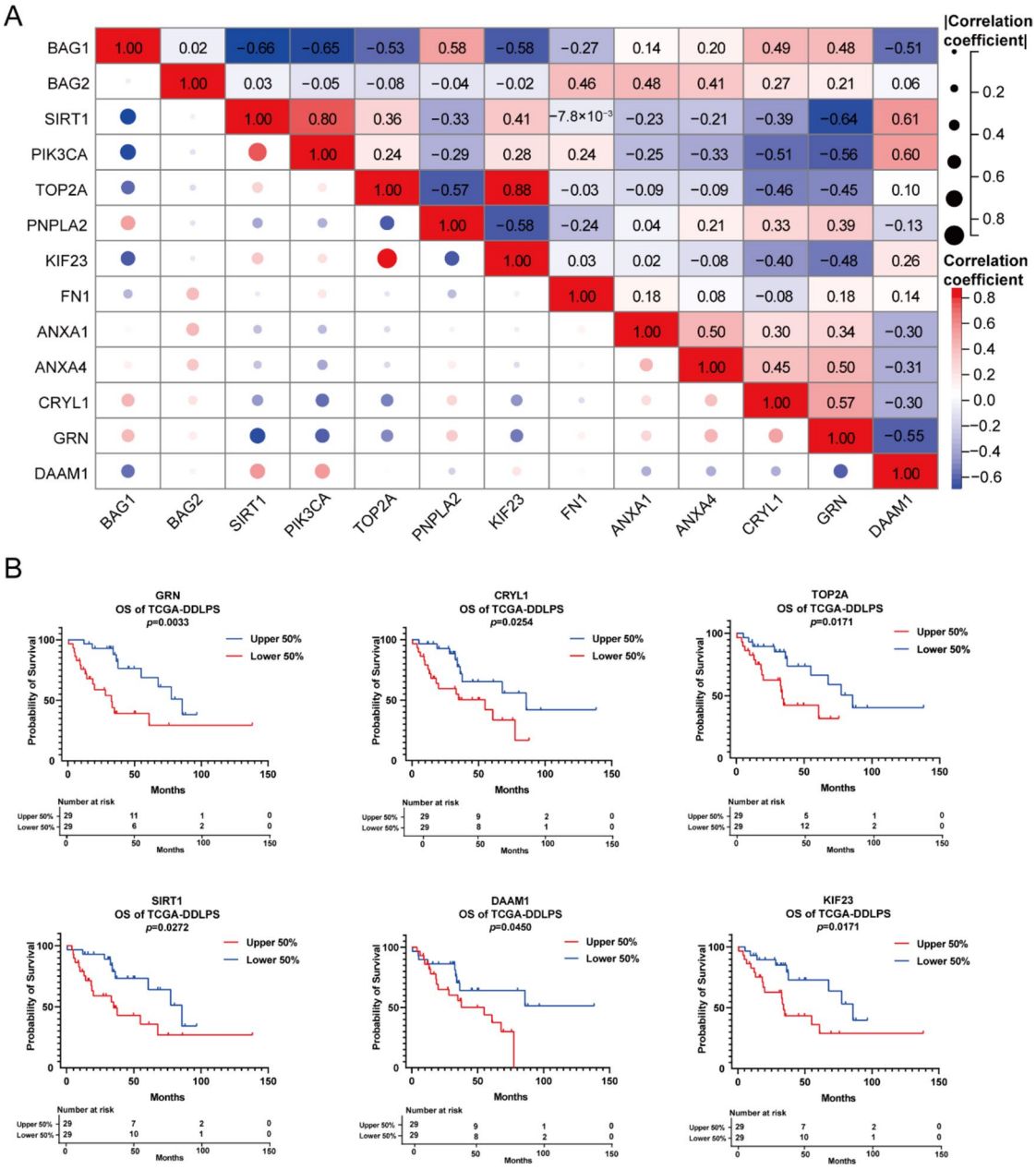


Figure 3. Correlation analysis between BAG1/BAG2 and previously reported prognostic biomarkers or DEGs of LPS. (**A**) Association of BAG1/BAG2 with the known biomarkers or DEGs of LPS was estimated by Pearson's rank correlation test with correlation coefficient marked on the matrix plot. Positive and negative correlations were indicated by red and blue boxes, respectively. Color intensity and absolute value are proportional to the correlation strength. (**B**) Survival curves were plotted to verify the prognostic abilities of the known biomarkers or DEGs in LPS.

Moreover, to determine the prognostic significance of BAG1 or BAG2, correlation analysis was performed among BAG1, BAG2 and previously reported biomarkers or DEGs in LPS[27–31]. The correlation matrix showed that BAG1 was negatively correlated with SIRT1, PIK3CA, TOP2A, KIF23 or DAAM1 (Fig. 3A), the high expression of which predicted the unfavorable survival of DDLPS patients (Fig. 3B). While BAG1 was positively correlated with PNPLA2, CRYL1 or GRN (Fig. 3A), the high expression of which predicted the favorable survival of patients (Fig. 3B). In addition, BAG2 showed the positive correlation with FN1, ANXA1 or ANXA4 (Fig. 3A). These results further confirmed the prognostic value of BAG1 or BAG2 in LPS, which exerted the opposite prediction effect on LPS survival.

Validation of the prognostic values of BAG1 and BAG2 for LPS patients

The relationship between BAG1 or BAG2 expression and clinicopathological parameters was assessed using chi-square test, including therapy outcome, recurrence, tumor size and gender (Table 1). The statistical analysis

Variables	No. of patient	BAG1 expression		X^2	*p*-value	Variables	No. of patient	BAG2 expression		X^2	*p*-value
		Upper 50%	Lower 50%					Upper 30%	Lower 70%		
Therapy outcome						Therapy outcome					
Progressive disease	15	4	11	5.241	**0.022**	Progressive disease	15	8	7	6.906	**0.009**
Complete response	18	12	6			Complete response	18	2	16		
Recurrence						Recurrence					
No	14	9	5	3.884	**0.049**	No	14	4	10	0.059	0.809
Yes	9	2	7			Yes	9	3	6		
Radiation therapy						Radiation therapy					
No	54	25	29	4.296	**0.038**	No	54	17	37	1.781	0.182
Yes	4	4	0			Yes	4	0	4		
Tumor status						Tumor status					
Tumor free	23	15	8	3.238	0.072	Tumor free	23	2	21	7.971	**0.005**
With tumor	32	13	19			With tumor	32	14	18		
Tumor size						Tumor size					
≥4500	13	2	11	1.666	0.197	≥4500	13	7	6	4.816	**0.028**
<4500	41	14	27			<4500	41	9	32		
Gender						Gender					
Female	19	8	11	0.704	0.401	Female	19	4	15	0.93	0.335
Male	39	21	18			Male	39	13	26		
Age at diagnosis						Age at diagnosis					
≥60	39	18	21	0.704	0.401	≥60	39	12	27	0.122	0.727
<60	19	11	8			<60	19	5	14		
New tumor after treatment						New tumor after treatment					
No	31	18	13	2.174	0.14	No	31	6	25	3.559	0.059
Yes	26	10	16			Yes	26	11	15		
Tumor multifocal						Tumor multifocal					
No	33	18	15	0.982	0.322	No	33	9	24	0.132	0.716
Yes	22	9	13			Yes	22	7	15		
Metastatic						Metastatic					
No	20	9	11	1.2	0.273	No	20	5	15	1.008	0.315
Yes	4	3	1			Yes	4	2	2		

