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MAFF Mediates the Antitumor Effects of Ginsenoside Rh2 via MAPK Signaling in Prostate Cancer

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ABSTRACT

Background: Prostate cancer (PCa) is a common malignancy in men characterized by high heterogeneity and frequent therapeutic resistance, particularly in advanced stages. Ginsenoside Rh2 (Rh2), a bioactive compound extracted from red ginseng, has demonstrated antitumor activity in various cancers, but its role and mechanisms in PCa remain unclear. Methods: We systematically investigated the effects of Rh2 on DU145 PCa cells through functional assays and transcriptome sequencing. Cell viability, proliferation, migration, invasion, and apoptosis were evaluated. RNA Sequencing and downstream analyses were performed to identify potential regulatory targets and pathways. MAFF knockdown, overexpression, and combined MAPK inhibition experiments to validate its functional relevance. Results: Rh2 treatment significantly suppressed DU145 cell proliferation, migration, and invasion while promoting apoptosis. Transcriptomic profiling revealed pronounced downregulation of the transcription factor MAFF. Western blot analysis confirmed that Rh2 reduced MAFF expression and inhibited phosphorylation of p38, ERK, and JNK. Silencing MAFF recapitulated the tumor-suppressive effects of Rh2, whereas MAFF overexpression enhanced malignant phenotypes, including proliferation, motility, and resistance to apoptosis. Importantly, the p38 MAPK inhibitor BIRB796 partially reversed the oncogenic effects driven by MAFF overexpression, underscoring a MAFF–MAPK regulatory axis in PCa. Conclusion: Rh2 may inhibit malignant phenotypes of PCa cells in vitro by downregulating MAFF and attenuating MAPK pathway activation. The identification of the Rh2–MAFF–MAPK axis provides novel mechanistic insight into Rh2´s anticancer effects and highlights MAFF as a potential molecular target for PCa.

KEYWORDS

prostate cancer, ginsenoside rh2, rna-seq, MAFF, MAPK

INTRODUCTION

Prostate cancer (PCa) is a prevalent malignant tumor in men and is the second most diagnosed cancer globally [1, 2]. Its incidence increases significantly with age, and a positive family history is a major risk factor. The

American Cancer Society projects around 299,010 new PCa cases and 35,250 related deaths in the United States for 2024 [1]. The incidence is typically higher in developed nations, likely due to the prevalent use of prostate-specific antigen (PSA) screening and longer life expectancy [2]. Despite significant advances in diagnostic technologies and therapeutic strategies, the high heterogeneity of PCa and the complexity of the tumor microenvironment continue to pose substantial challenges to effective treatment.

Current treatment options encompass radical prostatectomy, radiotherapy, androgen deprivation therapy (ADT), chemotherapy, and newer methods like targeted therapy and immunotherapy [3-5]. Despite initial effectiveness, these treatments often lead to resistance in many patients, resulting in disease progression and metastasis [6].

Traditional Chinese medicine (TCM) has gained attention in recent years for its potential in treating PCa due to its multi-target mechanisms and low toxicity. Research indicates that TCM can have tumor-suppressive effects by triggering apoptosis, autophagy, and cell cycle arrest; suppressing proliferation, migration, and angiogenesis; boosting antitumor immunity; and counteracting drug resistance [7]. In Taiwan, China, over 50% of PCa patients reportedly use TCM as an adjunct therapy, with those receiving treatment for more than 200 days showing a significantly reduced mortality risk [8]. Moreover, certain TCM formulas, such as Chaihu Jia Longgu Muli Decoction, have shown promising potential in improving survival and alleviating cancer-related pain in patients with metastatic PCa. Systematic reviews and meta-analyses have further confirmed the safety and efficacy of TCM in PCa management [9]. In recent years, natural bioactive compounds have also attracted increasing attention in the development of biomedical functional materials, including fiber-based and textile-derived biomaterials designed for biomedical and tissue engineering applications.

Ginsenoside Rh2 (Rh2), a pharmacologically active compound extracted from red ginseng, has demonstrated therapeutic potential across various conditions, including cardiovascular and neurodegenerative diseases, as well as multiple types of cancer [10, 11]. In oncology, Rh2 has been reported to induce apoptosis and inhibit proliferation through multiple pathways. Rh2 induces apoptosis in leukemia cells through the TNF-α pathway and inhibits triple-negative breast cancer cell growth by targeting the IL-6/JAK2/STAT3 axis [12, 13]. Preliminary studies suggest that Rh2 also exhibits antiproliferative and pro-apoptotic effects on PCa cells; however, the underlying mechanisms remain incompletely understood [14].

This research systematically assesses the impact of Rh2 on PCa cell behavior through in vitro experiments. Furthermore, we seek to elucidate its potential molecular mechanisms and identify candidate biomarkers, thereby providing experimental evidence for the application of TCM in PCa treatment.

