HSPA5 regulates the expression and alternative splicing of inflammatory and immune response genes.

Heng Fan

Department of Integrated Traditional Chinese and Western Medicine Union Hospital Tongji Medical College Huazhong University of Science and Technology

Linping Xue

Hubei University of Chinese Medicine

Yujin Liu

Department of Integrated Traditional Chinese and Western Medicine Union Hospital Tongji Medical College Huazhong University of Science and Technology

Dongmei Zuo (✉ zuo_345@126.com)

Department of Integrated Traditional Chinese and Western Medicine Union Hospital Tongji Medical College Huazhong University of Science and Technology <https://orcid.org/0000-0001-8701-9238>

Fei Gao

Department of Integrated Traditional Chinese and Western Medicine Union Hospital Tongji Medical College Huazhong University of Science and Technology

Huarong Li

Department of Integrated Traditional Chinese and Western Medicine Union Hospital Tongji Medical College Huazhong University of Science and Technology

Lijuan Zhang

Department of Integrated Traditional Chinese and Western Medicine Union Hospital Tongji Medical College Huazhong University of Science and Technology

Zhexing Shou

Department of Integrated Traditional Chinese and Western Medicine Union Hospital Tongji Medicine Huazhong University of Science and Technology

Research Article

Keywords: HSPA5, endoplasmic reticulum, inflammatory/immune, transcription, alternative splicing

Posted Date: April 28th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-397944/v1>

Abstract

HSPA5 encodes a chaperone protein, BIP/GRP78, which is also an RNA-binding protein with potential transcriptional and post-transcriptional regulatory functions. To explore the functions of HSPA5 on target genes, we over-expressed HSPA5 (HSPA5-OE) in HeLa cells. Then RNA-seq analysis found 928 genes were significantly differently expressed, among which 460 genes were up-regulated and 468 genes were down-regulated in the HSPA5-OE cells. GO analysis showed that the differently expressed genes were mainly enriched in inflammation and innate Immunity responses. In addition, the up-regulated genes were enriched in the process of cell proliferation and differentiation. Immune-related pathways were also found in the DEGs with KEGG analysis. Furthermore, a total of 659 different alternative splicing events were identified based on the splicing junction reads. And the related genes were enriched in apoptosis, TNF signaling, and NF-kappa B signaling pathways. RT-qPCR experiment proved that the expression of inflammatory/immune-related genes was significantly changed with HSPA5-OE and showed significant difference in alternative splicing of genes involved in the above pathways. Our results suggest that HSPA5 regulates the expression and alternative splicing of inflammatory and immune response genes, which makes a foundation for further exploring the function of HSPA5 as RBP.

Introduction

Endoplasmic reticulum (ER) is one of the vital organelles and responsible for many biological process [1]. ER stress is induced when cell homeostasis is disturbed, leading to the unfolded protein response (UPR) which is related with inflammation, obesity, and cardiovascular disease [2,3]. UPR maintains the cellular functions by stopping protein translation, degrading misfolded proteins, activating signaling pathways, and triggering apoptosis [4-6]. During the UPR signaling, molecular chaperone Binding immunoglobulin protein (BiP), also known as 78 kDa glucose-regulated protein (GRP78) plays an important role. Numerous studies about BiP/GRP78 have shown that it plays an important role in the pathology of many diseases [7-10].

BiP/GRP78 encoded by HSPA5 belongs to heat shock protein family A (Hsp70) member [11], which is a molecular chaperone that is mainly found in the ER. BiP/GRP78 contains two highly conserved domains, N-terminal nucleotide-binding domain (NBD) regulates the affinity of substrate binding and C-terminal substrate-binding domain (SBD) binds to hydrophobic stretches[12]. These two domains determine BiP/GRP78's ATPase activity and substrate binding ability, which help to fold and hold the newly protein in ER and participate in the unfolded protein response (UPR)[11]. GRP78 influences genes transcription as chaperone [13], which could bind to transmembrane receptors, including the kinase PERK, the kinase/endonuclease IRE1, and the transcription factor ATF6, inhibiting their activity [14]. With the misfolded proteins accumulating in ER, GRP78 is dissociated with these receptors, resulting the activation of them. Activated IRE1 splices the mRNA of transcription factor X-box Binding protein 1 (Xbp1) which induces the transcription of specific genes related to ER-stress. And activated ATF6 can induce the transcription of GRP78 and Xbp1 [15]. PERK undergoes autophosphorylation and active a series of downstream transcription factor signals.

