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Original Article

Gossypol induces apoptosis of multiple myeloma cells through the JUN-JNK pathway

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Abstract: Multiple myeloma (MM) is one of the most common hematologic neoplastic diseases. Gossypol was once used as a male contraceptive but is considered a novel antitumor agent. This study aimed to reveal the gossypol-induced apoptosis mechanism and its hub genes. Gossypol-induced MM cell apoptosis is concentration- and time-dependent. Of a total of 532 differentially expressed genes, 273 genes were upregulated and 259 genes were downregulated in gossypol-treated MM cells. Through KEGG and WGCNA analyses, the apoptosis-associated module was identified, and *JUN* was identified as the hub gene. The expression of the *JUN* protein product c-Jun was downregulated in MM cell lines compared to that in normal plasma cells. High-risk MM patients had a lower expression of *JUN*. High-expression *JUN* group patients had a lower risk of death. *JUN* overexpression in MM cells induced potent cell death and growth inhibition by a caspase-dependent apoptotic mechanism. *DR5* is one of the upstream receptors of the JNK pathway, and shRNA knockdown of *DR5* can partially reverse gossypol-induced apoptosis. A total of 1017 genes were coexpressed with *JUN* in MM patients. These genes are mainly involved in other JNK-associated signaling pathways, such as the *IL6*, *EGF* and *PDGF* signaling pathways. In conclusion, *JUN* is identified as the hub gene in gossypol-induced apoptosis, and gossypol can activate caspase-dependent apoptosis through the JNK pathway by targeting c-Jun and other JNK-associated pathways. *DR5* and *IL6* are also involved in this mechanism.

Keywords: Multiple myeloma, gossypol, apoptosis, JNK, JUN

Introduction

Multiple myeloma (MM) is one of the most common hematological neoplastic diseases, comprising 1% of all cancers and 10% of hematological malignancies [1]. In the United States, over 30,000 new cases occur each year, and more than 12,000 people die from the disease [2]. With the development of treatments, the survival of patients with multiple myeloma has improved significantly over the years [3]. However, most patients will relapse with refractory disease, develop resistance and eventually die [4]. The most advanced treatment for MM is multi-drug myeloablative chemotherapy, followed by autologous stem cell transplantation (ASCT) [5, 6]. However, not all MM patients are suitable for ASCT, and drug therapy remains the main treatment for multiple myeloma [7-10]. Therefore, it is of great significance to find more effective drugs to be used in MM therapy. Natural plant extracts, especially natural polyphenols, have shown potential as anticancer drugs [11, 12].

Gossypol is a polyphenolic compound extracted from cotton [13] and was originally used as a male contraceptive [14]. The research on gossypol has declined due to some side effects [15]. However, in recent years, gossypol has generated interest because it can inhibit many types of cancers [16-19]. People have found that gossypol-induced apoptosis is activated by an increased *Bax/Bcl-2* ratio, mitochondrial

transmembrane potential depolarization, and activation of the caspase cascade [20, 21]. Interleukin (IL)-6 plays a critical role in the proliferation of MM cells [22]. Gossypol can inhibit the phosphorylation of *JAK2* and the *IL-6* signaling receptor, *gp130*, as well as the downstream signaling effectors *STAT3*, *p38MAPK* and *ERK1/2* [23], which are major cell survival-associated signaling pathways regulated by *IL-6* [24].

However, the effect of gossypol in MM treatment is still unclear, and the existing studies have not been sufficient to elucidate its mechanism. In this study, we aimed to further reveal the critical genes and pathways of gossypol-induced apoptosis. We found that *JUN* is the hub gene in gossypol-induced apoptosis, whose protein product, c-Jun, is one of the main downstream targets of the *JNK* pathway. Gossypol activates the caspase cascade through c-Jun, causing MM cell apoptosis. In addition, death receptor 5 (*DR5*) can activate the JNK pathway [25], and knockdown *DR5* can partially reverse gossypol-induced apoptosis.

Materials and methods

Gossypol

Gossypol (CatLog# G8761) was purchased from Sigma, USA; RPMI1640 medium, fetal bovine serum, penicillin, and streptomycin were purchased from Gibco, USA.

Public data source

Gene expression data from multiple myeloma cell lines were download from The Cancer Cell Line Encyclopedia (CCLE). Survival data were from the University of Arkansas for Medical Sciences-TT2 (UAMS-TT2), UAMS-TT3.

Cell culture

The multiple myeloma cell lines used in this study included U266, MM1-144, OPM2, ARP-1, OCI-MY5, CAG, H929, KMS11, and ARK. These myeloma cell lines were cultured in flasks. Culture conditions: Cells in RPMI1640 liquid medium with 10% fetal bovine serum (FBS) were cultured in a constant temperature incubator at 37°C with 5% CO_2. The cells were passaged to reach the logarithmic growth phase for experiments.

Concentration effect of gossypol on cell inhibition

The myeloma cell lines were seeded in 96-well plates at a density of 1000 cells per well. After 12 hours, gossypol was added: 1) the negative control group was the serine group; 2) in the gossypol drug treatment group, the final gossypol concentrations in the wells were 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 μM, with five replicate wells in each group. Cell survival was tested using a CellTiter-Fluor™ Cell Viability Assay (Promega G6080). The CellTiter-Fluor™ Reagent was added to wells, and viability was measured after incubation for 30 minutes at 37°C. Caspase-Glo® 3/7 Reagent was added, and a microplate reader was used to measure luminescence at 490 nm. Based on the cell survival results, the cell survival curves were plotted, and the IC_{50} value and 95% confidence interval were calculated.

Time effect of gossypol on cell inhibition

Logarithmic-stage myeloma cells were seeded in 96-well plates at a density of 1000 cells per well. After 24 hours of culture, the drug was added to the appropriate groups. The drug group received gossypol at a 5 μM concentration and were treated for the following time lengths: 0, 24, 48, 72, 96, 120 h. Three replicate experiments were set up for each group. After drug treatment, a CellTiter-Fluor™ Cell Viability Assay (Promega G6080) was used to test cell survival. The time-dependent effect of gossypol at 5 μM on cell growth inhibition was determined [26].

RNA isolation, amplification and microarray hybridization

RNA isolation and amplification were performed as described in previously published articles. Briefly, RNA was isolated from drug-treated cells using an RNeasy Mini Kit (Qiagen, Hilden, Germany) and was reverse transcribed into cDNA. According to Affymetrix's protocols, half of the cDNA was used as a template for bacteriophage T7 RNA polymerase to synthesize biotinylated antisense RNA for hybridization to Affymetrix Human Genome U133A oligonucleotide microarrays (Affymetrix, Santa Clara, CA, USA) [27, 28].

