Research article

Identification of critical genes associated with oxidative stress pathways in benzene-induced hematotoxicity

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ABSTRACT

Background and aims: Bone marrow failure (BMF) is chronic benzene-induced hematotoxicity, which is associated with differential gene expression abnormality. Benzene-induced BMF is characterized by irreversible bone marrow depression. Despite extensive studies have been conducted, there is a lack of reliable, useful and simple diagnostic method for BMF. Previous studies have shown that the aberrant gene expression changes and reactive oxygen species production in bone marrow cells related to the development of BMF. Early detection of differentially expressed genes (DEGs) as potential biomarkers is important for diagnosis and treatment. However, the validation of effective biomarker through DEGs analysis in benzene-induced BMF still deserve to be clarified. This study aimed to identify target genes as potential biomarkers with benzene-induced BMF based on DEGs analysis.
Methods: First, we developed a benzene-induced BMF mouse model and obtained the DEGs in bone marrow cells of benzene-exposed CD1 mice. Next, after obtaining the DEGs via RNA-Sequencing (RNA-seq) analysis, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were also used, key genes associated with benzene-induced BMF were identified. Additionally, the key markers for benzene poisoning was evaluated using qRT-PCR technique.
Results: We identified DEGs for further KEGG functional analysis. Ten statistically significantly (up or down) regulated genes, namely Mapk11, Foxo1, Lefty1, Ren1, Bank1, Fgf3, Cdc42ep2, Rasgrf1, P2rx7, and Shank3 were found mainly associated with mitogen-activated protein kinases (MAPK) oxidative stress **pathway.** Further analysis using qRT-PCR identified that eight statistically significant DEGs associated with signaling pathways such as MAPK. We found that the level of mRNA expression of Mapk11, Foxo1, Bank1, Lefty1, Ren1, P2rx7, and Fgf3 genes were increased and Cdc42ep2 gene was decreased in BMF mice compared to control mice. Additionally, we validated the eight candidate genes for potential biomarkers in peripheral blood mononuclear cells of benzene poisoning patients by qRT-PCR.
Conclusion: Our results indicated that Mapk11 and Fgf3 were predominantly candidate genes linked to novel biomarkers for benzene hematotoxicity in human beings. Our study will provide new candidate genes as useful biomarkers involved in benzene-induced hematotoxicity.

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1. Introduction

Benzene is a well-known environmental chemical agent that causes hematological diseases such as bone marrow failure (BMF). Benzene-induced BMF is characterized by the anemia, leukocytopenia, and/or thrombocytopenia [1], which is associated with genes alternation [2]. Commonly, most BMF patients with benzene exposure lack significant and obvious early symptoms until severely anemia, fatigue, increasing infection and bleeding [3–5]. Patients with seriously BMF need to receive expensive immunosuppression drug intervention and hematopoietic stem cell transplantation treatment strategies [6]. The observation of therapeutic effect need to rely on bone marrow puncture procedure and biopsies technique which can be limited owing to the invasive nature [7]. A major gap that currently exists in non-invasive diagnostic technique is the lack of accurate biomarkers to identify benzene-induced BMF in the early stage. Therefore, identification of sensitive and specific early diagnostic biomarkers may overcome these potential complications such as invasive and patient's refusal. Additionally, the studying on alterations of genes related to hematotoxicity of the benzene exposure may give us a clue for prevention of progression of hematopoietic hazards [8]. However, the effective biomarkers in the peripheral blood of benzene poisoning and BMF have not been identified. Therefore, exploring potential biomarkers of benzene poisoning and BMF deserve further study.

Until today, the precise mechanisms of the benzene-induced hematotoxicity in human beings still remain to be elucidated [9]. Benzene, as a volatile organic compound and the main air pollutants in the environment, its hematotoxicity is associated with initiate immune oxidative stress responses trigged by reactive oxygen species (ROS) production [10,11]. Additionally, benzene as environmental pollutant and carcinogen induced aberrant gene alternation has been associated with exposure to benzene [12]. The changes of benzene-induced gene expression were observed after treatment with benzene exposure [13], which suggests that the impact of benzene exposure deserves further exploding. The suppression effect of benzene exposure on hematopoietic cells was associated with abnormal gene expression profile involved in inflammation and DNA damage selected signal pathways [11]. Benzene and its metabolites especially benzoquinone (BQ) induced myelotoxicity is associated with BQ-induced ROS production in HL-60 cells [14,15], which is considered to be important factors in the occurrence of hematotoxicity. Badham, H. J. et al. found that bioactivation pathway of chronic benzene exposure is associated with production of increased ROS maybe play a key role in the benzene induced toxicity [16, 17]. However, the precise oxidative stress pathways involved benzene-induced BMF remain to be determined.