MATERIALS AND METHODS

Cell Culture and Transfection

The DU145 human PCa cell line was sourced from the American Type Culture Collection (ATCC, USA). Cells were maintained at 37°C in a humidified incubator with 5% CO_2, using MEM supplemented with 10% FBS and 1% penicillin-streptomycin (Gibco, USA). Rh2 was purchased from MedChemExpress (USA). For gene silencing experiments, siRNA targeting MAFF (si-MAFF: GCGGCGCCGCACACTCAAAAACC) was synthesized. Transfections utilized a GenePharma siRNA kit (China) following the provided instructions.

CCK-8 Cell Proliferation Assay

DU145 cells were plated in 96-well plates at a concentration of 2,000 cells per well. At specified time intervals (days 1-5), 10 µL of CCK-8 solution (Dojindo, Japan) was added to each well and incubated at 37°C for 1 hour. A microplate reader (BioTek, USA) measured absorbance at 450 nm.

EdU Incorporation Assay

The EdU incorporation assay was employed to assess cell proliferation. DU145 cells underwent a 2-hour EdU incubation, followed by fixation with 4% paraformaldehyde and permeabilization using 0.5% Triton X-100. EdU incorporation was assessed with the BeyoClick™ EdU-594 Imaging Kit (Beyotime, China) following the manufacturer's instructions. Hoechst 33342 was used for nuclear counterstaining, and images were captured with a fluorescence microscope.

Wound Healing Assay

A wound healing assay was utilized to evaluate cell migration. DU145 cells were seeded at a density of 5×10^4 cells per well in 96-well plates and cultured until reaching full confluence. A pipette tip was used to create a scratch, after which the cells were gently washed with PBS to eliminate debris and then incubated in a serum-free medium. Wound closure was observed and documented with photographs at 0 and 24 hours using a light microscope. ImageJ software was utilized to quantify the wound area.

Transwell Invasion Assay

Cell invasion was evaluated using 24-well Transwell chambers equipped with 8.0 μm pore polycarbonate membranes (Corning, USA) coated with Matrigel (BD Biosciences, USA) to mimic the extracellular matrix. DU145 cells (5×10^4) were suspended in 100 μL of serum-free medium and placed in the upper chamber. The lower chamber contained 600 μL of medium with 30% fetal bovine serum (FBS) serving as a chemoattractant. Following a 24-hour incubation at 37°C, non-invading cells on the membrane's upper surface were meticulously eliminated using a cotton swab. Cells that invaded the lower surface were fixed using paraformaldehyde, stained with crystal violet, and observed under a light microscope. Invaded cells were quantified in five randomly chosen fields per well.

Apoptosis Assay

Apoptosis was assessed with the Annexin V-APC/PI detection kit (eBioscience, USA) following the manufacturer's guidelines. Stained cells underwent analysis via a Millipore flow cytometer.

RNA Isolation and Quantitative real-time PCR

Total RNA was extracted using TRIzol reagent (Invitrogen, USA). cDNA was synthesized using the PrimeScript RT Reagent Kit (Takara, Japan). Quantitative real-time PCR (qRT-PCR) was performed with SYBR Green Master Mix (Takara, Japan) on a Roche LightCycler 96 Real-Time PCR System. Each reaction was run in triplicate. GAPDH served as the internal control. Refer to Supplementary Table 1 for primer sequences.

Supplementary Table 1. The primers of the detected genes

Gene		Primer (5′–3′)
EXOC7	Forward	CCCAACAAGAGGAAAGACA
	Reverse	TTGACGAAGGCACTGACG
MAFF	Forward	GAGGTGACACGGCTCAAGCA
	Reverse	GCGCTCCAGCTCCGACTT
ZNF517	Forward	TGGAGAACTATGGGAACCTGG
	Reverse	CACGCTCTGTTCAGCCACC
ETS1	Forward	CCCACTATTAACTCCAAGC
	Reverse	ATTCATTCACAGCCCACA
CTSH	Forward	CAACTGGAGGAAGATAAACG
	Reverse	GCTGAGCAATTCTGAGGC
CCDC12	Forward	GGCAACTACGGCTGGTGT
	Reverse	GATGCTTGGTCTTTGGCTC
GAPDH	Forward	GACCTGACCTGCCGTCTA
	Reverse	AGGAGTGGGTGTCGCTGT

Western blotting

Protein extraction from cells was performed using the RIPA reagent (Beyotime, China). Protein concentration was measured using a BCA assay kit (Beyotime, China). Proteins were separated using 10% SDS-PAGE and then transferred to PVDF membranes. The PVDF membranes were incubated with primary antibodies at 4°C overnight, then exposed to secondary antibodies for 1 hour. Protein band intensity was quantified via ECL chemiluminescence and analyzed using Image Lab software. GAPDH was employed as an internal reference.