Identification of gossypol-induced differential expressed genes

Genes whose fold changes (FC) >2 between gossypol-treated and untreated cell lines, and these changes occurred in at least 4 cell lines (out of 5 total cell lines) were defined as differentially expressed genes (DEG).

Function enrichment analysis

Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were implemented for function enrichment analysis. GO terms were divided into "cellular component", "biological process" and "molecular function". GO and KEGG terms whose adjusted *p*-values <0.05 were considered significant. These terms were visualized by the "GOplot" package. Ingenuity Pathway Analysis (IPA) was also used to analyze the signaling pathways.

WGCNA

Weighted correlation network analysis (WGCNA) was implemented to analyze the coexpression network. The 532 apoptosis-associated genes in the CCLE dataset were used. The expression values were transformed by log2 (n+1), where n is the expression value of the genes. The Pearson product-moment correlation coefficient between each gene was calculated. R-square was set to 0.85. The genes were clustered into several modules. Gray color means that the genes were not clustered into any modules. The correlation between modules was also calculated. A topological overlap matrix (TOM) was calculated, and a TOM plot was constructed for visualization.

Survival analysis

The median of *JUN* expression values was the cutoff value to divide MM patients (TT2 or TT3 project) into high- and low-expression groups. As previously reported [29], patients in the TT2 and TT3 projects were divided into high- and low-risk groups. A Kaplan-Meier curve was used to analyze patients' survival.

Western blotting

Cells were treated using lysis buffer with protease inhibitor and PMSF on ice. Loading buffer was then added, and samples were boiled at 95°C for 30 minutes. Then, 20 μl of cell lysate was separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a PVDF membrane. Membranes were incubated overnight at 4°C with primary anti-c-Jun, STAT1, AIF, Caspase 3, Caspase 7, Caspase 8, Caspase 9, PARP or β-ACTIN antibody (Cell Signaling Technology, Inc., Massachusetts, US). After overnight culture, blots were washed with 0.5% PBST and then incubated with an HRP-conjugated secondary antibody for 1 h at room temperature. The signal was visualized using an ECL detection reagent. β-Actin was used as a loading control.

Results

Effect of gossypol anti-multiple myeloma

To test gossypol's anticancer effect on MM cell lines, a total of 9 different multiple myeloma cell lines (U266, ARK, MM1, H929, OCI-MY5, OPM2, ARP-1, KMS11, CAG) were tested. We found that the proportions of living cells of all MM cell lines decreased rapidly as the concentration of gossypol increased, indicating that gossypol has a concentration-dependent anticancer effect. All MM cell lines have an IC_{50} of less than 6 μM. When the concentration of gossypol was greater than 6 μM, the effect of increasing the concentration on cell death becomes smaller, and the curve becomes relatively flat (**Figure 1B**). We selected a gossypol concentration of 5 μM to test the time-dependent effect on MM cell survival. When the gossypol concentration was constant at 5 μM, the number of viable cells gradually decreased with time (**Figure 1C**). After 3 days, the proliferation rates of gossypol-treated MM cell lines were obviously lower than those of untreated MM cell lines. Furthermore, the inhibitory effect of gossypol on fast-proliferating MM cells, such as ARP-1 and OCI-MY5 cells, was stronger than the effect on slow-proliferating MM cells, such as OPM2 and KMS11 (**Figure 1D**). One of the most important clinical manifestations of MM is bone destruction. MM can break the osteoblast-osteoclast balance, leading to bone destruction and severe bone pain in patients [30]. Therefore, we also use gossypol to treat osteoclasts, and then found that gossypol can also inhibit the differentiation and growth of osteoclasts, with the increase of the concentration, the inhibition is enhanced (**Figure 1E**). This