In addition to chaperone function, HSPA5 could also act as an RNA binding protein (RBP) [16]. Numerous and diverse RBPs could ensure that different mRNA regions, including 5' and 3' untranslated (UTRs) and the coding region, are covered or exposed accurately, regulating the fate or function of the bound RNAs [17,18]. RBPs also have the post-transcriptional functions including regulating alternative splicing, editing RNA, maintaining mRNA stability, and influencing translation by interacting with pre-mRNAs and mRNAs [19]. Besides, RBPs could act as transcription factor or interacts with other proteins to regulate genes transcription[20]. The functions of RBPs were widely studied in many diseases such as neurodegenerative disease, immune disease, and cancer[21-24]. While, the transcriptional and post-transcriptional regulatory functions of HSPA5 still need to be further studied.

In this study, to decipher the functions of HSPA5 on whole transcriptome profile, we over-expressed HSPA5 in HeLa cells, with two biological replicates in treatment group and control group. Next-generation transcriptome sequencing was performed with the HSPA5-OE cells. Transcriptome data were used to study the differences in transcription and splicing levels of genes caused by changes in HSPA5 expressions. This research deepens our understanding of the transcriptional and post-transcriptional regulatory functions of HSPA5, which makes a foundation for future researches about HSPA5.

Materials And Methods

Plasmid and cell transfect

The plasmid vector pIRES-hrGFP-1a-HSPA5 was reconstructed and transfected in HeLa cells as previously described[25]. The inserted fragment was amplified (primer forward: agcccgggcgcatccgaattcATGAAGCTCTCCCTGGTGGC, and reverse: gtcaccttgtagtctctcgagCAACTCATCTTTTCTGCTGTATCCT) from HeLa cells cDNA.

RNA sequencing and data processing

Two biological replicates of the treatment group and the control group were set. Total RNA with qualified concentration was submitted to 'Appreciate the Beauty of Life, Inc' for the construction of sequencing library. Illumina Hiseq X Ten sequencing platform was used to 150 nt paired-end sequencing. FASTX-Toolkit (version 0.0.13) were used to clean reads. Tophat2 software were used to align clean reads to human genome (GRch38) allowing 4 mismatches [26]. Uniquely mapped reads were used for gene reads number counting and FPKM calculation by Cufflinks software.

Differentially expressed genes (DEGs) and function enrichment analysis.

Differentially expressed analysis was performed by edgeR [27], and a cut-off value (fold change $\geq$ 2 or $\leq$ 0.5, false discovery rate (FDR) < 0.05) was set for picking DEGs. To get the function of DEGs, Gene Ontology (GO) and KEGG functional enrichment analysis were applied using KOBAS 2.0 server [28].

Alternative splicing (AS) analysis.

A total of ten types of alternative splicing events (ASEs) were identified based on the splicing junction reads, including intron retention (IR), alternative 5' splice site (A5SS), alternative 3' splice site (A3SS) exon skipping (ES) et al [29]. The T-test method was used to compare the variation of splicing level between two samples of the same splicing type. $P \leq 0.05$ was set as a criterion with significant differences (RASE). T-value was used to determine the percentage of ASEs that occur between the treatment and control groups. Similarly, GO, KEGG functional enrichment analysis was used to obtain the function of genes that had different ASE (RASGs).

RT-qPCR

Total RNA extracted from HeLa cells or the same batch of RNA for sequencing were reversely transcribed into cDNA. RT-qPCR reactions were performed on a Bio-Rad S1000 (DBI Bioscience, Shanghai, China), and all data were normalized to GAPDH mRNA expression. For RASGs validation, we used a boundary-spanning primer contained the junction of constitutive exon and alternative exon as well as a reverse primer in a constitutive exon. The primer pairs are showed in Supplemental Material 1.

Western blotting

Western blotting analysis was performed as described previously[30]. Flag antibody (1:1,000, 2368S, CST) was used to Western blot as primary antibodies.

Availability of data and materials

The data discussed in this publication are available under GEO Series accession number GSE165235

Results

The high quality data were obtained for subsequent analysis.