The analysis of differentially expressed genes (DEGs) is an important tool thought to identify potential biomarkers and is a rapid non-invasive diagnosis technique for blood diseases [18,19].Studies have shown that DEGs analysis methods as an important tool was used to gain hug genes of disease [20]. Moreover, Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis were functional analysis method that can help to predict target genes for effective treatment [21]. Based on using quantitative real-time PCR (qRT-PCR) can identify candidate genes expression levels in human peripheral blood [22], the combined application of DEGs, KEGG and (qRT-PCR) to validate potential biomarkers will help to find target genes associated with diagnosis and therapeutic of disease. The gene expression analysis between normal and diseased animal models may help to find effective and specific biomarker in the hematological diseases [23]. Benzene-induced BMF mouse model was an important research tool for understanding the biological progress and molecular pathways in etiology. Here, we established a mouse model of BMF by benzene subcutaneous injection route at a dose of 2 ml/kg 3 days a week for 8 weeks. Based on the DEGs analysis in the bone marrow cells from benzene-induced BMF mouse model, the aim of this study was to investigate the expression level of DEGs responsible for the induction of BMF and to reveal whether ROS-related biology pathways involved in benzene-induced hematotoxicity.

2. Materials and methods

2.1. Establishment and identification of benzene induced BMF mouse model

Male specific pathogen-free CD1 mice aged 6–8 weeks were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd (SCXK2012-0001). CD1 mice were randomly divided into two groups: the control group (Control) and benzene-treated BMF group (BMF) (n = 6/each group). Benzene induced BMF mouse model was carried out according to the previous study [24]. Briefly, as our previous study [25], mice in the BMF group were subcutaneously injected with 2 ml/kg body weight of benzene with a purity of 99.7 % (MERCK, German) dissolved in corn oil, and mice in the control group were treated with the equal dose corn oil (Golden dragon fish, China) once every day, 3 days per week for 8 weeks. The changes of body weight and peripheral blood were observed weekly. All other chemicals with analytical grade or the highest commercial grade available were from Beijing Chemical Works (Beijing, China). Animal handing was conducted in accordance with the general requirements for laboratory animals and approved by the Research Ethics Committee in the Shandong Academy of Occupational Health and Occupational Medicine. The study was approved by the ethics commission (Approval number: W202203070197).

Mice were anesthetized and sacrificed after 8 weeks of benzene-treatment completion. To evaluate BMF mouse model development, the sections of paraffin-embedded femur were cut at 5 mm serially and stained with hematoxylin and eosin (H&E) for morphological analysis. All of the smears were observed by microscopy (Olympus, Japan), The peripheral blood cells in each group of mice were counted by hematology analyzer (Mindray autohematology analyzer, China).

2.2. Collection of bone marrow mononuclear cells and RNA-sequencing analysis

According to the results of the bone marrow smears changes and femur histological changes, 3 benzene-exposed **CD1 mice** and 3

control mice were selected for DEGs profiles analysis. DEGs profiles were investigated using RNA-sequencing (RNA-seq) in bone marrow mononuclear cells (BMMNCs). Bone marrow cells were extracted from the femur with saline buffer and were suspended in 2 ml of red blood cell lysis buffer (Cat. No. R1010, Solarbio Science Technology, Beijing, China). BMMNCs were isolated from bone marrow cells removal of erythrocytes according to our previous study [26]. Total RNA was extracted from BMMNCs in 1 ml of TRIzol reagent (Invitrogen, CA, USA) and stored at $-80\ ^\circ$C. Next, RNA integrity was evaluated using the high-throughput RNA-seq analysis (Agilent 2100 Bioanalyzer RNA-6000 Nano kit coupled to DNA-1000 kit, USA), and further sequencing according to the manufacture's specifications. Samples were analyzed with RNA-seq data sequenced by Illumina (San Diego, USA). DEGs were obtained based on the expression profile datasets in benzene treated BMF mice compared with non-treated benzene control mice. DEGs were identified using the fold change $\geq \pm 1.5$ and adjusted p value ≤ 0.05.

2.3. Identified key pathways associated with DEGs using GO and KEGG enrichment analysis

The enrichment of the RNA-associated binding sites target genes was analyzed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. The function of RNA-associated binding sites target genes was analyzed using the Gene Ontology (GO) enrichment analysis. We used GO combined with KEGG analysis to elucidate the potential genes function annotation and pathways enrichment linked to the common DEGs in the BMMNCs. Subsequently a transcription aligned reads were generated using cufflinks software. Detailed experimental methods were performed in according to the associated previous study [27]. The GO analysis contains molecular function, biological process and cellular component. These analyses allowed us to predict the biological pathway and functional classification in which the enrichment DEGs. Next, we used the online STRING database to investigate gene-regulatory network for the interesting DEGs.

2.4. Collection of mononuclear cells from peripheral blood and RNA extraction

To identify the expression of the genes selected from RNA-seq data, we collected peripheral blood from chronic benzene poisoning patients (n = 10) and healthy controls (n = 10). This study has received approval for the Research Ethics Committee in the Shandong Academy of Occupational Health and Occupational Medicine, Shandong First Medical University (Approval number: R202203040080). After obtaining appropriate informed consent, the study population included 10 patients with chronic benzene poisoning diagnosed at Shandong Provincial Occupational Diseases Hospital. Diagnosis was assigned according to the GBZ68-2013. Excluding criteria involved (A: Age less than 18 or more than 60. B: history of defined organ function abnormality. C: History of cancer or blood disease. D: Patients or families unwilling to participate.) Patients characteristics are summarizes in Table 1. Venous blood (10 ml) samples were collected with ethylenediamine tetra-acetic acid (EDTA) as an anticoagulant. Mononuclear cells from peripheral blood (PBMNC) were collected using Ficoll-Paque (Cat. No. P9011, Solarbio Science Technology, Beijing, China) as a densitu-gradient mediumas following the instructions of the vendors. After that, all samples were preserved in 1 ml of TRIzol reagent (Invitrogen, CA, USA) and stored at $-80\ ^\circ$C for further study.