Table 1. The relationship between BAG1 or BAG2 expression and clinicopathological features in LPS patients. Significant values are in bold.

results showed that the proportion of progressive disease (11/17 vs. 4/16; $p = 0.022$) and recurrence rate (7/12 vs. 2/11; $p = 0.049$) were significantly higher in BAG1 low-expression group compared to high-expression group (Table 1), indicating that BAG1 expression is significantly relevant to these clinical characteristics of LPS patients. In contrast, the expression pattern of BAG2 was different from that of BAG1. The proportion of progressive disease rate (8/10 vs. 7/23; $p = 0.009$), tumor-bearing status (14/16 vs. 18/39; $p = 0.005$) and tumor size (7/16 vs. 6/38; $p = 0.028$) was significantly higher in BAG2 high-expression group than that in low-expression group (Table 1), indicating the significant relevance between BAG2 expression and these clinicopathological features of LPS patients. However, BAG1 and BAG2 exhibit the contrary expression pattern, and may exert the opposite influence on tumorigenesis and development of LPS.

To investigate whether the expression of BAG1 or BAG2 could be an independent predictor in LPS patients, univariate and multivariate analyses were performed. The univariate analysis showed that the decreased BAG1 expression ($p = 0.007$, HR = 0.276, 95% CI [0.108–0.705]), elevated BAG2 expression ($p = 0.036$, HR = 2.327, 95% CI [1.056–5.130]), and therapy outcome (progressive disease) ($p = 0.006$, HR = 17.402, 95% CI [2.245–134.874]) were significantly associated with a higher risk of LPS progression (Table 2). The following multivariate analysis showed that the reduced BAG1 expression ($p = 0.043$, HR = 0.231, 95% CI [0.056–0.955]) and therapy outcome (progressive disease) ($p = 0.016$, HR = 13.549, 95% CI [1.631–112.560]) were regarded as independent prognostic indicators for LPS (Table 2), while BAG2 was not significant in multivariate analysis ($p = 0.623$), thus, when more datasets with follow-up information will be available, analyses are needed to demonstrate the possibility that it can be regarded as an independent prognostic biomarker for LPS.

Exploration of function and molecular mechanism of BAG1 or BAG2 in LPS

GeneMANIA and STING databases were applied to analysis the co-expressed genes and PPI of BAG1 or BAG2. As illustrated, BCL2L11 was most closely related to BAG1 (Fig. 4A), which acts as an inducer of apoptosis[32]. Moreover, the interacting proteins of BAG1 was displayed, including HSPA4, HSPA8 and BAG2 (Fig. 4B). AHNAK in the co-expressed genes was strongly associated with BAG2 (Fig. 4C), which is involved in tumor

Subgroup	Univariate analysis		Multivariate analysis (BAG1)		Multivariate analysis (BAG2)	
	Hazard ratio (95% CI)	*p* value	Hazard ratio (95% CI)	*p* value	Hazard ratio (95% CI)	*p* value
All patients (N = 59)						
BAG1 expression high vs. low (N = 58)	0.276 (0.108–0.705)	**0.007**	0.231 (0.056–0.955)	**0.043**		
BAG2 expression high vs. low (N = 58)	2.327 (1.056–5.130)	**0.036**			1.354 (0.404–4.536)	0.623
Therapy outcome: progressive disease vs. complete response (N = 33)	17.402 (2.245–134.874)	**0.006**	13.549 (1.631–112.560)	**0.016**	17.402 (2.245–134.873)	**0.013**
Female vs. male (N = 59)	1.997 (0.919–4.338)	0.081				
Age ≥ 60 vs. < 60 (N = 59)	1.860 (0.700–4.944)	0.213				
Metastatic yes vs. no (N = 25)	2.218 (0.569–8.646)	0.251				
Subgroup	**Multivariate analysis**					
	Hazard ratio (95% CI)	***p* value**				
All patients (N = 59)						
BAG1 expression high vs. low (N = 58)	0.231 (0.055–0.964)	**0.044**				
BAG2 expression high vs. low (N = 58)	1.301 (0.392–4.313)	0.667				
Therapy outcome: progressive disease vs. complete response (N = 33)	11.758 (1.306–105.843)	**0.028**				

Table 2. Univariate and multivariate analysis of risk factors associated with LPS survival. Significant values are in bold.