The efficiency of HSPA5 over-expression in HeLa cells were tested with WB and RT-qPCR which showed that HSPA5 was successfully over-expressed (Figure 1A). The raw data obtained from sequencing of each sample was over 12G, and over 11G was left after data clean (Supplemental Material 2). Then the clean reads were mapped back onto the human genome (GRCh38), and over 90% of reads were uniquely mapped (Supplemental Material 3). Compared with the control group, FPKM showed that HSPA5 expression was significantly increased in over-expressed HeLa cells (Figure 1B). Cluster analysis showed that the experimental two group were obviously distinguished, and the two groups were clustered into different branches. And a total of 19178 genes were expressed in at least one sample of the treatment/control group (FPKM > 0) (Figure 1D). $FPKM \geq 1$ was set as the threshold value to analyze the distribution of gene expressions. The distribution was consistent between the two groups, and more than half of the gene expressions were greater than 7 (Figure 1E).

HSPA5 regulated the expression of genes enriched in Calcium signaling pathway, inflammatory, and immune response.

Differential expression analysis found a total of 928 significantly differentially expressed genes (DEGs), including 460 up-regulated genes and 468 down-regulated genes (Figure 2A). The heatmap cluster of DEGs expression showed that the samples of the two groups were significantly different, and some genes expression were inconsistent (Figure 2B). GO enrichment showed that the up-regulated genes were enriched in the inflammatory response (GO: 0006954) and innate immune response (GO: 0045087). Besides, the pathways associated with cell proliferation differentiation, including negative regulation of cell proliferation (GO: 0008285), cell differentiation (GO:0030154), and signal transduction (GO:0007165) were also enriched. Immune-related GO enrichment also appeared in the down-regulated genes including innate immune response (GO: 0045087) (Figure 2C). Furthermore, KEGG analysis directly found the pathways related with HSPA5. The up-regulated genes were concentrated in the Calcium signaling pathway, Complement and coagulation cascades, and Influenza A pathway, while the down-regulated genes were related with Systemic Lupus erythematosus (figure 2D). All the results of functional enrichment have been reported to be related to HSPA5, suggesting that HSPA5 can be involved in biological activities or diseases by regulating the expression of genes in related pathways.

HSPA5 regulated the expression levels of genes related to inflammatory/immune process.

DEGs analysis found HSPA5 was closely related to inflammatory/immune process. RT-qPCR were performed to validate the inflammatory/immune genes expression were significant changed. Genes were picked from GO and KEGG enrichment results. We successfully verified the expression levels of three genes in HeLa cells (Figure 2E). ADAM8 and CSF2 were involved in inflammatory signaling and HSPA1A was participated in Influenza A. The RNA-seq results were consistent with that of RT-qPCR, indicating HSPA5 regulated the genes expression related with inflammatory/immune process.

Abnormal splicing events were mainly enriched in immune associated pathway in HSPA5 over-expression HeLa cell.

After excluding ASEs with high false positives, we found that A5SS and A3SS events with significant differences were the most numerous, followed by ES events, cassette exon events and intron retention (IR) events. Among these events, except IR events, the number of up-regulated events was more than down-regulated events (Figure 3A). A total of 5 genes showed different expression and different alternative splicing simultaneously, indicating that the change in splicing level affected the genes expression (Figure 3B). GO and KEGG functional enrichment analysis were performed on RASG to obtain the related biological processes and signaling pathways. GO showed that the most enriched genes are related to apoptosis and immunity, such as apoptotic process (GO: 0006915), positive regulation of I-kappaB kinase/NF-kappaB cascade (GO: 0043123) (Figure 3C). These signaling pathways were consisted with KEGG enrichment results. The top 5 KEGG enriched pathways were related with TNF signaling pathway, apoptosis, NF-kappa B signaling pathway (Figure 3D). All the results indicated HSPA5 may regulate AS of genes in the signaling pathway about immunity.

HSPA5-regulated alternative splicing of key genes in apoptosis, TNF signaling pathway, and NF-kappa B signaling pathway.

In order to verify the authenticity of ASEs, we randomly selected genes in these pathways for quantitative experiments. We designed primers at near the splicing site to detect the relative splicing levels of different transcripts. The ASEs of two genes (MAP3K7 and RIPK1) were successfully verified. MAP3K7 produced the two types of transcripts by occurring cassetteExon splicing which was significantly up-regulated in the treatment groups (Figure 4A). While RIPK1 experienced the 5pMXE splicing which declined in the treatment groups (Figure 4B). The results of RNA-seq identification were consistent with those of RT-qPCR. Our result proved that the HSPA5 regulated the AS of genes involved in apoptosis, TNF signaling pathway, and NF-Kappa B signaling pathway.