2.5. Validation potential target genes using quantitative real-time polymerase chain reaction analysis (qRT-PCR)

RNA was isolated from bone marrow cells using an adaptation of the Trizol method (Invitrogen, CA, USA) and RNA quality was assessed using the RNA Kit (Agilent 2100 Bioanalyzer, USA). Related mRNA expression was measured by qRT-PCR. The primers of genes and the internal control GAPDH were designed and synthesized by Oebiotech, China. RNA from bone marrow cells was reverse-transcribed into cDNA with the PrimeScript RT reagent kit with gDNA Eraser (TaKaRa, Dalian, China). qRT-PCR was carried out in a 20 μl reaction volume with SYBR Green PCR Master Mix (Toyobo, Osaka, Japan) on a real-time PCR thermocycler (IQ5 Real-Time PCR cycler, Bio-Rad). PCR samples were activated at 95 °C for 30 s followed 45 cycles that were performed at 95 °C for 10 s, 60 °C for 30 s. The mRNA expression of related genes was calculated by the method of $2^{-\triangle\triangle CT}$. Relative expression was normalized to that of GAPDH. A list of target primer sequence analyzed in presented in Tables 2 and 3.

Table 1
The characteristics of patients for benzene-induced BMF and healthy populations. Mean $\pm$ SD.

	Healthy controls (n = 10)	Chronic benzene poisoning (n = 10)
Age (years)	46.40 ± 8.13	50.30 ± 6.38
Sex (male/female)	4/6	4/6
WBC	5.01 ± 1.11	2.76 ± 0.79[a]
RBC	4.85 ± 0.36	3.13 ± 0.91[a]
PLT	261.40 ± 63.53	95.80 ± 26.92[a]

Peripheral blood cell number of white blood cell (WBC), red blood cell (RBC) and platelet (PLT). Compared to the patients with healthy.

[a] $P < 0.01$.

2.6. Statistical analysis

Statistical analysis was performed by paired *t*-test using GraphPad Prism 5 (San Diego, CA, USA) software for comparison between two groups. Data are presented as means ± SD. Significance differences were taken when $P < 0.05$.

3. Results

3.1. Weight loss and blood cells decreased in mice exposed to benzene

The cumulative loss of body weight is an important symptom in benzene-induced BMF animal models. In the study, we examined the body weight and hematological alternations in CD1 mice between the control and benzene-exposed groups succeed 8 weeks. Apparently, a significant weight loss in the benzene-induced BMF model group (Fig. 1A), and a decrease of white blood cells (WBC), red blood cells (RBC), and platelets (PLT) in peripheral blood (Fig. 1B) compared to the control group. The data suggest that a mouse model of benzene-induced BMF was established.

3.2. Histopathological and peripheral blood parameter evaluation

Histological changes of femur tissues and peripheral blood smears in the mice were examined under microscope. Benzene-induced decreases in blood cells and bone marrow cells using hematoxylin-eosin (HE) staining from the control and BMF group were observed. Bone marrow smears showed a decrease of bone marrow cells in benzene-treated BMF group (Fig. 2B) in comparison with the control group (Fig. 2A). Histological analysis of mice femur showed an increase of fat cells in benzene-treated BMF group (Fig. 2D) in comparison with the control group (Fig. 2C). These histological and blood changes suggest a successful BMF mouse model was developed by 8-week benzene subcutaneous injection, which confirmed previous study of BMF mouse model.

3.3. DEGs in the bone marrow cells

We designed an experimental approach that allowed us to examine the DEGs in response to benzene exposure scheme (Fig. 3A). The first step in determining target genes was to compare differential expressed genes between BMF and control in BMMNCs. To identify target genes involved in benzene-induced BMF mice, 378 DEGs were identified from the bone marrow cells using RNA-seq (Fig. 3B). We randomized our samples in all samples (3 mice from the BMF and the control groups, respectively). As a result, transcriptional expression pattern of the BMF group compared to control groups was distinct (Fig. 3C), suggesting that the datasets were sufficiently robust at the group level to distinguish benzene-induced BMF from the control.

3.4. Enriched gene ontology terms associated with benzene-induced hematotoxicity

We performed Gene Ontology (GO) enrichment analysis on the DEGs and found they are strongly enriched in Biological Process GO terms related to cell adhesion as well as in Cellular Component GO terms related to extracellular region, and Molecular Function GO terms related to TAP binding between control group and benzene-treated BMF group in Fig. 4, whereas the Biological Process related to immune response, and the Cellular Component related to plasma membrane and cell surface were similarly revealed both in control and in benzene-treated BMF two groups.