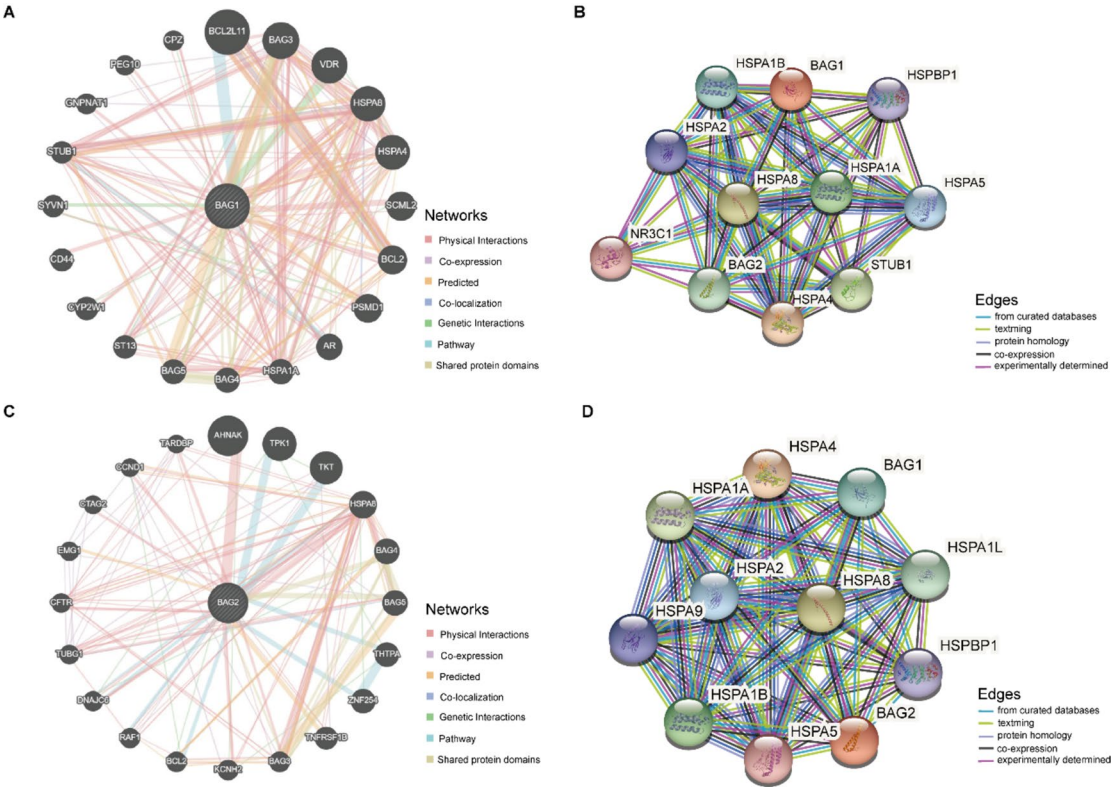


Figure 4. Co-expressed gene and protein–protein interaction (PPI) analysis of BAG1 or BAG2. (**A**,**C**) Co-expressed genes of BAG1 and BAG2, respectively. (**B**,**D**) The comprehensive PPI network of BAG1 and BAG2, respectively.

metastasis[33]. The top three proteins interacted with BAG2 were listed as follows: HSPA4, HSPA8 and HSPA1A (Fig. 4D). Based on these results, GO and KEGG functional enrichment analyses were performed by Hiplot using the data derived from co-expressed genes of BAG1 or BAG2. In brief, it is noteworthy that both BAG1 and BAG2 are involved in regulation of cellular response to heat, protein folding, ubiquitin protein ligase binding and chaperone binding[16] (Fig. 5), consistent with the results of PPI analysis. In addition, the enriched functional items of BAG2 are relevant to 'cancer' (Fig. 5H). To further elaborate the function and mechanism of BAG1 or BAG2 exerting on LPS progression, GSEA was conducted to perform the functional enrichment analysis using the data derived from two subgroups of DDLPS patients divided according to the expression level of BAG1 or BAG2 (Fig. 6A, D and E). In detail, gene sets clustered in BAG1 high-expression group were related to 'antigen procession and presentation' (Fig. 6B), while gene sets clustered in BAG1 low-expression group were involved in

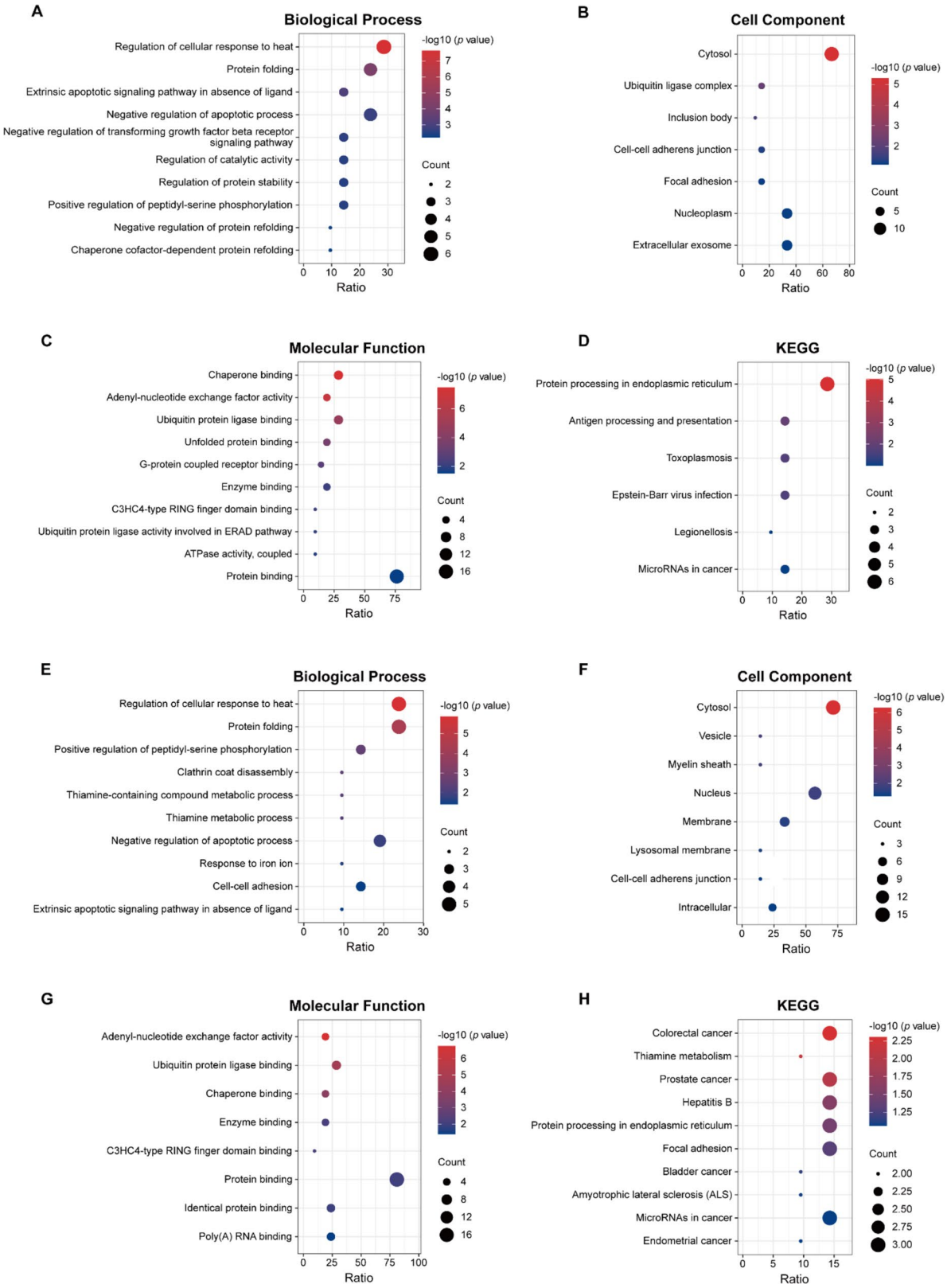
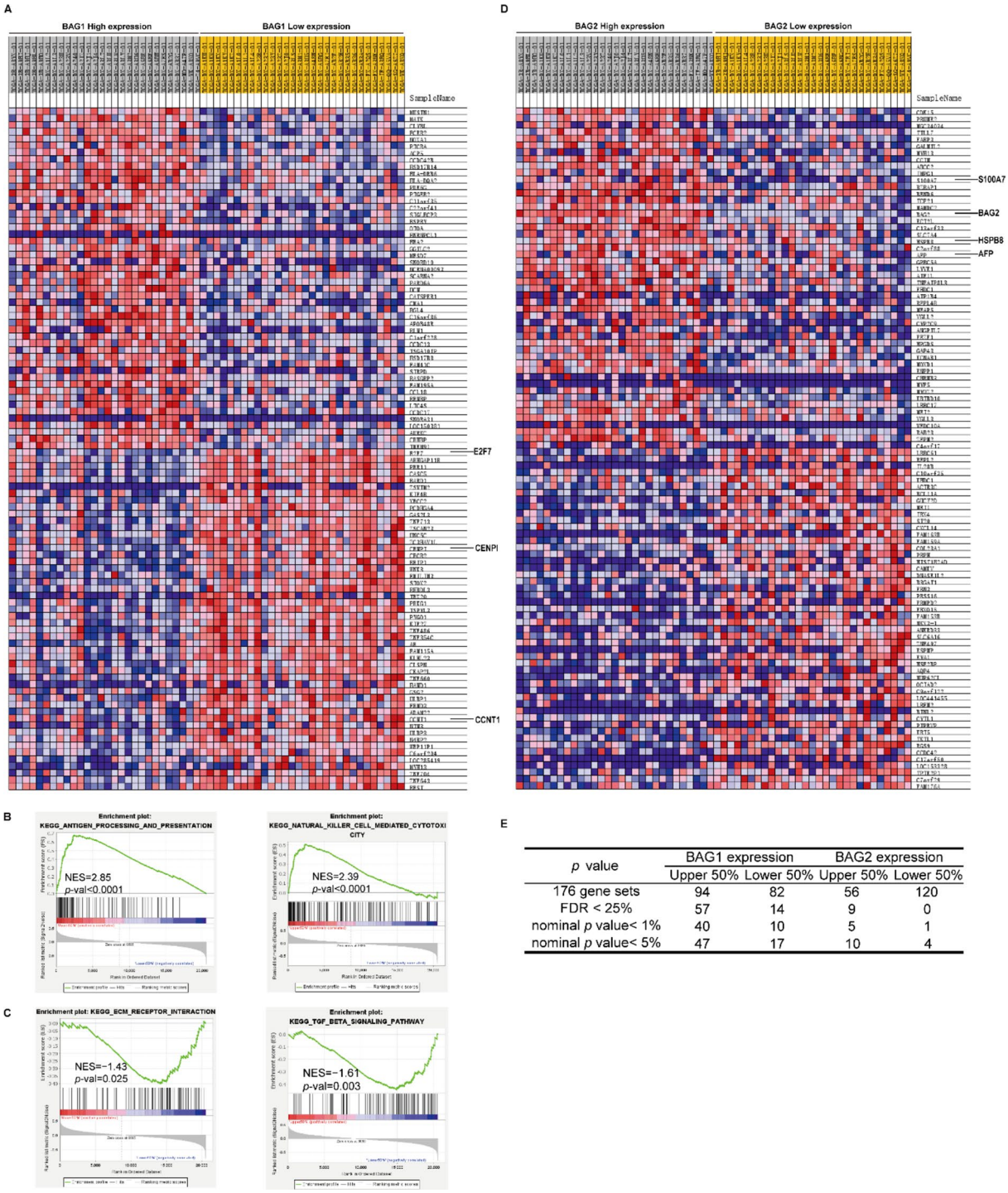