Discussion

The purpose of this study was to explore the transcriptional and splicing regulation of HSPA5. Firstly, HSPA5 gene was successfully over-expressed in HeLa cells. High-quality sequencing reads were obtained for subsequent analysis. Different expression genes analysis showed that a total of 928 genes expression were significantly changed in HSPA5-OE HeLa cells, and functional analysis showed that DEGs were enriched in the inflammatory/immune process. AS analysis showed that RASEs were mainly derived from A5SS, A3SS, and ES type events, which were mainly involved in key apoptosis-related signaling pathways. Experimental verification results suggested that HSPA5 might regulate gene transcription and splicing of genes involved in inflammation/immunity.

HSPA5 encodes a long-existing protein-BiP/GRP78 in the ER, which acts as a molecular chaperone to assist the folding of new proteins and the degradation of misfolded proteins [13]. In the process of ER-stress, a series of downstream responses are activated to restore ER functions and control cell fate [4]. This study found that changes in HSPA5 expression caused a series of changes in genes expression. It has been reported that HSPA5 can exert transcriptional regulatory functions by activating transcription factors and play an important role in inflammation/immunity [31,32]. In this study, HSPA5 was found closely related with many biological processes or signaling pathways with the functional enrichment analysis of DEGs. Genes involved in inflammatory/immune processes were found in both up-regulated and down-regulated genes.

HSPA5 is a key protein in UPR, which interacts with inflammatory pathways at different pathways, such as ROS production, NF-KB, Jnk, and IRF3 activation [33]. In addition, BiP is highly expressed in Rheumatoid Arthritis and is a major Target of B and T cells [34]. KEGG enrichment analysis showed the changed genes were involved in virus infection, such as Influenza A. HSPA5 has been reported as a therapeutic target for viral and bacterial infections [35,36]. Furthermore, HSPA5 was critical for Ebola infection and disease replication [37], and participated in the innate immune response of hepatocytes to HCV virus [38]. In addition, DEGs from this study were involved in calcium signaling pathways and cell proliferation and differentiation, which are related to the function of HSPA5. HSPA5 is a Ca^{2+} binding protein that affects the transfer of Ca^{2+} between the ER and mitochondria and maintains mitochondrial function [39]. Besides, HSPA5 is one of the GRPs which could be actively trans-located to other cellular locations and perform additional functions about cellular signaling, proliferation, and invasion[32]. The

function analysis of DEGs was consistent with the role of HSPA5 in the study of a variety of diseases, suggesting that HSPA5 might achieve its role by regulating the transcription level of related genes.

In addition, HSPA5 is also an RBP and involved in post-transcriptional processing of many genes, including alternative splicing. We identified 659 RASEs with transcriptome sequencing analysis. There were significant differences about these 659 RASEs between the two groups, suggesting the influence of HSPA5 on genes AS. RASGs were mainly enriched in the apoptotic process, TNF signaling, and NF-KB signaling pathways. Previous studies have demonstrated that GRP78 is a key regulator of ER-related apoptosis[32]. Over-accumulation of abnormal proteins in the cell could trigger cells apoptosis. PERK undergoes auto-phosphorylation and inhibit alpha subunit of the eukaryotic initiation factor 2 (eIF2 α) that block the production of new proteins [40]. The over-phosphorylated eIF2 activates transcription factor ATF4 which induces transcription factor CHOP/GADD134 expression[41], inducing pro-apoptotic proteins Bax and Bim expression[42] and suppressing anti-apoptotic protein Bcl-2 expression[43]. GRP78 could activate IRE1 and lead to splicing of XBP1 mRNA during the UPR [35,44]. TNF signaling pathway and NF-KB signaling pathway were both related to inflammation/immune processes and apoptotic [45-48].

Our results indicated that HSPA5 regulated the expressions and alternative splicing of inflammatory/immune-related genes, which suggested the functions of HSPA5 from another perspective. As a molecular chaperone, HSPA5 affects gene transcription and alternative splicing in ER-stress, but whether it directly targets the proteins or not to regulate the gene expression still needs further exploration. Based on the important role of HSPA5 in various diseases, more studies on HSPA5 as an RBP are worthy of further study.

Declarations

Acknowledgements

This study was funded by the National Natural Science Foundation of China (No. 81904018 and 81774093).

Conflicts of interest

The work was original research that has not been published previously, and not under consideration for publication elsewhere, in whole or in part.

Availability of data and material

The original RNA-seq data has been update in GEO, and the GEO number is GSE165235

Code availability

Not applicable for this section

Authors' contributions

DMZ, HF, LPX and YJL developed the idea of the study, participated in its design and coordination and helped to draft the manuscript. FG and HRL contributed to the acquisition and interpretation of data. LJZ and ZXS provided critical review and substantially revised the manuscript. All authors read and approved the final manuscript.