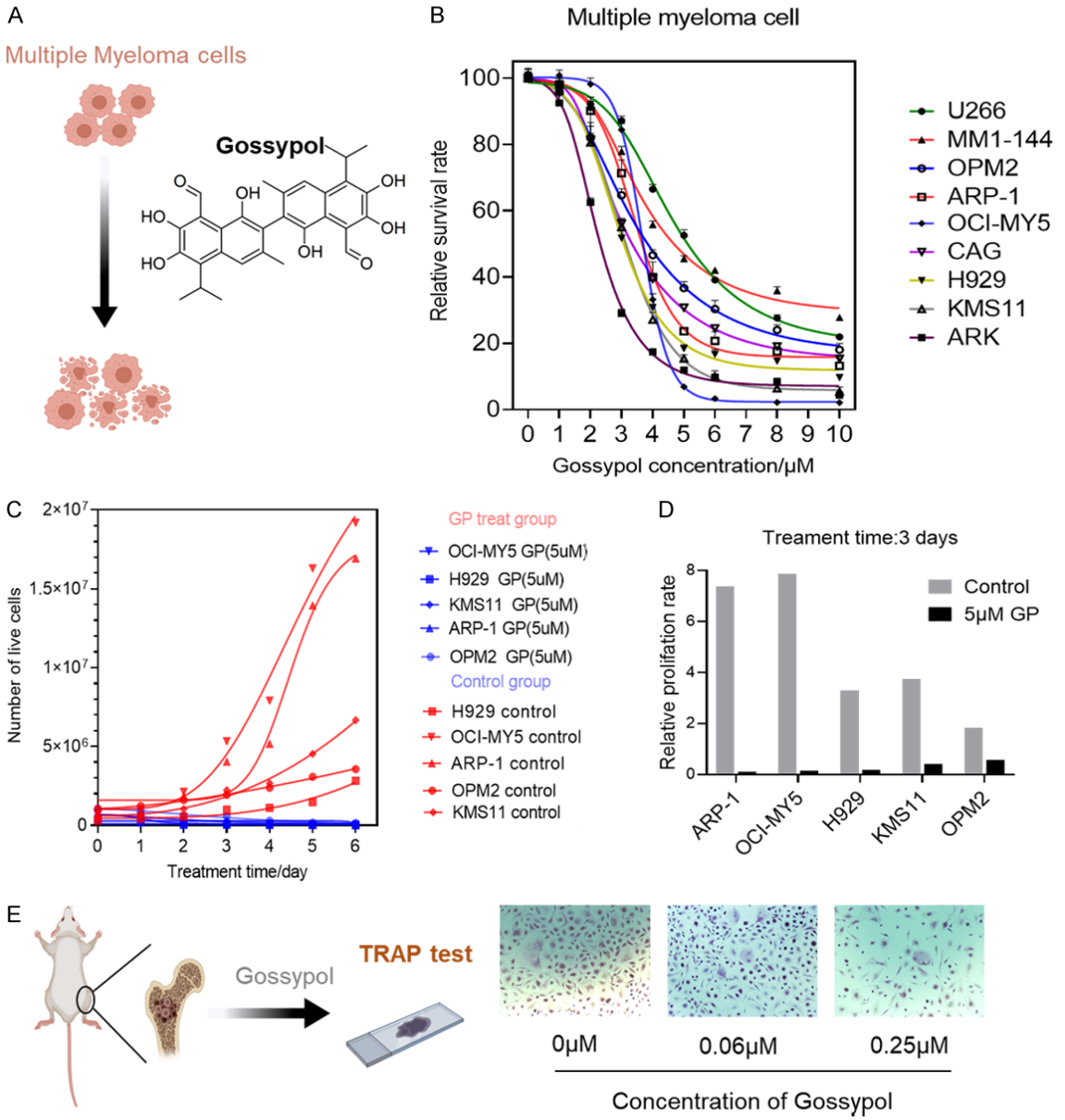

Figure 1. Gossypol can inhibit multiple myeloma. (A) flowchart of gossypol treatment to MM cells. (B) Gossypol-induced MM cell death is concentration-dependent (C) Gossypol-induced MM cell death is time-dependent (D) Gossypol has a better inhibitory effect on faster proliferating MM cells. (E) Trap test showed that gossypol can inhibit osteoclast cell differentiation.

shows that the anti-MM effect of gossypol is not only by promoting MM cell apoptosis, but also by inhibiting osteoclasts.

Identification of gossypol-induced differentially expressed genes (DEGs)

To identify the mechanism of gossypol-induced inhibition, we used a gene chip to test the transcriptome change. According to our selection criteria, a total of 532 genes regulated by gossypol were defined as differentially expressed genes (DEGs) (**Figure 2A**). In total, 273 genes were upregulated, and 259 were downregulated. To fully show the relationship between these genes and MM cell chromosomes and their changes in different cell lines, we use circos plot to display these genes. It shows that these genes are widely distributed on all human chromosomes. And heatmaps inside the circos plot shows these genes' expression change in five MM cell lines are similar (**Figure 2B**). From

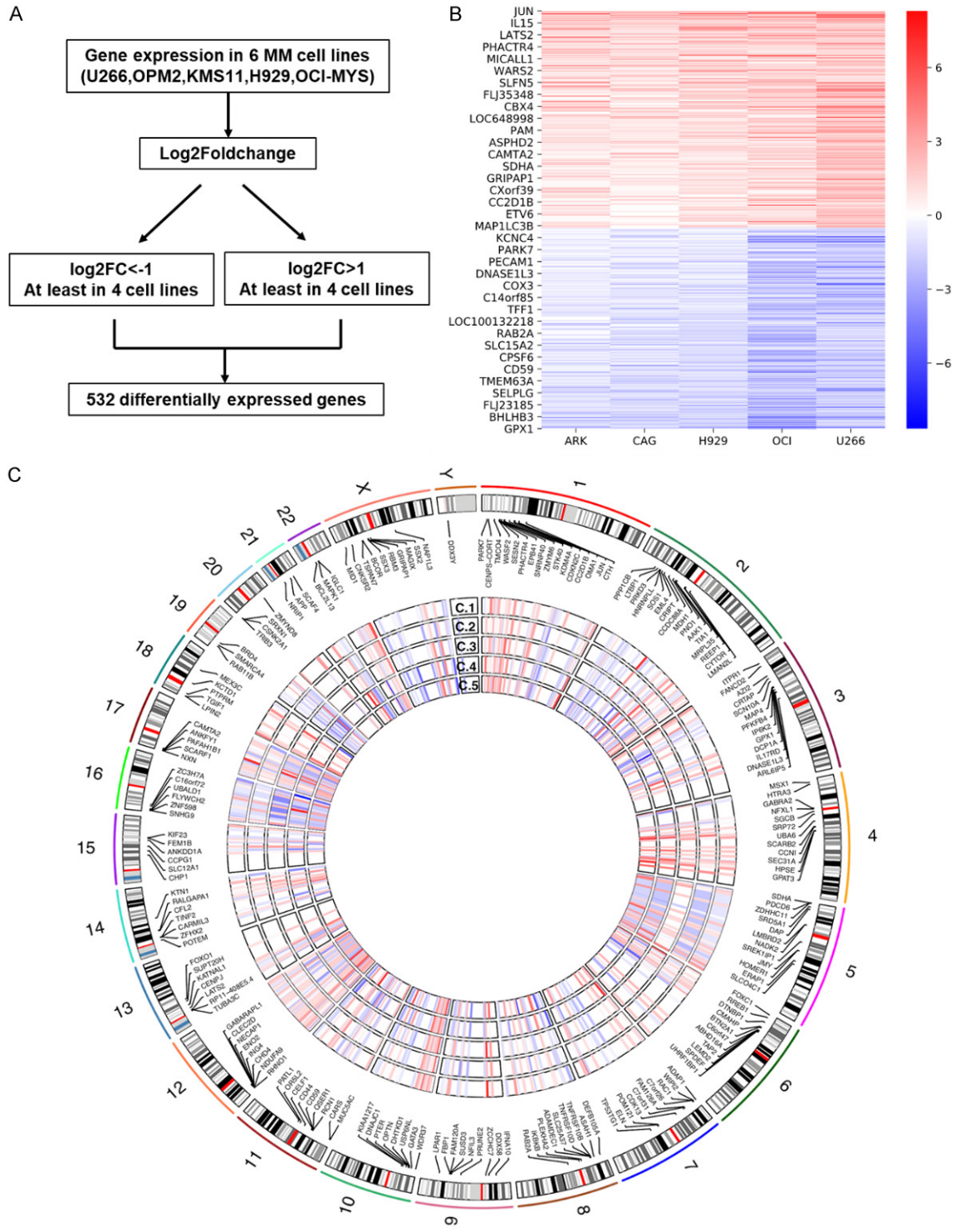

Figure 2. Identification of 532 gossypol-induced differentially expressed genes. A. Schematic diagram of selecting significantly regulated genes. genes whose expression fold changes >2 after gossypol treated and occur in at least 4 cell lines (total 5 cell lines) are selected; B. Heatmap of 532 differentially expressed genes in 5 cell lines. Bar plot on the right of heatmap means the fold change of genes expression before and after gossypol treated. C. Circos 2D track plot of gossypol-induced apoptosis-associated genes. It was generated by RCircos package on R. The outermost loop represents the distribution of the genes on the chromosome band. The inner five circular heatmaps of representing the expression of genes on five cell lines: C.1: ARK; C.2: CAG; C.3: H929; C.4: OCI; C.5: U266.

the circos plot, we found that DEGs were the most abundant on chromosome 1, including many apoptosis-associated genes, such as *AKT3*, *JUN*, *PSEN2*, and *CTSS*.