Figure 5. GO and KEGG analysis of the co-expressed genes of BAG1 or BAG2. (**A**,**E**) Biological process, BP. (**B**,**F**) Cell component, CC. (**C**,**G**) Molecular function, MF. (**D**,**H**) KEGG analysis. KEGG pathways were obtained from KEGG software (www.kegg.jp/kegg/kegg1.html). Each point on the graph represents a group of genes. The point size represents the number of genes. The redder the point color, the smaller the p value, the more statistically significant.



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p value	BAG1 expression		BAG2 expression	
	Upper 50%	Lower 50%	Upper 50%	Lower 50%
176 gene sets	94	82	56	120
FDR < 25%	57	14	9	0
nominal *p* value< 1%	40	10	5	1
nominal *p* value< 5%	47	17	10	4

Figure 6. Gene set enrichment analysis of BAG1 or BAG2. (**A**,**D**) Heat map of the top 50 DEGs enriched in BAG1 or BAG2 differential expression group generated by GSEA software (version 4.2.2; https://www.gsea-msigdb.org/gsea/index.jsp). (**B**,**C**) The gene sets enriched in BAG1 high (**B**) and low (**C**) expression group, respectively. (**E**) Ranked gene list correlation profile, a summary of GSEA results.

'TGF beta signaling pathway' (Fig. 6C). Through estimating the genes clustered in heatmap (Fig. 6A and D), we found that E2F7, CENPI and CCNT1 were overexpressed when BAG1 mRNA was at low level (Fig. 6A), which were significantly negatively correlated with BAG1 (Fig. S2A–C). On the other hand, HSPB8 and AFP were overexpressed when BAG2 mRNA was at high level (Fig. 6D) and HSPB8 was significantly positively correlated with BAG2 (Fig. S2D). In addition, high expression of CENPI, CCNT1 or AFP denoted the worse prognosis of LPS patients (Fig. S2E and F). It is noteworthy that E2F7, CENPI, CCNT1, HSPB8 or AFP was regarded as

oncogene involved in cancer recurrence and metastasis[34–38]. These indicated the involvement of BAG1 or BAG2 in tumor progression.

Evaluation of the correlation between BAG1 or BAG2 expression and immune cell infiltration or therapeutic targets in LPS

Furthermore, we also delved into the relationship between BAG1 or BAG2 expression and immune infiltration level to estimate the effects of BAG1 or BAG2 on LPS TME. By employing CIBERSORT algorithm, we systematically evaluated the percentage of immune cells in LPS samples. In accordance with BAG1 or BAG2 expression, LPS patients were categorized into low- and high-expression group. By comparison, BAG1 low-expression group was significantly associated with high abundance of Tregs ($p < 0.05$, Fig. 7A and B). As known, Tregs suppress the anti-cancer immunity[39], thus low expression of BAG1 may be associated with Tregs-mediated immunosuppressive effect. In contrast, high expression of BAG2 was correlated with the low abundance of 'T cells follicular helper' or the high proportion of 'Macrophages M2' (Fig. S3). The correlation analysis by ESTIMATE suggested that there was a significantly positive correlation between immune score and BAG1 expression in LPS ($p = 1.1e\text{-}4$, $r = 0.49$, Fig. 7C), indicating that high expression of BAG1 may predict a higher immune score. Furthermore, the correlation between BAG1 expression and immune cells enriched in TME was verified by xCell algorithm in LPS samples. As illustrated, the enrichment of 'CD4 + Tem', 'CD8 + naïve T-cells', 'Monocytes' and 'Th1 cells' displayed positive correlations with BAG1 expression (Fig. 7D). These results suggested that BAG1 may enhance the anti-cancer immunity of patients to exert protective effect, thus contributing to the improved prognosis.