Ethics approval

Not applicable for this section

Consent to participate

Not applicable for this section

Consent for publication

Not applicable for this section

References

- [1] Z. Liu, Y. Lv, N. Zhao, G. Guan, J. Wang, Protein kinase R-like ER kinase and its role in endoplasmic reticulum stress-decided cell fate, *Cell Death Dis* 6 (2015) e1822. 10.1038/cddis.2015.183.
- [2] O.M. Amen, S.D. Sarker, R. Ghildyal, A. Arya, Endoplasmic Reticulum Stress Activates Unfolded Protein Response Signaling and Mediates Inflammation, Obesity, and Cardiac Dysfunction: Therapeutic and Molecular Approach, *Front Pharmacol* 10 (2019) 977. 10.3389/fphar.2019.00977.
- [3] T. Minamino, I. Komuro, M. Kitakaze, Endoplasmic reticulum stress as a therapeutic target in cardiovascular disease, *Circ Res* 107 (2010) 1071-1082. 10.1161/CIRCRESAHA.110.227819.
- [4] C. Hetz, The unfolded protein response: controlling cell fate decisions under ER stress and beyond, *Nat Rev Mol Cell Biol* 13 (2012) 89-102. 10.1038/nrm3270.
- [5] I. Kim, W. Xu, J.C. Reed, Cell death and endoplasmic reticulum stress: disease relevance and therapeutic opportunities, *Nat Rev Drug Discov* 7 (2008) 1013-1030. 10.1038/nrd2755.
- [6] G. Liu, Y. Sun, Z. Li, T. Song, H. Wang, Y. Zhang, Z. Ge, Apoptosis induced by endoplasmic reticulum stress involved in diabetic kidney disease, *Biochem Biophys Res Commun* 370 (2008) 651-656. 10.1016/j.bbrc.2008.04.031.
- [7] M. Cerezo, S. Rocchi, New anti-cancer molecules targeting HSPA5/BIP to induce endoplasmic reticulum stress, autophagy and apoptosis, *Autophagy* 13 (2017) 216-217. 10.1080/15548627.2016.1246107.
- [8] K.W. Park, G. Eun Kim, R. Morales, F. Moda, I. Moreno-Gonzalez, L. Concha-Marambio, A.S. Lee, C. Hetz, C. Soto, The Endoplasmic Reticulum Chaperone GRP78/BiP Modulates Prion Propagation in vitro and in

vivo, *Sci Rep* 7 (2017) 44723. 10.1038/srep44723.

[9] Y. Xu, X. Wang, Y. Zhang, Myocardial Infarction-Related Transcripts (MIAT) Participate in Diabetic Optic Nerve Injury by Regulating Heart Shock Protein 5 (HSPA5) via Competitively Binding to MicroRNA-379, *Med Sci Monit* 25 (2019) 2096-2103. 10.12659/MSM.911930.

[10] A.B. Enogieru, S.I. Omoruyi, D.C. Hiss, O.E. Ekpo, GRP78/BIP/HSPA5 as a Therapeutic Target in Models of Parkinson's Disease: A Mini Review, *Adv Pharmacol Sci* 2019 (2019) 2706783. 10.1155/2019/2706783.

[11] J. Wang, J. Lee, D. Liem, P. Ping, HSPA5 Gene encoding Hsp70 chaperone BiP in the endoplasmic reticulum, *Gene* 618 (2017) 14-23. 10.1016/j.gene.2017.03.005.

[12] A. Zhuravleva, L.M. Gierasch, Substrate-binding domain conformational dynamics mediate Hsp70 allostery, *Proc Natl Acad Sci U S A* 112 (2015) E2865-2873. 10.1073/pnas.1506692112.

[13] C. Roller, D. Maddalo, The Molecular Chaperone GRP78/BiP in the Development of Chemoresistance: Mechanism and Possible Treatment, *Front Pharmacol* 4 (2013) 10. 10.3389/fphar.2013.00010.

[14] M. Wang, S. Wey, Y. Zhang, R. Te, A. Lee, Role of the Unfolded Protein Response Regulator GRP78/BiP in Development, Cancer, and Neurological Disorders, *Antioxid Redox Signal* 11 (2009) 2307-2316.

[15] H. Yoshida, T. Matsui, A. Yamamoto, T. Okada, K. Mori, XBP1 mRNA Is Induced by ATF6 and Spliced by IRE1 in Response to ER Stress to Produce a Highly Active Transcription Factor., *cell* 107 (2001) 881-891.