3.5. KEGG enrichment analysis suggest DEGs mainly involved in MAPK signaling pathway regulated inflammatory response in mice

Cell adhesion and immune response were related to benzene-induced hematotoxicity by GO enrichment analysis. To investigate the different significant pathways associated with benzene-induced hematotoxicity, we also performed KEGG pathways analysis of the DEGs. Pathway enrichment analysis identified the enriched pathways from the downregulated DEGs. The top 20 signaling pathways

Table 2
The qRT-PCR. primers of related genes in mice.

Gene	Forward	Reverse	Product length (bp)
GAPDH	GCAAGGACACTGAGCAAGA	GGATGGAAATTGTGAGGGAG	76
Mapk11	TACCATGACCCTGACGAT	GCTTGAAGCTAAGGACTTCT	118
Foxo1	AATGCCACTGAATGACTAGG	GACAACTGCCCATGATTCACA	111
Lefty1	TCTGAAGTAGCCTCATCCCTAA	GCATACAGTGCAGTGAACAATA	114
Ren1	CAGATGGACAGAAGGAGGAT	TTCGTTCAAAGGTAGCGGT	97
Bank1	ACTGGAAATCGGTCTTTCATTA	TTTGTTGGAACACTTGAGCTAT	112
Fgf3	GGGCTACAATACATATGCTTCC	GACCCTTGCCATTCACCGA	113
Cdc42ep2	ATGGGTGCTGGCTAGTGA	GCTCCTCTGTGGATGTTGTTA	101
Rasgrf1	GATGCCTCTCTGAGATGTAAGT	CATCGTTAAGCCGGATGG	80
P2rx7	TAGCAGAGGTGACGGAGAA	CCTGCAAAGGGAAGGTGTA	93
Shank3	GACTGGCTGGAGAGCATC	CACGAAGTCTTCCTTGGTGA	102

Table 3
The qRT-PCR. primers of related genes in patients for benzene-induced BMF.

| Gene	Forward	Reverse	Product length (bp)
GAPDH	CGGAACTCGCTATCCCTGTT	CAGCTTGTGCTGGTTTCAC	71
Mapk11	CAACATCGTCAAGTGCCAGG	AGCTCACAGTCCTCGTTCAC	150
Foxo1	TTCACCCAGCCCAAACTACC	GAGTCCAGGCGCACAGTTAT	139
Lefty1	CTACAGGTGTCGGTGCAGAG	CGAGGCAAAGCGGACCA	75
Ren1	GGGCTCCTGTACCTTTGGTC	TTCGTTCAAAGGTAGCGGT	146
Bank1	CATACGGGCAGAGTGCAGAT	TCTTCATGGGTTGGCTCCAC	94
Fgf3	GGGGACGACTCTATGCTTCG	CCGTTCACAGACACGTACCA	172
Cdc42ep2	CCACCAAGGTGCCCATCTAT	TGAATGGTGTGGCGGAAGTC	121
Rasgrf1	ATTACACGGAAGACGGCCTG	GCGTTACCTTTGCTTGGTGC	120
P2rx7	GGAGCCAAAGCCGACATTAAA	TGTGAAGTCCATCGCAGGTC	136
Shank3	GTGCTGAAGAATGGTGGTGC	GTGGTAGAGGGGTGTCAAGC	165
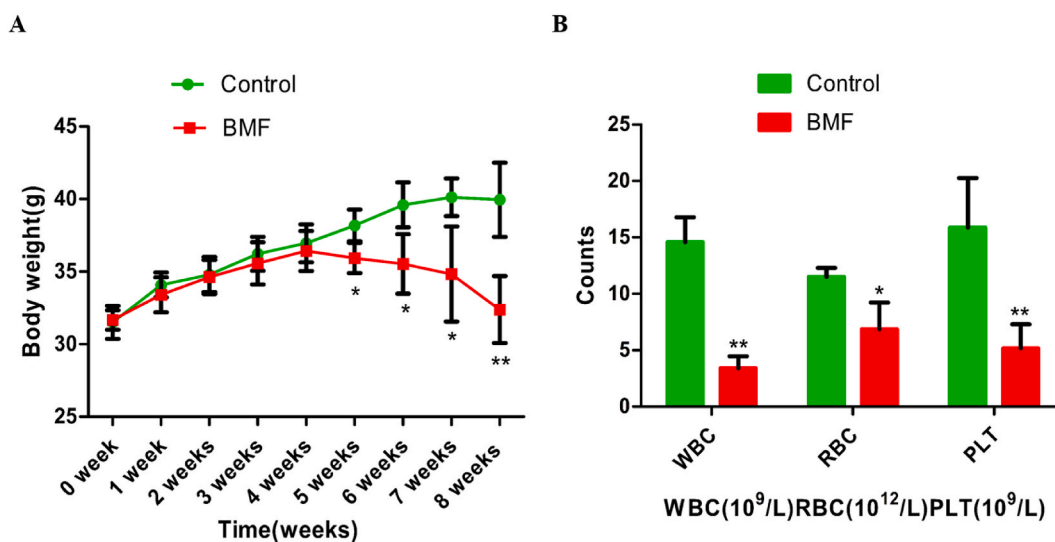
Fig. 1. The changes of the body weight and blood parameters of mice after benzene injection (n = 6). **A.** The weekly body weight of mice exposed to 2 ml/kg benzene. **B.** The counts of blood cells at the end of benzene treatment. *P < 0.05 and **P < 0.01 compared to control group.
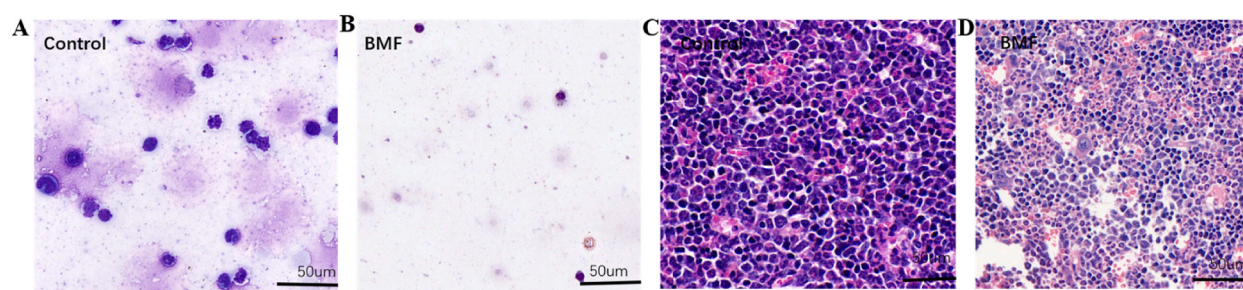