The correlation between BAG1 or BAG2 expression and immune infiltration level in sarcomas (including liposarcoma) was evaluated on TIMER2.0 database. The expression of BAG1 was significantly positively associated with tumor purity and the infiltration level of CD4 + T cell (Fig. S4A). In contrast, the expression of BAG2 was significantly negatively related to the infiltration level of CD4 + T cell (Fig. S4B). BAG1 and BAG2 may play opposite roles to alter TME of sarcomas patients by affecting immune cells infiltration level in distinctive manners, thereby exerting contrary effects on anti-tumor immunity.

As summarized in previous research, several therapeutic drugs for LPS were related to DNA replication, microtubule binding, cell cycle arrest and adipogenic differentiation, including epirubicin, doxorubicin, ribociclib, efatutazone, pioglitazone, etc.[26]. In the present study, BAG1 and BAG2, with distinct predictive effects

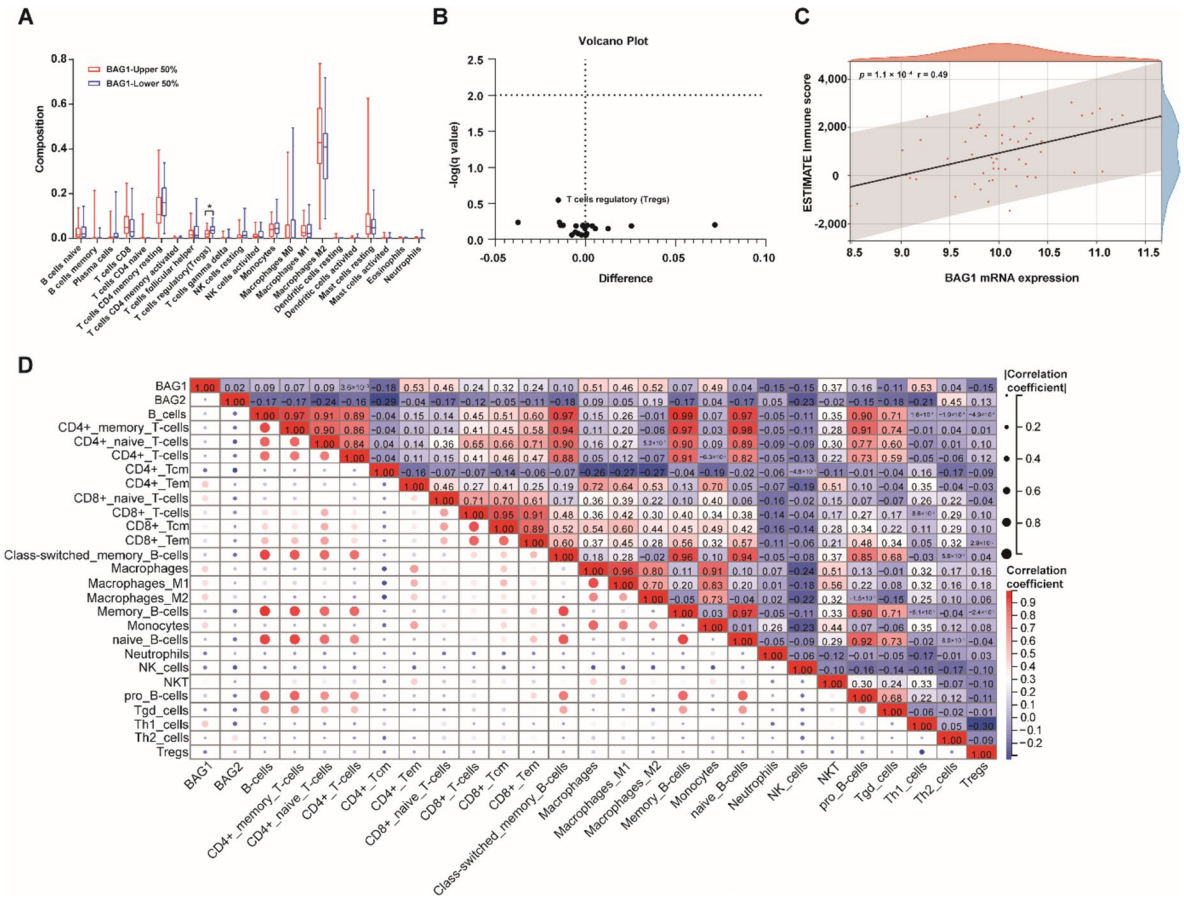

Figure 7. Analysis of immune infiltration in TME of LPS tissues. (**A**) Comparison of immune cell compositions between BAG1 high-expression (Upper 50%) and low-expression group (Lower 50%). (**B**) Volcano map of (**A**). (**C**) The association between BAG1 expression and immune score. (**D**) The correlation between BAG1 expression and enrichments of immune cells.

(protective vs. risk factor), were identified as potential prognostic biomarkers and promising therapeutic targets for LPS. To probe this prospect, Pearson's correlation analysis of BAG1/BAG2 with these therapeutic targets for LPS was performed (Fig. S5). BAG1 was significantly positively associated with PPARG ($r = 0.48$, $p = 1.6 \times 10^{-5}$), on the contrary, BAG2 was significantly negatively associated with PPARG ($r = -0.63$, $p < 2.2 \times 10^{-16}$) (Fig. S5B and E). Additionally, BAG1 was significantly negatively associated with TOP2A ($r = -0.4$, $p = 1.1 \times 10^{-6}$), while BAG2 was significantly positively associated with NFKB1 ($r = 0.5$, $p = 3.1 \times 10^{-10}$) (Fig. S5C and D).

Establishment and evaluation of a BAG1-BAG2-based prognostic signature

To further confirm the prognostic values of BAG1 or BAG2, a 2-gene signature was established by Lasso regression analysis and evaluated by survival or ROC curve (Fig. 8). In this risk assessment model, patients were stratified into high risk and low risk groups based on the median of risk scores (Fig. 8A and B), as shown, patients with distant recurrence mainly included in high-risk group, indicating this 2-gene signature might be a risk indicator