[16] E. Sebestyen, B. Singh, B. Minana, A. Pages, F. Mateo, M.A. Pujana, J. Valcarcel, E. Eyras, Large-scale analysis of genome and transcriptome alterations in multiple tumors unveils novel cancer-relevant splicing networks, *Genome Res* 26 (2016) 732-744. 10.1101/gr.199935.115.

[17] M.W. Hentze, A. Castello, T. Schwarzl, T. Preiss, A brave new world of RNA-binding proteins, *Nat Rev Mol Cell Biol* 19 (2018) 327-341. 10.1038/nrm.2017.130.

[18] G. Singh, G. Pratt, G.W. Yeo, M.J. Moore, The Clothes Make the mRNA: Past and Present Trends in mRNP Fashion, *Annu Rev Biochem* 84 (2015) 325-354. 10.1146/annurev-biochem-080111-092106.

[19] T. Glisovic, J.L. Bachorik, J. Yong, G. Dreyfuss, RNA-binding proteins and post-transcriptional gene regulation, *FEBS Lett* 582 (2008) 1977-1986. 10.1016/j.febslet.2008.03.004.

[20] R. Xiao, J.Y. Chen, Z. Liang, D. Luo, G. Chen, Z.J. Lu, Y. Chen, B. Zhou, H. Li, X. Du, Y. Yang, M. San, X. Wei, W. Liu, E. Lecuyer, B.R. Graveley, G.W. Yeo, C.B. Burge, M.Q. Zhang, Y. Zhou, X.D. Fu, Pervasive Chromatin-RNA Binding Protein Interactions Enable RNA-Based Regulation of Transcription, *Cell* 178 (2019) 107-121 e118. 10.1016/j.cell.2019.06.001.

- [21] S. Hong, RNA Binding Protein as an Emerging Therapeutic Target for Cancer Prevention and Treatment, *J Cancer Prev* 22 (2017) 203-210. 10.15430/JCP.2017.22.4.203.
- [22] J. Guo, H. Qu, Y. Chen, J. Xia, The role of RNA-binding protein tristetraprolin in cancer and immunity, *Medical Oncology* 34 (2017). 10.1007/s12032-017-1055-6.
- [23] B. Maziuk, H.I. Ballance, B. Wolozin, Dysregulation of RNA Binding Protein Aggregation in Neurodegenerative Disorders, *Front Mol Neurosci* 10 (2017) 89. 10.3389/fnmol.2017.00089.
- [24] M.D. Diaz-Munoz, M. Turner, Uncovering the Role of RNA-Binding Proteins in Gene Expression in the Immune System, *Front Immunol* 9 (2018) 1094. 10.3389/fimmu.2018.01094.
- [25] Q. Wang, R. Shu, H. He, L.I. Wang, Y. Ma, H. Zhu, Z. Wang, S. Wang, G. Shen, P. Lei, Co-silencing of Birc5 (survivin) and Hspa5 (Grp78) induces apoptosis in hepatoma cells more efficiently than single gene interference, *International Journal of Oncology* 41 (2012) 652-660. 10.3892/ijo.2012.1471.
- [26] D. Kim, G. Pertea, C. Trapnell, H. Pimentel, R. Kelley, S.L. Salzberg, TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions, *Genome Biol* 14 (2013) R36. 10.1186/gb-2013-14-4-r36.
- [27] M.D. Robinson, D.J. McCarthy, G.K. Smyth, edgeR: a Bioconductor package for differential expression analysis of digital gene expression data, *Bioinformatics* 26 (2010) 139-140. 10.1093/bioinformatics/btp616.
- [28] C. Xie, X. Mao, J. Huang, Y. Ding, J. Wu, S. Dong, L. Kong, G. Gao, C.Y. Li, L. Wei, KOBAS 2.0: a web server for annotation and identification of enriched pathways and diseases, *Nucleic Acids Res* 39 (2011) W316-322. 10.1093/nar/gkr483.
- [29] H. Keren, G. Lev-Maor, G. Ast, Alternative splicing and evolution: diversification, exon definition and function, *Nat Rev Genet* 11 (2010) 345-355. 10.1038/nrg2776.
- [30] J. Yang, X.X. Liu, H. Fan, Q. Tang, Z.X. Shou, D.M. Zuo, Z. Zou, M. Xu, Q.Y. Chen, Y. Peng, S.J. Deng, Y.J. Liu, Extracellular Vesicles Derived from Bone Marrow Mesenchymal Stem Cells Protect against Experimental Colitis via Attenuating Colon Inflammation, Oxidative Stress and Apoptosis, *PLoS One* 10 (2015) e0140551. 10.1371/journal.pone.0140551.
- [31] S. Janssens, B. Pulendran, B.N. Lambrecht, Emerging functions of the unfolded protein response in immunity, *Nat Immunol* 15 (2014) 910-919. 10.1038/ni.2991.
- [32] A.S. Lee, Glucose-regulated proteins in cancer: molecular mechanisms and therapeutic potential, *Nat Rev Cancer* 14 (2014) 263-276. 10.1038/nrc3701.
- [33] S.Z. Hasnain, R. Lourie, I. Das, A.C. Chen, M.A. McGuckin, The interplay between endoplasmic reticulum stress and inflammation, *Immunol Cell Biol* 90 (2012) 260-270. 10.1038/icb.2011.112.