Fig. 2. Representative images of bone marrow smear (A–B) and femur histopathology(C-D) were shown in control group and benzene-treated BMF group (n = 3). Scale Bars = 50μm. HE staining (40 ×).

presented in Fig. 5 show that interesting pathways such as Cell adhesion molecules and Inflammatory response pathways. Related genes such as Mapk11C and Cdc42ep2 were presented in these gene sets. We named these gene sets as "interesting sets". Interesting sets analysis showed that contained abnormal represented genes mainly enriched in the MAPK signaling molecule pathway. These findings suggest that the members in interesting set have strong associations with MAPK signaling pathway to regulate oxidative stress response. Notably, MAPK signaling pathway related genes could probably act as upstream and downstream components of the oxidative stress signaling pathway during benzene induced hematotoxicity. To further investigate gene-regulatory network for the genes in interesting set, we used the STRING database, which provides co-localization, as well as direct (physical) and indirect (functional) associations. The interconnectivity of these potential candidate genes predicated by STRING was showed in Fig. 5.
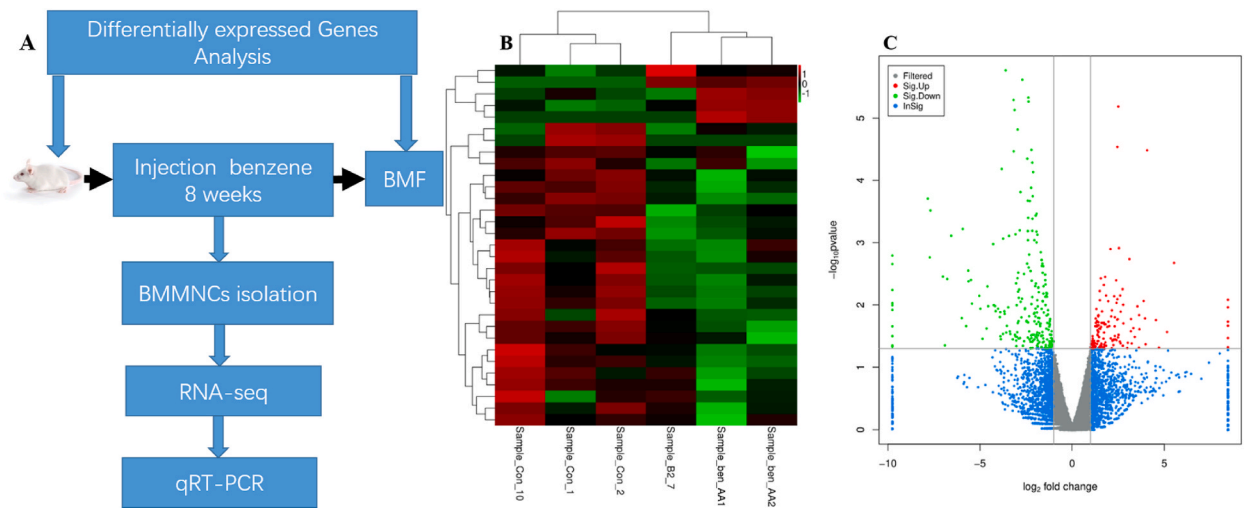
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Fig. 3. The benzene-treated BMF experiment scheme to identify DEGs playing the important role in the hematotoxicity effects of benzene. After the benzene injection 8-weeks duration, 3 mice from BMF group were randomly used. BMMNCs were collected from each individual sample of mice and from pooled internal reference samples (A). Differential expressed genes were determined through group comparisons in edgeR and further characterized through gene enrichment and network analysis. Representative genes were further validated with qRT-PCR. Heat map showed significant differentially expressed genes in BMMNCs of BMF mice model (B). Con1, Con2, Con6 represent control samples and BZ1, BZ2, and BZ6 represent BMF samples. Dendrograms produced by clustering analysis of the samples are present on the top. Heatmap of gene expression across all samples with their 3 independent biological replicates was clustered using the Pearson correlation method. The heat-map showed DEGs identified as potential genes involved in benzene-induced BMF. The color scale from green to red illustrated relative expression levels according to normalized Z-scores: red represents upregulated genes and green represents downregulated genes. The bar graph on the left and top of the heat-map show the hierarchical clustering of the transcripts for gene names and for all different samples (3 benzene-treated and 3 control groups), respectively. Volcano plots between fold change ($\log_2$ Fold Change) of genes vs. significance of change (-$\log_{10}$ P value). Red plots and green plots represent up-regulated ($\log_2$ Fold Change>1 and -$\log_{10}$ P value > 1.3) and down-regulated ($\log_2$ Fold Change < -1 and -$\log_{10}$ P value > 1.3) genes significantly different between BMF and control group. A total of 378 DEGs (139 up- and 239 down-regulated genes) were identified (C). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
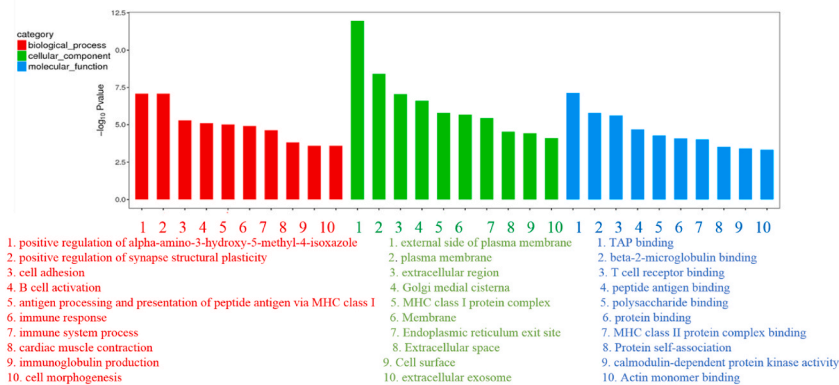