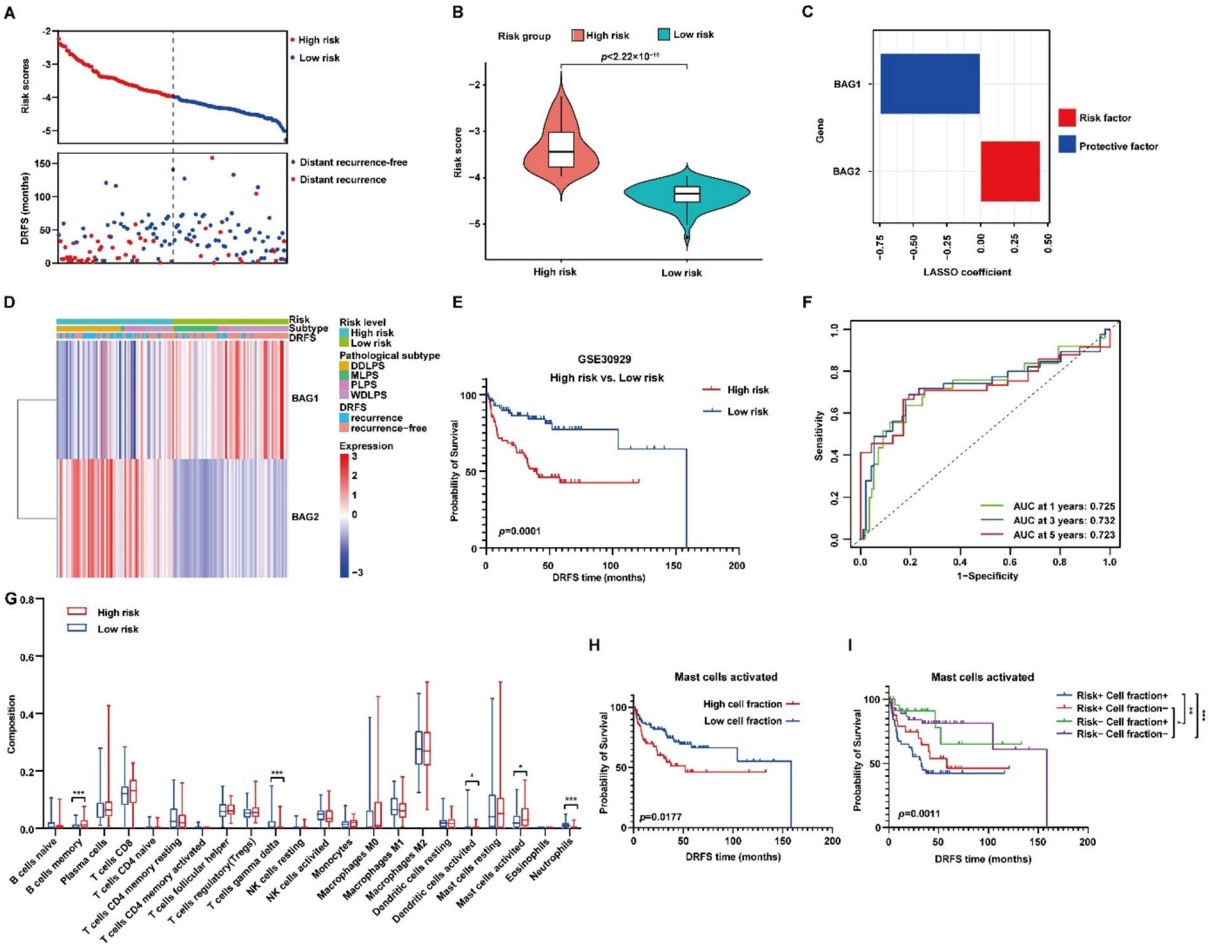

Figure 8. Establishment of a BAG1-BAG2-based signature and evaluation of its prognostic ability for LPS using data from GES30929 dataset. (**A**) Distribution of risk scores based on a 2-gene signature, and scatter plots of survival status (distant recurrence risk) of LPS patients with high or low risk scores. (**B**) Comparison of risk scores between high and low risk group. (**C**) Distribution of Lasso regression coefficients for BAG1 and BAG2. Red represents the risk factor and blue denotes the protective factor. (**D**) A comprehensive heatmap plotted by R (version 4.2.3; https://www.r-project.org/) including risk level, pathological subtype, DRFS as well as expression of BAG1 and BAG2. DDLPS, dedifferentiated liposarcoma; WDLPS, well differentiated liposarcoma; *PLPS* pleomorphic liposarcoma; *MLPS* myxoid liposarcoma. (**E**) Kaplan–Meier survival curve of DRFS based on the risk scores (cut-off: 50%). (**F**) Time-conditioned ROC curves for evaluating the prognostic ability of the 2-gene risk assessment model at 1, 3, 5 years. (**G**–**I**) Analysis of risk score-related immune infiltration in TME of LPS tissues. (**G**) Comparison of immune cell proportion between high and low risk group. (**H**) Survival curves based on immune cell infiltration level. Kaplan–Meier plots of DRFS for LPS patients classified by the proportion of activated mast cells. (**I**) Survival curves were plotted to compare DRFS of patients with high or low risk and high or low proportion of activated mast cells: patients were subdivided into four groups. Risk + Cell fraction + : the group with high risk and high proportion of activated mast cells; Risk + Cell fraction−: the group with high risk but low proportion of activated mast cells; Risk- Cell fraction + : the group with low risk but high proportion of activated mast cells; Risk- Cell fraction−: the group with low risk and low proportion of activated mast cells; cut-off: 50%. *DRFS* distant recurrence-free survival.

of LPS recurrence. Consistent with Cox regression results (Table 2), BAG1 is a protective factor, yet BAG2 is a risk factor (Figs. 8C and S6A). A heat map illustrated the differential expression profiles of BAG1 and BAG2 in patients at different risk level (high vs. low risk); with different pathological subtype; with recurrence or not (Fig. 8D). As shown, the high risk group was more inclined to relapse and included most DDLPS (more malignant subtype) patients, while more WDLPS patients were distributed in the low risk group. As survival curve depicted, patients in high risk group displayed a significantly poor prognosis than low risk group (GSE30929: $p = 0.0001$; TCGA: $p = 0.0492$) (Figs. 8E and S6B), indicating the improved predictive performance of this 2-gene prognostic model. Then, ROC curve of BAG1 or BAG2 was plotted based on risk scores and the value of AUC reached 0.725 at 1 year, 0.732 at 3 years and 0.723 at 5 years, respectively (Fig. 8F), which was verified in validation set (Fig. S6C). These results confirmed the robust prognostic abilities of BAG1 and BAG2 in LPS.

Additionally, the relationship between risk score and immune infiltration level was estimated to further evaluate the effects of this 2-gene risk model on LPS TME. According to risk scores, LPS patients were categorized into low and high risk group. By comparison, high risk group was significantly associated with high abundance of 'Mast cells activated' ($p < 0.05$, Fig. 8G), and high proportion of 'Mast cells activated' indicated a adverse prognosis of LPS patients (Fig. 8H). To comprehensively consider the influences of risk score and immune cell infiltration level on patients' prognosis, survival curve with these factors was plotted, and patients were divided into 4 subgroups according to risk score and cell proportion (Fig. 8I). As illustrated, patients with high risk and proportion of 'Mast cells activated' deciphered the worst DRFS, while patients with low risk and proportion of 'Mast cells activated' interpreted the best DRFS (before 100 months) (Fig. 8I), indicating a profound influence of this 2-gene prognostic model on LPS TME and risk assessment.