Function enrichment analyses of gossypol-induced DEGs

To obtain the functions of the above genes and their involved signaling pathways, these DEGs were then chosen for GO and KEGG analyses. A gene may have multiple functions and participate in multiple signaling pathways. Genes involved in multiple pathways are often critical, so we use chord plots to show GO and KEGG analyses results. In the cellular component GO terms, two significant GO terms were "cell leading edge" and "adherents' junction" which are related to cellular movement functions (**Figure 3A**). In the biological process terms, "endomembrane system organization" and "regulation of cysteine-type endopeptidase activity involved in the apoptotic process" were the most enriched (**Figure 3B**). In the molecular function GO terms, "DNA binding"-associated GO terms were the most enriched, such as "proximal promoter sequence-specific DNA binding" and "RNA polymerase II proximal promoter sequence-specific DNA binding" (**Figure 3C**). In the KEGG analysis results, there were five significant pathways: "Apoptosis" was the most enriched pathway, and the remaining four pathways were "Osteoclast differentiation", "B cell receptor signaling pathway", "PDL1 expression and PD1 checkpoint pathway in cancer" and "AGE-RAGE signaling pathway in diabetic complications". In the KEGG chord plot we found that *JUN* is involved with above 5 significant KEGG pathways. Thus, *JUN* may be a very critical gene (**Figure 3D**).

Identification of the apoptosis-associated module and hub genes in gossypol-induced DEGs

To identify the hub genes related to apoptosis in 532 DEGs, we first found the gene modules related to apoptosis. The cluster map of the gene correlation of the 532 DEGs shows that these genes could be clustered into two main modules (Left module and Right module) (**Figure 4A**). The Left module has a greater intra-module correlation than the Right module. To further identify the apoptosis-associated module in the gossypol-induced differentially expressed genes, we utilized RNA-Seq data from the CCLE MM cell lines to perform WGCNA analysis (**Figure 4B-D**). We identified 3 modules: blue, turquoise, and brown modules; gray color means the genes were not clustered into any module (**Figure 4B**). The blue and brown modules are more related and can be classified as one large module (**Figure 4C**). The cluster maps in **Figure 4A** and **4D** are the same. We can learn from this that most genes in the Left module are from the turquoise module (**Figure 4A**, **4D**). Each module, including the gray module, was used to perform KEGG analysis. Only the turquoise module had significant pathways, and the most significant and enriched pathway was apoptosis (**Figure 4E**). Thus, we identified the turquoise module as the apoptosis-associated module in gossypol-induced DEGs. We found that *JUN* was on the turquoise gene list, and *JUN* is one of the genes enriched in the apoptosis signaling pathway. And as shown in previous KEGG results, *JUN* was involved in all pathways, including apoptosis. These results indicated that *JUN* is very likely to play a critical role in gossypol-induced multiple myeloma cell apoptosis.

JUN induces cell apoptosis of multiple myeloma

In the MM cell lines, *JUN* is expressed at low levels compared to those in normal plasma cells, monoclonal gammopathy of undetermined significance (MGUS) cells, and MM tissue (**Figure 5A**). Patients in the UAMS-TT2 and UAMS-TT3 projects were divided into high- and low-risk groups; high-risk group patients have a significantly lower *JUN* expression level (**Figure 5B**, **5C**), and low *JUN* expression group patients have a significantly higher survival risk (**Figure 5D**, **5E**), which indicates that JUN may inhibit MM development.

To further test the function of *JUN*, we overexpressed *JUN* in MM cell lines by transfection (**Figure 6A**). MM cell proliferation of *JUN*-overexpressing cell lines was significantly inhibited compared to wild-type and empty vector-transfected cell lines (**Figure 6B**), and cell viability was also significantly reduced in *JUN*-overexpressing cell lines compared to wild-type and empty vector-transfected cell lines (**Figure 6C**). In *JUN*-overexpressing MM cell lines, the expression levels of apoptosis-associated pro-