Fig. 4. Gene Ontology enrichment analysis of differentially expressed genes in control group versus benzene-treated BMF group. Red represents Biological Process, green represents Cellular Component, blue represents Molecular Function. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.6. Target genes were validated in human samples as promising biomarkers for benzene-induced BMF

To identify potential biomarkers, we examined the related genes expression associated with MAPK signaling pathway in bone marrow cells in BMF mice using qRT-PCR. We constructed a gene interactions network (Fig. 6A) by transcriptional fold changes of the genes in interesting sets. Among the DEGs, 10 genes were selected candidate genes, including Mapk11, Foxo1, Lefty1, Ren1, Bank1, Fgf3, Cdc42ep2, Rasgrf1, P2rx7, and Shank3 with a Log2 fold change >1.0 (Table 4). The genes may serve as promising biomarkers of benzene-induced BMF. The results showed that there are significant differences in the target genes including Mapk11, Foxo1, Bank1, Lefty1, Ren1, Cdc42ep2, P2rx7, and Fgf3 (Fig. 6B) by qRT-PCR. The level of mRNA expression of Mapk11, Foxo1, Bank1, Lefty1, Ren1,
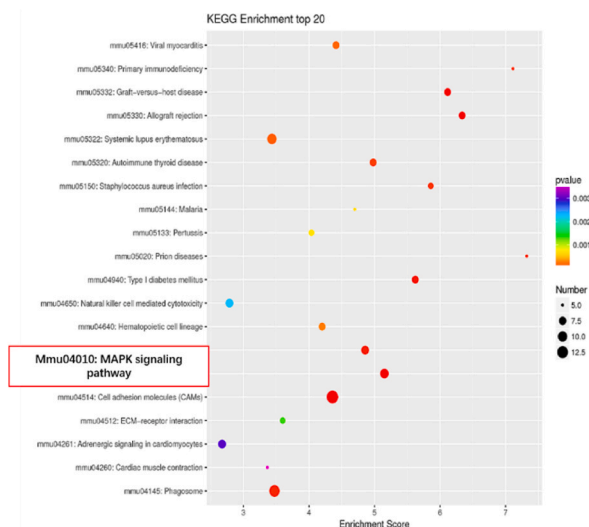
Fig. 5. KEGG pathway enrichment analysis of differentially expressed genes in control group versus benzene-treated BMF group.

P2rx7, and Fgf3 genes were increased and Cdc42ep2 gene was decreased in BMF mice compared to control mice. We further validated the potential biomarkers using qRT-PCR in benzene poisoning patients. The results showed that there are significant differences in the target genes including Mapk11, Fgf3 and Rasgrf1 (Fig. 6C). The genes mRNA expression of the peripheral blood mononuclear cells between the healthy and benzene poisoning patients has a significant different. The level of mRNA expression of Mapk11and Fgf3 genes were increased and Rasgrf1 gene was decreased in benzene poisoning patients compared to healthy.