Discussion

As a common type of soft tissue sarcoma, LPS is a malignant tumor with a disease mortality rate of up to 30%[3,40]. Due to the high risk for recurrence and metastasis of LPS, it is of great significance to develop prognostic biomarkers for LPS. Although some biomarkers for LPS have been reported[10,41], it is necessary to explore promising potential predictors for risk assessment of LPS patients followed by effective molecular targeted therapy. Thus, BAG1 and BAG2 were identified as potential predictors for LPS survival through screening ARGs, the roles of which in malignancies, especially LPS, have been less studied and the molecular mechanisms of their actions in tumor progression are still unclear. In the present research, we investigated the expression, prognostic significance and potential roles of BAG1 or BAG2 in LPS. Firstly, the expression level of BAG1 or BAG2 in LPS and normal tissues was compared to assess whether BAG1 or BAG2 is involved in LPS oncogenesis. With the analysis results, BAG1 and BAG2 displayed the distinct gene expression pattern: BAG2 was overexpressed in LPS, especially in DDLPS, on the contrary, BAG1 was low expressed in that. As is known, DDLPS is more malignant than WDLPS[42–44], indicating that BAG1 and BAG2 may exert distinct effects on LPS tumorigenesis and development, in particular, BAG1 may function as a protective gene to inhibit LPS progression, on the contrary, BAG2 may act as an oncogene to promote progressive disease. In addition, the results of ROC curves also verified the differential expression of BAG1 and BAG2 in DDLPS and normal adipose tissues. Subsequently, survival curves were plotted. In detail, patients with low BAG1 expression exhibited poor OS and DSS, while patients with high BAG2 expression exhibited adverse OS and DSS, confirming the opposite potential roles of BAG1 and BAG2 in LPS progression, in accordance with the expression analysis results. As reported, elevated BAG1 predicted a better prognosis in various cancers, including kidney renal clear cell carcinoma and early-stage breast cancer[21,45], while the contrary effect of BAG1 was observed in several cancers[18,46], and this contradiction might due to the diseases derived from different tissue and organ types. Moreover, KM survival curves plotted by integrating the expression of BAG1 and BAG2 showed that patients with BAG1- plus BAG2 + suffered from the worst survival, on the contrary, patients with BAG1 + plus BAG2- underwent the best survival, further confirming the opposite role of BAG1 and BAG2 in LPS progression. In addition, a possibility is proposed: the combined application of BAG1 and BAG2 as a robust prognostic gene signature for LPS patients risk assessment. Furthermore, the results of correlation analysis between BAG1/BAG2 and the known LPS biomarkers or DEGs, including SIRT1, PIK3CA, TOP2A and PNPLA2[27–31], further verified the predictive ability of BAG1 or BAG2 in LPS survival outcome. As previously reported, high expression of SIRT1, PIK3CA or TOP2A is associated with adverse prognosis of LPS patients, while for PNPLA2, exactly the reverse applies.

The opposite role of BAG1 and BAG2 in LPS progression make us forward to deeply explore the mechanism of this distinction. PPI analysis showed that BAG1 or BAG2 was closely related to heat shock proteins (HSPs), confirming that they are both involved in the regulation of cellular response to heat and function synergistically with HSPs[47–49]. Additionally, BAG1, BAG2 and most of their co-expression genes participated in the regulation of protein stability (protein folding, ubiquitin protein ligase and chaperone binding). More importantly, BAG proteins bind to HSP70 through a specific domain and regulate their activities as molecular chaperones[49–51], which play an important role in maintaining protein homeostasis, and are involved in autophagy/mitophagy[52]. BAG proteins are important for Hsp70/Hsc70 functions[53,54]. According to previous research, both human and canine cells displayed less BAG1 after VER155008 (Hsp70 inhibitor) treatment[55]. Therefore, activating Hsp70/Hsc70 and BAG1 may be related to the inhibition of LPS. Research on HSP70/Hsc70 activators may be a potential research direction for the treatment of LPS. And more research is needed to fully elucidate the therapeutic potential of targeting the BAG-Hsp70/Hsc70 interaction, our findings provide a valuable foundation for future studies in this area. What's more, the results of GSEA showed that TGF-β signaling pathway was enriched in BAG1 low-expression group, which promotes proliferation and metastasis of cancer cells[56,57]. In addition, in a recent study, single-cell sequencing of tumor cells from DDLPS patients revealed that the process by which tumor cells differentiate into the adipocyte lineage was inhibited by TGF-β. Treatment of DDLPS tumor cells with TGF-β inhibitors restores their adipocyte phenotype in vitro and in vivo. Therefore, it can be known that

the activation of TGF-β signaling will reduce the degree of differentiation of tumor cells, leading to further tumor deterioration[58]. Thus, we supposed that BAG1 low-expression may affect TGF-β signaling to promote tumor relapse and metastasis, as well as inhibit the differentiation of LPS cells, thereby leading to the unfavorable prognosis for LPS. To demonstrate the importance of these pathways in LPS, we also explored the possible role of MDM2/MDM4/TP53 according to the results of GSEA. MDM2 is a well-known prognostic marker and key oncogene for LPS[59–62]. MDM4 and MDM2 are homologues, and they both are negative regulators for p53[63]. When MDM2 is overexpressed, it will promote the degradation of wild-type p53 instead of mutant p53[64,65]. On the one hand, mutant functional p53 cause abnormal activation of TGF-β signaling pathway[66], thus influencing the differentiation of tumor cells as the role of BAG1. On the other hand, after p53 mutation, the ability of NK cells to kill tumors is reduced[67]. However, when activating p53 with MDM2 inhibitors, immune cells' ability to process and present antigens is improved[68]. At present, there are few studies on the interaction between MDM2/MDM4/TP53 and ECM receptor interaction. p53 may control the expression of ECM components and regulate the adhesion ability between cells[69], affecting the migration of tumor cells. The above proves that the pathways where BAG1/BAG2 is located affect the occurrence and development of LPS by influencing immunity and cell differentiation, suggesting the possibility of BAG1/BAG2 as a therapeutic target. As a result, activating BAG1 or/and repressing BAG2 may be intended to provide a feasibility for prospective therapeutic strategies of LPS. At present, surgical resection followed by radiotherapy and chemotherapy still remains the main therapy option for LPS, however, due to the molecular heterogeneity of LPS, the treatment effect is not ideal[70], as some patients would be tolerant or resistant to the uniform treatment and progress to recurrence. Therefore, it is necessary to develop prognostic biomarkers for LPS patients to make it possible that the molecular targeted therapy provides effective therapeutic strategies. In recent years, drug targeted therapy has been applied to clinical treatment. DDLPS has higher chemotherapy sensitivity than WDLPS, since the chemotherapy sensitivity of WDLPS is almost 0%[4]. Trabectedin and pazopanib, as tyrosine kinase inhibitors, were employed to treat DDLPS patients aiming to target vascular endothelial growth factor[5,71], and the underlying mechanism is to suppress cell signaling and angiogenesis thus leading to tumor growth inhibition. As potential prognostic biomarkers, BAG1 or BAG2, might be regarded as promising therapeutic targets of drugs (agonists or inhibitors) to explore the feasibility of combined application of BAG1/BAG2-targeted drugs (BAG1 agonists/BAG2 inhibitors), for the sake of improving the clinical outcomes of LPS patients.