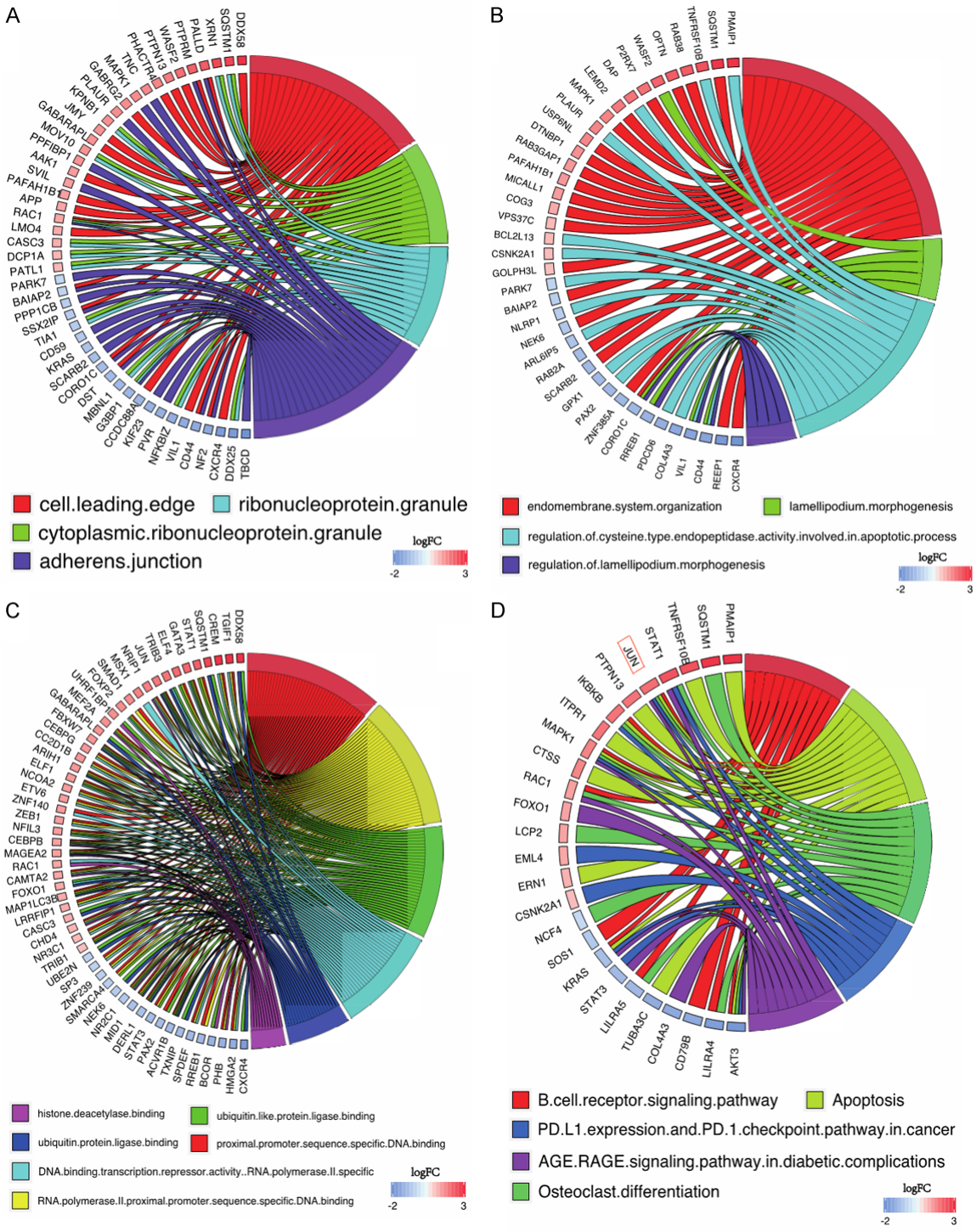

Figure 3. Chord diagram of function enrichment analyses of 532 gossypol-induced differentially expressed genes. A. Chord diagram of GO analyses results of 5 cellular components GO terms. B. Chord diagram of GO analyses results of biological process GO terms. C. Chord diagram of GO analyses results of molecular function GO terms. D. Chord diagram of KEGG analyses results. Only significant GO terms or KEGG pathways are showed.

teins, such as Caspase 3, Caspase 7, Caspase 8, Caspase 9, and PARP, were increased (**Figure 6D**). When the Caspase inhibitor Z-VAD-FMK was added to the *JUN*-overexpressing cell lines, the cell proliferation capacity (**Figure 6E**) and viability (**Figure 6F**) were significantly increased.

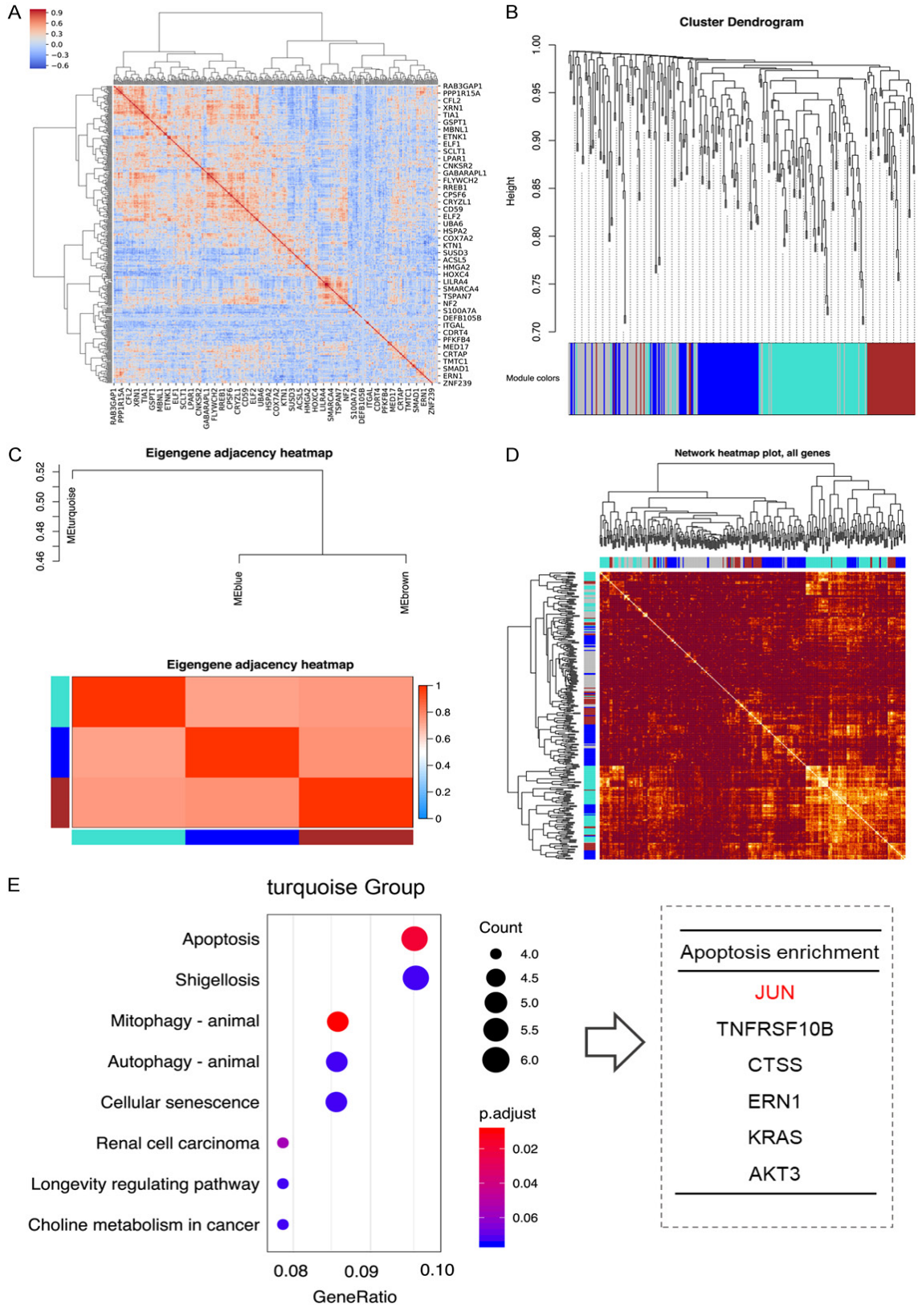

Figure 4. Identification of apoptosis-associated module and hub genes. A. Map of unsupervised clustering of 532 gossypol-induced differentially expressed genes. B. Dendrogram of all 532 gossypol-induced differentially expressed genes clustered based on a dissimilarity measure. C. Clustering and heatmap of modules eigengenes.