4. Discussion

Benzene-induced hematotoxicity is causatively associated with oxidative stress-activated signaling pathway in bone marrow cells [28]. In human, chronic exposure to benzene may lead to the onset of irreversible bone marrow depression, which is characterized by a decrease in all three cell types (WBC, RBC and PLT) in peripheral blood that is ultimately termed BMF [1]. Using mouse models mimicking the human conditions of benzene-induced BMF development, we assessed differentially expressed genes (DEGs) changes with focus on oxidative stress response aiming to identify the potential biomarkers for benzene-induced BMF. The mice display benzene-induced the decrease in peripheral blood cells and bone marrow depression which is characterized by pancytopenia in BMF mouse model. We calculated the relative DEGs level by using BMF mice group compared with control mice group without benzene exposure. By performing RNA-sequencing (RNA-Seq) and statistical evaluation, we narrowed over 378 promising candidate DEGs to 10 genes as potential diagnosis biomarkers. The target genes associated with benzene-induced hematotoxicity were gathered from previous studies based on oxidative stress-activated signaling pathways such as NF-KappaB, MAPK in peripheral blood mononuclear cells [28,29]. Studies have also reported that elevated levels of ROS in bone marrow cells are tied to abnormal gene expression [30]. The aim of the current study was to verify potential target genes by RNA-Seq for different benzene-induced hematotoxicity.

A whole transcriptome approach was used to identify DEGs in bone marrow cells of BMF mice. A total of DEGs were identified by RNA-seq and 378 DEGs (139 up- and 239 down-regulated genes) were identified and enriched in a number of associated benzene induced BMF pathways and GO terms. Stress-activated signaling pathway especially mitogen-activated protein kinases (MAPK) plays a critical role in benzoquinone-induced ROS response in HL-60 cells [14]. Differential expression of the selected 10 genes was confirmed by qRT-PCR analysis of BMF mouse models further step. We next examined whether related genes expression through the regulation transcription factors associated with selected signaling pathways. We found that compared to control, there are 8 genes (Mapk11, Foxo1, Bank1, Lefty1, Ren1, Cdc42ep2, P2rx7, and Fgf3) were abnormally expressed in bone marrow cells from BMF mice. The mRNA level of Mapk11, Foxo1, Bank1, Lefty1, Ren1, P2rx7 and Fgf3 genes were found to demonstrate a significant increase in BMF group mice in compared with control mice. Previous study showed that the oxidative stress can induced protective response through activation of Mapk11 in HeLa cells [31], and increased ROS production appear to be tied to benzene-induced BMF development and an activation of MAPK signal transduction pathway involved in cell growth [5,14,28]. Activation of MAPK signaling pathway is involved in cellular redox stress response to ROS generation [32]. Studies have also reported that elevated levels of Foxo1 and P2rx7 were observed in vivo with inflammatory and oxidative stress-mediated diseases [33–35]. P2rx7 as a major driver of NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome activation [36] is connected to the pains in patients [37]. In light of these findings, we support increased Mapk11, Foxo1, and P2rx7 expression in bone marrow cells is associated with inflammatory signaling pathway in response to benzene exposure, and suggest that these genes might inhibit anti-infection ability after benzene exposure and promote abnormal bone marrow dysfunction development.