It is well known that immunity is closely related to tumor progression. Some immune cells are related to prognosis and treatment of soft-tissue sarcomas (STS)[72]. Tregs can inhibit the function of effector T cell, thereby accelerating tumor escape and growth[73]. The effective tumor immunity is suppressed by Tregs, which infiltrate into TME and is relevant to the adverse prognosis. Thus, the depletion of Tregs improves anti-tumor immunity[74,75]. In the present study, we evaluated the correlation between BAG1 or BAG2 expression and immune cell infiltration in LPS, and found that BAG1 expression was significantly associated with Tregs abundance in TME of LPS. In detail, more abundant Tregs infiltrated into TME of LPS patients with BAG1 low expression, which predicted the worse survival as KM curves illustrated, demonstrating the low-expression of BAG1-mediated high infiltration of Treg into TME of LPS (as a risk factor) and the consequential unfavorable prognosis of LPS patients. On the other hand, BAG1-high-expression might be a protective factor, as low infiltration of Treg and the resultant favorable prognosis of LPS patients were observed upon BAG1 overexpression. Thus, it further verified the immuno-prognostic values of BAG1 that patients with BAG1-high-expression exhibited the enhanced anti-tumor immunity and prolonged survival. Additionally, as an anti-cancer factor[76,77], PPARG presented the significantly positive correlation with BAG1, which was identified as a protective factor by our findings. On the contrary, PPARG exhibited the significantly negative correlation with BAG2, which was regarded as a risk factor by our findings. As a type of thiazolidinedione, efatutazone or pioglitazone, acts as a PPARγ agonist, exerts its anti-cancer activity in preclinical studies by specifically and effectively activating PPARγ-mediated transcription and subsequent inhibition of tumor growth, showing promise in treating advanced malignancies[76–79]. Thus, it is speculated that there might be a certain connection between PPARG and BAG1 or BAG2: patients with high BAG1 or/and low BAG2 expression might display higher PPARγ expression and anti-cancer activity, and be more sensitive to therapeutic drugs of PPARγ agonist (efatutazone or pioglitazone) for LPS, deriving more clinical benefits. In prospective view, it is expected that targeting BAG1/BAG2 (agonists/antagonists) combined with efatutazone or pioglitazone might become a potential molecular targeted therapeutic strategy for LPS treatment.

Recently, gene signature has been widely applied for survival prediction and risk assessment in various cancers[80–82]. Based on gene expression profiling and survival data, gene signatures were established by Lasso-Cox regression. To further validate the prognostic significance of BAG1 and BAG2, a 2-gene signature was constructed to stratify LPS patients according to risk scores. As illustrated, the higher the risk score was, the higher recurrence risk would be for LPS patients, the more malignant subtype distributed. Survival and ROC curves further validated the accuracy and sensitivity of this 2-gene prognostic model. Patients at high risk were inflicted with the adverse prognosis, indicating the robust risk assessment performance of this 2-gene signature model and that BAG1/BAG2 could be potential prognostic biomarkers for LPS. Additionally, this 2-gene model has a profound influence on LPS TME, as evidenced by the infiltration level difference of activated mast cells between high and low risk group and the survival time difference of LPS patients with high or low risk plus high or low infiltration level of activated mast cells. It is well-known that activated mast cells are able to release pro-angiogenic factors and cytokines (including CSF2, CCL3 and IL-6), which promote tumor growth by stimulating angiogenesis or recruiting tumor-associated macrophages[83–85]. And higher infiltration level of mast cells reflects poor responses to anti-tumor immunotherapy[86]. Here, we found that the 2-gene prognostic model exerts a significant influence on LPS TME and risk assessment: patients with high risk and proportion of activated mast cells indicate the worst prognosis, confirming the prognostic significance of this 2-gene risk model for LPS patients, which might guide the molecular targeted therapy and immunotherapy for LPS. What's more, B cells are important indicators for predicting the therapeutic effect of tumors. A study of soft tissue sarcoma found that the group

with high infiltration of B cells often corresponded to a better prognosis. Analysis of the results indicated that B-cell infiltration was a key set of differential features to improve the survival time of patients[87]. In combination with PD-L1 blocking (one type of immunotherapy) and VEGF inhibition, high B-cell lineages and vascular density showed the highest response and improvement in progression-free survival in patients with advanced soft tissue sarcomas[88]. Therefore, the number of B cells in sarcoma is a key indicator of the prognosis of patients. A study also established a prognostic model of osteosarcoma with good predictive performance, revealing the indispensable role of B cells in the development of osteosarcoma, and that B cell activation can contribute to better prognosis of patients with osteosarcoma[89]. The abundance of B cells may be an indicator to evaluate the prognosis of LPS patients.

Conclusion

In conclusion, the present study demonstrates that decreased BAG1 or/and increased BAG2 expression in patients suffered with LPS is associated with poor prognosis. Although BAG1 and BAG2 belong to the same BAG family and function as regulators of chaperone, they may exert opposite influences on liposarcomagenesis and TME, and have distinct predictive performances for LPS prognosis and anti-tumor immunity. Thus, as their valuable performances on the risk assessment of LPS survival, BAG1 and BAG2 may be considered as the potential targets for the treatment of LPS patients (developing BAG1 agonists/BAG2 inhibitors).

Data availability

Publicly available datasets were analyzed in this study. This data can be found here: The datasets analyzed for this study can be found in TCGA database or UCSC Xena (https://xenabrowser.net/datapages/?cohort=TCGA%20Sarcoma%20(SARC)&removeHub=https%3A%2F%2Fxena.treehouse.gi.ucsc.edu%3A443) and GEO database (https://www.ncbi.nlm.nih.gov/gds/?term=) (accession numbers: GSE159659, GSE21122 and GSE30929).

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Author contributions

YL and JC: methodology, data analysis, writing-original draft preparation; JH and BZ: methodology, data analysis; JW and XL: data collection and curation; MY, MH and TW: formal analysis, validation; TY, XH and TS: investigation, validation; MT, KZ and GL: visualization, software; YA: conceptualization, design, funding acquisition, project administration, writing-original draft preparation, review and editing. All authors consent to publish this manuscript.

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Competing interests

The authors declare no competing interests.

Additional information

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