D. The topological overlap matrix plot of 532 gossypol-induced differentially expressed genes. E. KEGG results dot plots of the turquoise module. Apoptosis is the most enrichment signaling pathway. Genes enriched in the apoptosis signaling pathway are listed in the right.

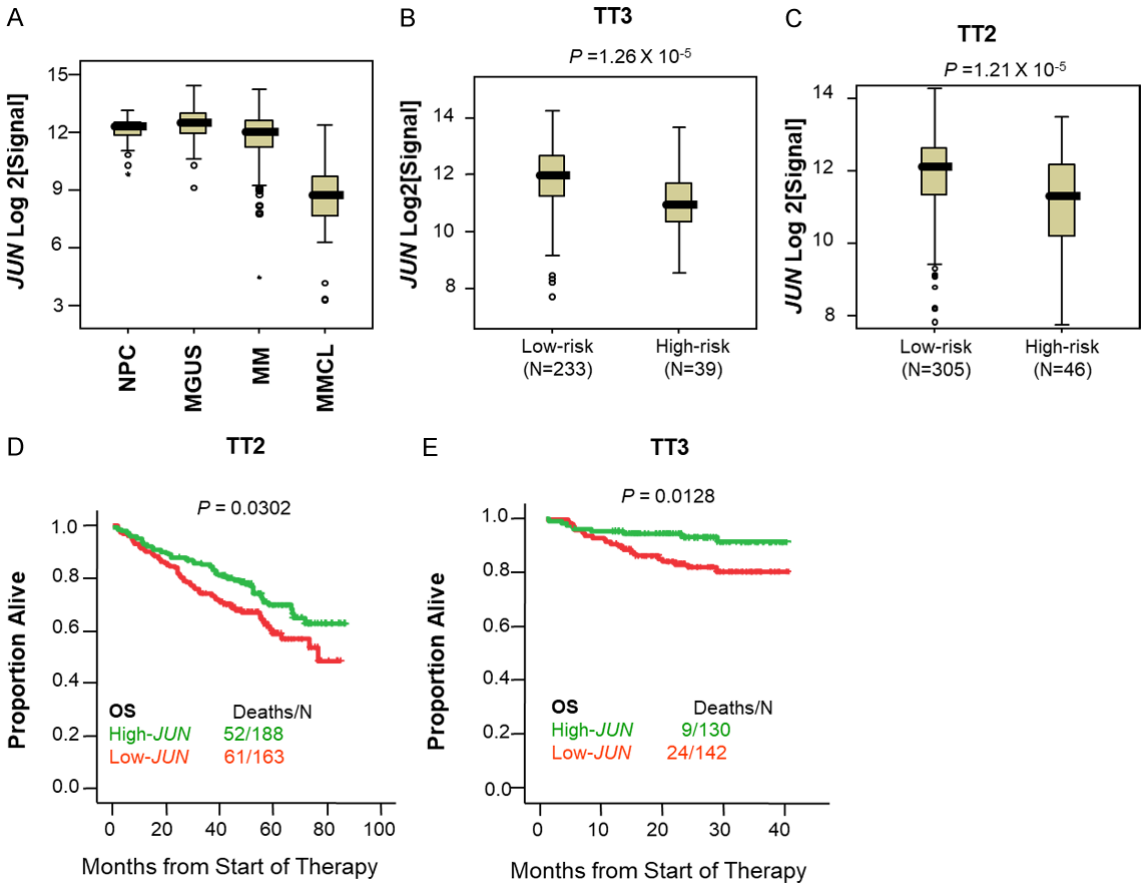

Figure 5. *Jun* showed significantly high expression in both gossypols treated multiple myeloma cells and low-risk multiple myeloma patients. A. *Jun* showed significantly high expression in MM tissue and MM cell lines than normal plasma cell and MGUS. B. Low-risk multiple myeloma patients in the TT3 cohort have significantly higher *Jun* expression; C. (Low-risk multiple myeloma patients in TT2 cohort have significantly higher *Jun* expression; D, E. High *Jun* expression group patients show a significantly shorter overall survival time in both TT2 and TT3 cohort.

When Z-VAD-FMK was added into the *JUN*-overexpressing cell line, the expression levels of Caspase proteins and PARP were decreased, similar to the empty vector cell line (**Figure 6G**).

Gossypol induces MM cell apoptosis through the JUN-JNK pathway

From the above results, it is apparent that *JUN* overexpression can promote apoptosis of MM cells through the caspase cascade. c-Jun, the protein product of *JUN*, is an important downstream target of the JNK pathway. We examined some known important upstream and downstream molecules on the JNK pathway: *DR5*, *STAT1* [27], and *AIF*. We found that *DR5* shows overexpression after gossypol treatment in MM cell lines (**Figure 7A**). Performing the shRNA knockdown of *DR5* can partially reverse gossypol-induced apoptosis (**Figure 7B**). Therefore, we suggested that *DR5* is only one of the pathways by which gossypol can induce MM cell apoptosis. Gossypol should also be able to regulate c-Jun through other pathways to induce apoptosis (**Figure 7C**).