In addition, previously studies have proved the mechanism of benzene hematotoxicity is associated with the immunotoxicity [38]. Recent study showed MAPK11 maybe as target gene was substantially involved in infection patients [39], and Foxo1 abnormal
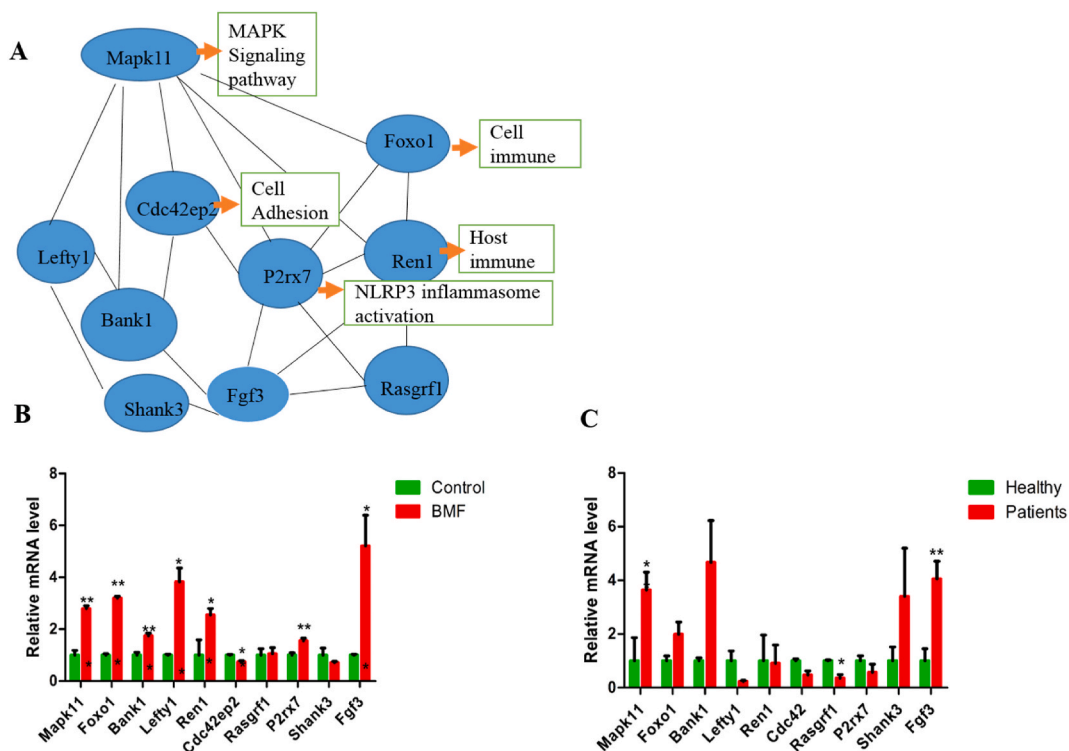
Fig. 6. Correlation gene-regulatory network involved inflammatory signaling pathway. Correlation DEGs network and signaling pathways for negative and positive regulator of MAPK signaling pathway (see Table 4) for benzene-induced BMF mice (A). The differential expression of target genes associated with benzene-induced BMF in mice were confirmed by qRT-PCR in different groups (B). The differential expression of target genes were confirmed by qRT-PCR between in healthy and benzene poisoning patients(C). *$P < 0.05$ and **$P < 0.01$ compared to control group.

Table 4
Differentially expressed genes significantly changed in BMF mice.

Gene name	Genomic result	Pathway	Log2 fold change (Control vs. BMF)
Mapk11	Down-regulated	MAPK signaling pathway	−1.99
Foxo1	Down-regulated	MAPK signaling pathway	−1.47
Lefty1	Down-regulated	MAPK signaling pathway	−1.93
Ren1	Down-regulated	MAPK signaling pathway	−4.28
Bank1	Down-regulated	MAPK signaling pathway	−1.87
Fgf3	Down-regulated	MAPK signaling pathway	−5.43
Cdc42ep2	Down-regulated	Rho protein signal transduction	−1.93
Rasgrf1	Up-regulated	MAPK signaling pathway	2.60
P2rx7	Up-regulated	MAPK signaling pathway	1.74
Shank3	Up-regulated	MAPK signaling pathway	1.39

expression is linked to T-cell immune function in lymphoma patients [40], and Ren1gene differential expression is revealed to host complex in overall population [41]. Cdc42ep2 is a member of the CDC42 subfamily that belongs to Rho family and **plays** an important role in aberrant activation of cellular proliferation processes [42,43], it was down-regulated in the human periodontal ligament cells which may be related to Rho-mediated cell differentiation [44,45]. Our study **supports** that increased Foxo1 and Ren1 genes expression may active immune signaling pathways, and decreased Cdc42ep2 gene expression may inhibit proliferative signaling pathways in BMMNCs in the development of benzene-induced BMF.

Peripheral blood is an accessible biological sample and could be detected for early diagnosis. We identify the candidate genes mRNA expression of the peripheral blood mononuclear cells between the healthy and benzene poisoning patients for potential biomarkers. Our results demonstrate that increased Mapk11 and Fgf3 gene mRNA expression may be associated with benzene hematotoxicity. In addition, we observed that decreased Rasgrf1 expression was associated with an increased risk of benzene hematotoxicity. Rasgrf1 have been shown to modulate activation of the Ras signaling pathway [46], which may also be relevant for activation of inflammation and oxidative stress by regulation of the proteins of the Ras family [34]. We found the benzene can induce a suppression of Rasgrf1 gene expression, which suggest that Rasgrf1 has a role in benzene hematotoxicity. In light of genes validation of animal and human, we provide new potential target genes such as Mapk11, Foxo1, and Cdc42ep2 for benzene-induced hematotoxicity

diagnosis, the genes involved inflammatory signaling pathway and cellular proliferative signaling.

5. Conclusions

Conclusively, our study demonstrated that new candidate genes such as Mapk11, Foxo1, and Cdc42ep2 might be useful biomarkers for providing potential diagnosis method and therapeutic strategies of benzene-induced hematotoxicity. Our data suggest that further study is needed to confirm these potential biomarkers.

Availability of data and materials statement

The information for genomics including the raw data files etc. has been deposited into NCBI BioSample (https://www.ncbi.nlm.nih.gov/sra/PRJNA1135369) under accession number PRJNA1135369.

CRediT authorship contribution statement

Jin He: Writing – original draft, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Cheng Peng:** Writing – review & editing, Conceptualization. **XiaoHan Yang:** Software, Project administration, Investigation. **Peng Li:** Resources, Project administration, Investigation. **Jin Bai:** Software, Resources, Investigation. **Qiang Jia:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Cunxiang Bo:** Writing – review & editing, Project administration, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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