- [34] S. Bläß, U. A., J. Raymackers, F. Schumann, U. Ungethüm, S. Müller-Steinbach, K.F. D., E.J. M., G.R. Burmester, The Stress Protein BiP Is Overexpressed and Is a Major B and T cell target in Rheumatoid Arthritis, *Arthritis & Rheumatology* 44 (2001) 761-771.
- [35] L. Booth, J.L. Roberts, D.R. Cash, S. Tavallai, S. Jean, A. Fidanza, T. Cruz-Luna, P. Siembiba, K.A. Cycon, C.N. Cornelissen, P. Dent, GRP78/BiP/HSPA5/Dna K is a universal therapeutic target for human disease, *J Cell Physiol* 230 (2015) 1661-1676. 10.1002/jcp.24919.
- [36] J.L. Roberts, M. Tavallai, A. Nourbakhsh, A. Fidanza, T. Cruz-Luna, E. Smith, P. Siembida, P. Plamondon, K.A. Cycon, C.D. Doern, L. Booth, P. Dent, GRP78/Dna K Is a Target for Nexavar/Stivarga/Votrient in the Treatment of Human Malignancies, Viral Infections and Bacterial Diseases, *J Cell Physiol* 230 (2015) 2552-2578. 10.1002/jcp.25014.
- [37] S.P. Reid, A.C. Shurtleff, J.A. Costantino, S.R. Tritsch, C. Retterer, K.B. Spurgers, S. Bavari, HSPA5 is an essential host factor for Ebola virus infection, *Antiviral Res* 109 (2014) 171-174. 10.1016/j.antiviral.2014.07.004.
- [38] D. Wei, N.L. Li, Y. Zeng, B. Liu, K. Kumthip, T.T. Wang, D. Huo, J.F. Ingels, L. Lu, J. Shang, K. Li, The Molecular Chaperone GRP78 Contributes to Toll-like Receptor 3-mediated Innate Immune Response to Hepatitis C Virus in Hepatocytes, *J Biol Chem* 291 (2016) 12294-12309. 10.1074/jbc.M115.711598.
- [39] Y.B. Ouyang, L.J. Xu, J.F. Emery, A.S. Lee, R.G. Giffard, Overexpressing GRP78 influences Ca²⁺ handling and function of mitochondria in astrocytes after ischemia-like stress, *Mitochondrion* 11 (2011) 279-286. 10.1016/j.mito.2010.10.007.
- [40] C. Koumenis, C. Naczki, M. Koritzinsky, S. Rastani, A. Diehl, N. Sonenberg, A. Koromilas, B.G. Wouters, Regulation of protein synthesis by hypoxia via activation of the endoplasmic reticulum kinase PERK and phosphorylation of the translation initiation factor eIF2 α , *Mol Cell Biol* 22 (2002) 7405-7416. 10.1128/mcb.22.21.7405-7416.2002.
- [41] H. Yamaguchi, H.G. Wang, CHOP is involved in endoplasmic reticulum stress-induced apoptosis by enhancing DR5 expression in human carcinoma cells, *J Biol Chem* 279 (2004) 45495-45502. 10.1074/jbc.M406933200.
- [42] H. Puthalakath, L.A. O'Reilly, P. Gunn, L. Lee, P.N. Kelly, N.D. Huntington, P.D. Hughes, E.M. Michalak, J. McKimm-Breschkin, N. Motoyama, T. Gotoh, S. Akira, P. Bouillet, A. Strasser, ER Stress Triggers Apoptosis by Activating BH3-Only Protein Bim, *Cell* 129 (2007) 1337-1349. 10.1016/j.cell.2007.04.027.
- [43] K.D. McCullough, J.L. Martindale, L.O. Klotz, T.Y. Aw, N.J. Holbrook, Gadd153 sensitizes cells to endoplasmic reticulum stress by down-regulating Bcl2 and perturbing the cellular redox state, *Mol Cell Biol* 21 (2001) 1249-1259. 10.1128/MCB.21.4.1249-1259.2001.