Discussion

Gossypol exhibits excellent anticancer activity in various types of tumors [31-34]. For exam-

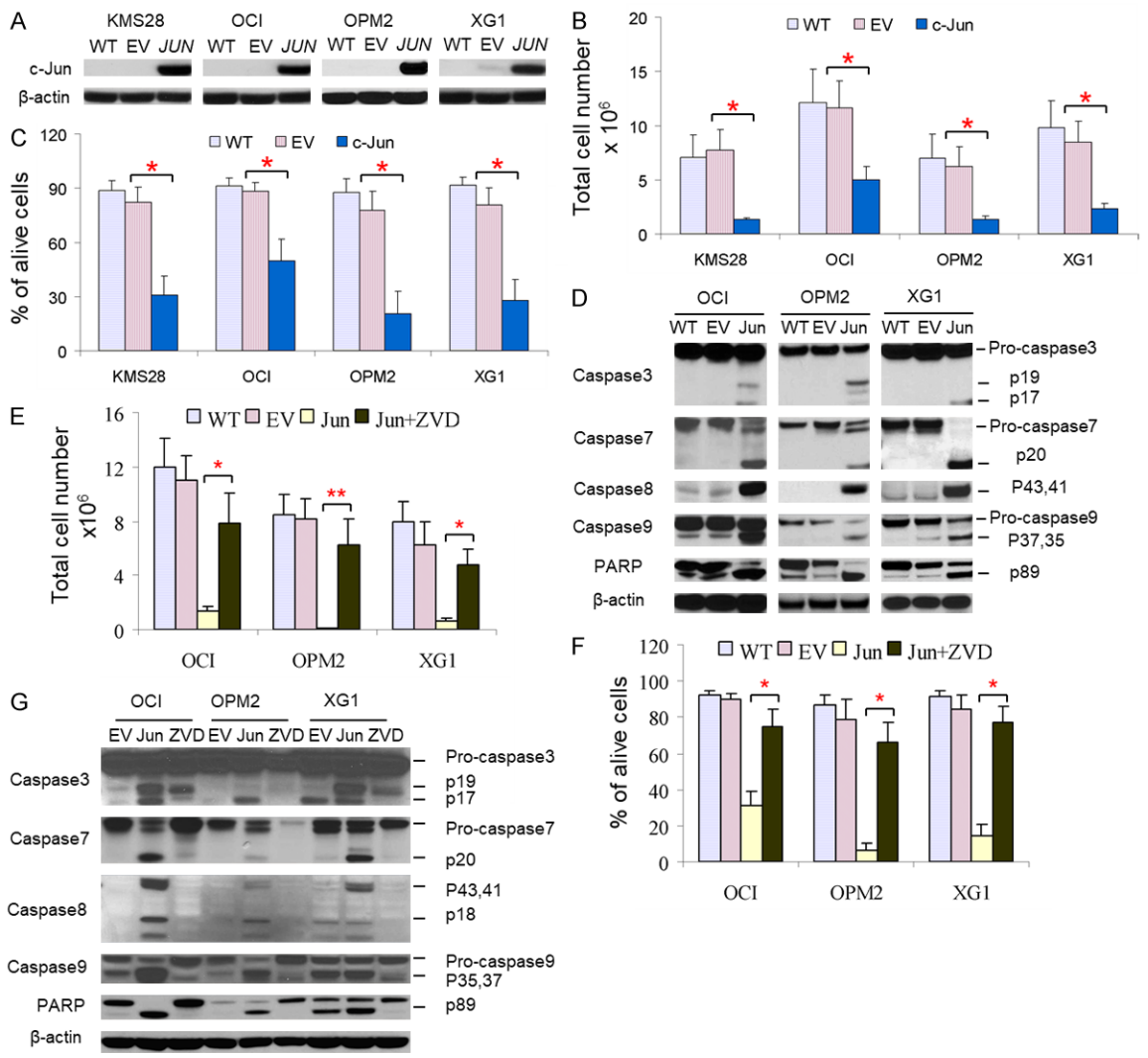

Figure 6. Overexpression of *c-jun* induced potent cell death and growth inhibition through caspase-dependent apoptotic mechanism. A. Western blot show *c-jun* was overexpressed in multiple myeloma cells. B, C. Overexpression of *c-jun* in MM cells can induced cell death and growth inhibition. D. Overexpressing *c-jun* can activate caspase-dependent apoptotic pathway. E-G. Caspase inhibitor ZVD can rescue *c-jun* overexpression effect in cell death and growth inhibition.

ple, gossypol retards the cell cycle, as determined by cell cycle regulatory proteins or *TGF-β* [33]. Gossypol inhibits the proliferation of cancer cells by inhibiting the synthesis of DNA through DNase inhibition [35]. Many common pathways in tumor apoptosis, such as *Bcl-2* [36], *p53* [37], *c-Myc* [38], and *NF-κB* [39], are also involved. There are also studies of the pro-apoptotic mechanism of gossypol in MM. Immunologically related proteins, DNA mismatch repair proteins, DNA replication proteins, and *Bcl-2* family proteins were significantly differentially expressed between gossypol-treated and untreated cells [20]. Michael P. Kline, et al. [21] reported that gossypol increased the *Bax/Bcl-2* ratio and activated the caspase cascade.

In our study, we identified 532 differentially expressed genes between gossypol-treated MM cell lines and the control. In the GO analysis results, two significant cell components GO terms, “cell leading edge” and “adherents junction”, were both related to cell movement. These findings were consistent with previous reports that gossypol could inhibit tumor cell invasion and migration [40, 41]. The biological pathway GO term “regulation of cysteine-type

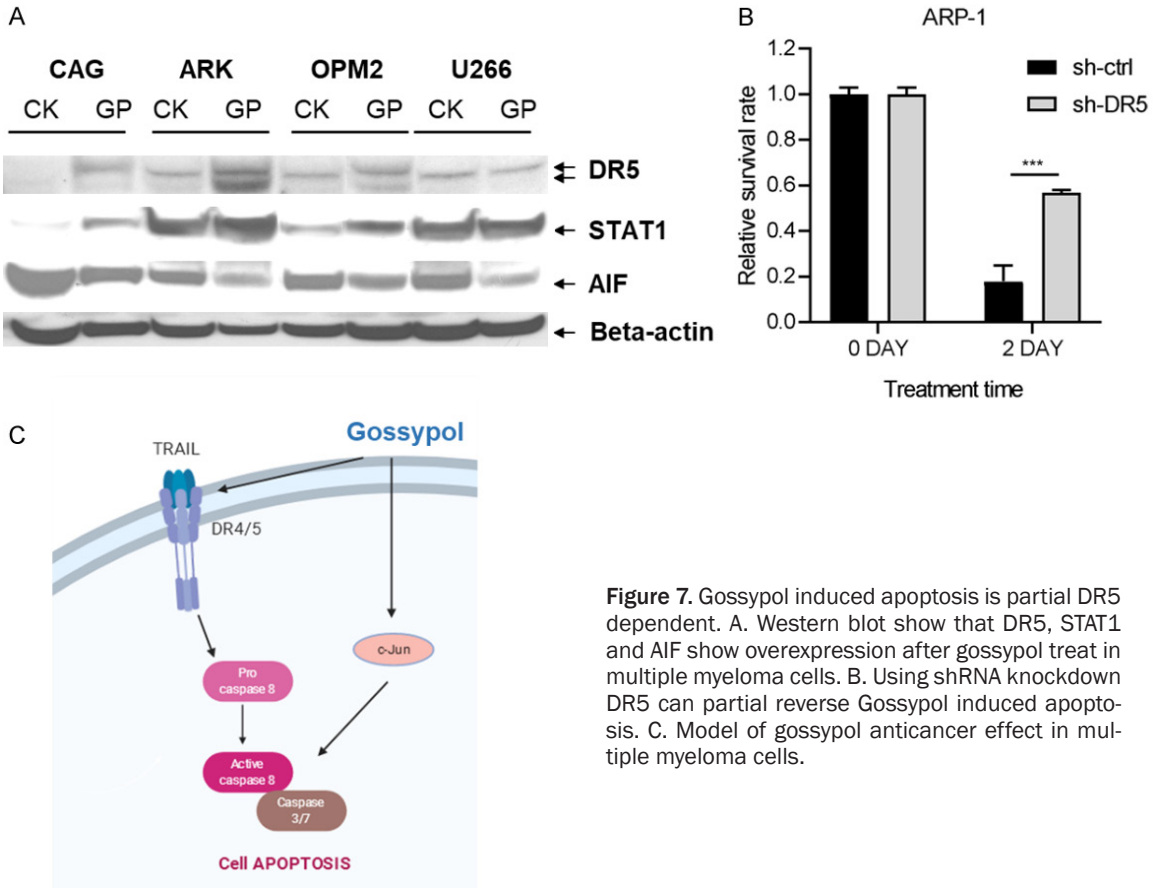

Figure 7. Gossypol induced apoptosis is partial DR5 dependent. A. Western blot show that DR5, STAT1 and AIF show overexpression after gossypol treat in multiple myeloma cells. B. Using shRNA knockdown DR5 can partial reverse Gossypol induced apoptosis. C. Model of gossypol anticancer effect in multiple myeloma cells.

endopeptidase activity involved in the apoptotic process" is enriched, which indicates that caspase-dependent apoptosis plays an import role [42]. Furthermore, DNA regulation-associated GO terms, such as "proximal promoter sequence-specific DNA binding", are also enriched which is also consistent with previous reports that gossypol can inhibit DNA synthesis [43]. KEGG results also indicated that the apoptosis pathway is the most import pathway by which gossypol-induced differentially expressed genes are involved. Our research confirms this conjecture and is consistent with previous findings [20, 21].

JUN is an oncogenic transcription factor [44] and encodes the protein c-Jun, the most prominent downstream target of the c-Jun N-terminal kinase (JNK) pathway [45]. c-Jun plays a key role in cell cycle progression by regulating cyclin D1 [46] and *p53* [45]. It exerts antiapoptotic activity in many tumors [47, 48]. However, c-Jun has also been reported to be a key mediator of tumor promotion [49-52]. In the present study, we identified *JUN* as a hub gene because it was significantly upregulated after gossypol treating and was significant in all five pathways in the KEGG results of 532 DEGs. *JUN*'s protein product, c-Jun, shows a significant anticancer property and was upregulated in gossypol-treated MM cell lines. MM cell lines have lower c-Jun expression compared to normal plasma cells, MGUS cells and MM tissue. *JUN* overexpression induced cell death and proliferation inhibition. High-risk MM patients have significantly lower *JUN* expression levels than low-risk patients. Patients with lower *JUN* expression experienced earlier disease-related death. These results indicated that *JUN* is a pro-apoptosis factor and a good prognostic factor in MM cells. As a prominent downstream molecule of the JNK pathway, c-Jun overexpression should lead to activation of the JNK pathway. Thus, it is reasonable to speculate that the JNK pathway plays an extremely important role in gossypol-induced apoptosis of MM cells by regulating c-Jun.

DR5 can promote cancer cell apoptosis by activating the JNK pathway and increasing the

c-Jun level [25, 53]. In this study, *DR5* was significantly upregulated in gossypol-treated MM cell lines (**Figure 4A**). Gossypol-induced apoptosis is partially reversed with the *DR5* knockdown (**Figure 4B**). Therefore, the mechanisms of gossypol-induced apoptosis may also include the death receptor signaling pathway (**Figure 4C**). However, it is also indicated that *DR5* is just one of the pathways by which gossypol can induce MM cell apoptosis. Gossypol should also be able to regulate c-Jun through other pathways to induce apoptosis. In the KEGG results of 532 DEGs, osteoclast differentiation was also significant. Thus, we performed a TARP test and found that gossypol not only inhibited MM cell proliferation but also inhibited osteoclast cell differentiation (**Figure 1E**). These findings indicate that gossypol may have a potential effect on MM treatment from both MM cells and osteoclasts. These findings deserve further study. To further analyze what signal pathways other than *DR5* can cause MM cell apoptosis through *JUN*, we utilized mRNA expression data from the UAMS-TT2 project, and 1017 *JUN*-correlated genes were selected. The results of IPA indicated that the major canonical pathways include the *IL-6* signaling pathway, the glucocorticoid receptor signaling pathway, the aryl hydrocarbon receptor signaling pathway, the *EGF* signaling pathway and the *PDGF* signaling pathway. The *IL-6* signaling pathway is the most significant. The *IL6R* expression level was significantly downregulated in gossypol-treated MM cell lines in our study, consistent with a previous report that *IL-6* plays an important role in gossypol-induced apoptosis [23]. Other significant signaling pathways such as EGF signaling and glucocorticoid receptor signaling pathways, have interactions with the JNK pathway [54-57]. Therefore, these significant signaling pathways identified in the IPA results may be all worthy of further study.

In conclusion, *JUN* is a hub gene that plays a critical role in gossypol-induced apoptosis through the JNK pathway. Gossypol activates the caspase cascade through c-Jun, causing MM cell apoptosis. *DR5* is one of the pathways by which gossypol regulates c-Jun. Thus, the mechanism of gossypol-induced apoptosis of MM cells is very complicated and involves multiple biological signaling pathways.

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Disclosure of conflict of interest

None.

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