[44] G.D. Pavitt, D. Ron, New insights into translational regulation in the endoplasmic reticulum unfolded protein response, Cold Spring Harb Perspect Biol 4 (2012). 10.1101/cshperspect.a012278.

[45] Y. Dondelinger, S. Jouan-Lanhuet, T. Divert, E. Theatre, J. Bertin, P.J. Gough, P. Giansanti, A.J. Heck, E. Dejardin, P. Vandenabeele, M.J. Bertrand, NF-kappaB-Independent Role of IKKalpha/IKKbeta in Preventing RIPK1 Kinase-Dependent Apoptotic and Necroptotic Cell Death during TNF Signaling, Mol Cell 60 (2015) 63-76. 10.1016/j.molcel.2015.07.032.

[46] N. Silverman, T. Maniatis, NF-kappaB signaling pathways in mammalian and insect innate immunity, Genes Dev 15 (2001) 2321-2342. 10.1101/gad.909001.

[47] C.J. Kearney, S.J. Vervoort, S.J. Hogg, K.M. Ramsbottom, Tumor immune evasion arises through loss of TNF sensitivity, Science Immunology 3 (2018) eaar3451.

[48] S. Naserian, M.E. Abdelgawad, M. Afshar Bakshloo, G. Ha, N. Arouche, J.L. Cohen, B.L. Salomon, G. Uzan, The TNF/TNFR2 signaling pathway is a key regulatory factor in endothelial progenitor cell immunosuppressive effect, Cell Communication and Signaling 18 (2020). 10.1186/s12964-020-00564-3.

Figures

Figure 1

High quality sequencing data were obtained for subsequent analysis. (A) WB (left) and RT-qPCR (right) results of HSPA5 in two groups. (B) FPKM values of HSPA5 expression reflected by RNA-seq data. (C) The heatmap showed the treatment groups and control groups were distinguished obviously. (D) Veen plot of genes numbers detected in the two groups. (E) The violin diagram of gene expression about the two groups. Error bars represent mean $\pm$ SEM. ***p < 0.001.

Figure 2

The identification of DEGs and analysis of functional enrichment analysis. (A) The number of differently expressed genes (DEGs) based on the standard P-value < 0.01 and fold change ≥ 2 or ≤ 0.5 . Up-regulated genes are labeled in red, whereas down-regulated are labeled in blue in the volcano plot. (B) The expression heatmap of all DEGs and reflected change of genes expression. (C) The top 10 representative GO biological processes of up- and down-regulated genes. (D) The top 10 representative KEGG pathways of up- and down-regulated genes. (E) The expression of ADAM8, CSF2, and HSPA1A quantified with RT-qPCR. Error bars represent mean $\pm$ SEM. ***p < 0.001, **p < 0.01, *p < 0.05.

Figure 3

Identification of RASGs and functional enrichment analysis. (A) The bar chart showed the number of HSPA5-regulated RASEs. Up represented the splicing pattern occurred more frequently in treatment groups than control, and vice versa. (B) Veen plot showed the common and unique genes between RASGs and DEGs. (C) The top 10 GO analysis and (D) KEGG analysis of the RASGs.

Figure 4

The alternative splicing events validation of genes involved in inflammation/immunity process by RT-qPCR. (A-B) Validation of HSPA5-regulated alternative splicing events of genes involved in inflammation/immunity process. The schematic diagrams depict the structures of ASEs, AS1 and AS2. The exon sequences are denoted by boxes and intron sequences by the horizontal line (top panel). RNA-seq quantification and RT-qPCR validation of ASEs are shown in the bottom panel. Error bars represent mean $\pm$ SEM. * $p < 0.05$. The altered ratio of AS events in RNA-seq were calculated using the formula: AS1 junction reads / (AS1 junction reads + AS2 junction reads); while the altered ratio of AS events in RT-qPCR were calculated using the formula: AS1 transcripts level / AS2 transcripts level.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementalMaterial1.xlsx](#)
- [SupplementalMaterial2.xlsx](#)
- [SupplementalMaterial3.xlsx](#)