RNA Sequencing (RNA-seq) and Data Analysis

RNA was extracted from DU145 cells, and its integrity was evaluated before sequencing. Library construction and high-throughput sequencing were performed on the Illumina platform. Raw sequencing reads were first assessed using FastQC, and low-quality sequences and adapters were trimmed with Trimmomatic. Clean reads were aligned to the human reference genome (GRCh38) using HISAT2, and alignment quality was evaluated using RSeQC, Qualimap, and BEDTools to assess metrics such as genome coverage, insert size distribution, sequence redundancy, and chromosomal mapping. Transcript assembly was performed using StringTie, and novel transcripts were identified by comparison to known gene models using GffCompare. Alternative splicing events were analyzed with ASprofile. Gene expression levels were quantified using StringTie. Differential expression analysis between Rh2-treated and control groups was conducted using DESeq2, with results visualized through volcano plots. Functional enrichment analyses, encompassing Gene Ontology (GO), Kyoto

Encyclopedia of Genes and Genomes (KEGG) pathway, and Eukaryotic Orthologous Groups (KOG) classification, were conducted utilizing topGO and clusterProfiler.

Statistical analysis

All experimental data were analyzed and visualized using GraphPad Prism (version 10). An unpaired t-test compared differences between the two groups. Data are expressed as mean ± standard deviation (SD) unless noted otherwise. Statistical significance was established at $p < 0.05$, with levels denoted as * * * $p < 0.001$, * * $p <0.01$, and * $p < 0.05$.

RESULTS

Ginsenoside Rh2 Inhibits Malignant Phenotypes of PCa Cells in Vitro

We conducted a CCK-8 assay to assess the impact of Rh2 on DU145 PCa cell viability and to establish a dose-response curve. The results demonstrated that Rh2 significantly inhibited DU145 cell viability, with a half-maximal inhibitory concentration (IC_{50}) of 0.125 mg/ml (Figure 1A). No non-specific cytotoxicity was observed in all subsequent functional assays at this concentration. Growth curve analysis demonstrated that Rh2 treatment significantly decreased the proliferation rate of DU145 cells relative to the control group (Figure 1B). EdU incorporation assays revealed a marked reduction in EdU-positive cells after Rh2 treatment, demonstrating effective inhibition of DNA synthesis activity (Figure 1C, D). Wound healing and Transwell assays were performed to evaluate the impact of Rh2 on cell motility. The wound healing assay demonstrated that Rh2 significantly impaired cell migration within 24 hours (Figure 1E, F). Transwell invasion assays consistently demonstrated a significant decrease in cell penetration through the membrane following Rh2 treatment, highlighting a strong inhibition of DU145 cell invasiveness (Figure 1G, H). This inhibition was not due to cytotoxicity, as 24 h Rh2 treatment did not induce significant apoptosis (Figure 1I, J). Annexin V-APC/PI double-staining and flow cytometry analysis demonstrated that Rh2 significantly increased apoptosis in DU145 cells, resulting in a notably higher total apoptotic rate compared to the control group (Figure 1I, J). Overall, the findings suggest that Rh2 mitigates the aggressive characteristics of DU145 PCa cells by curbing proliferation, migration, and invasion, and enhancing apoptosis.

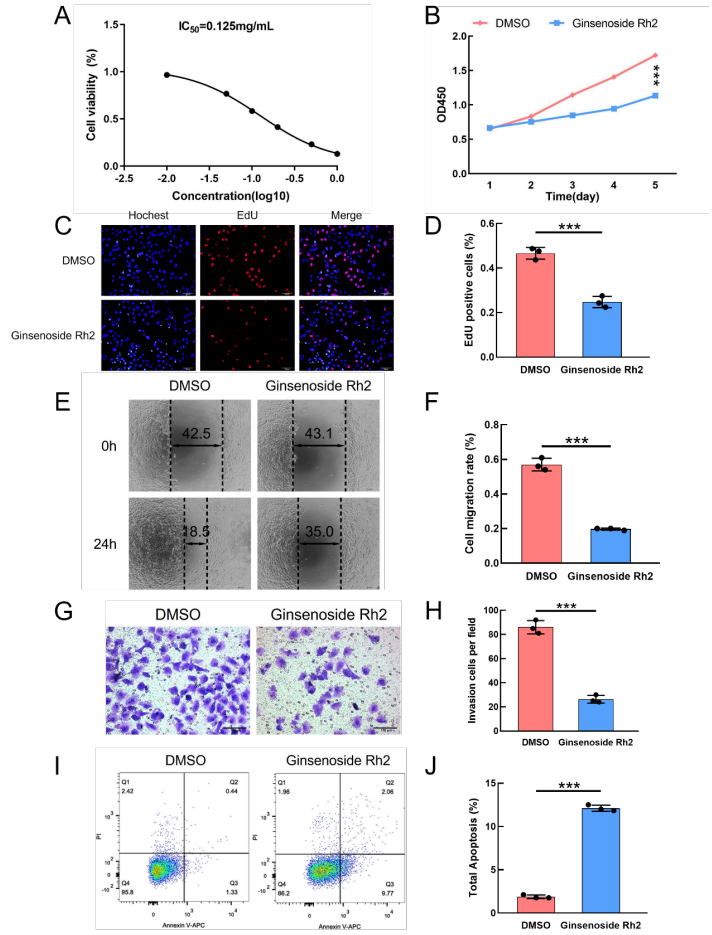

Figure 1. Ginsenoside Rh2 exerts inhibitory effects on proliferation, migration, and invasion, while promoting apoptosis in DU145 PCa cells: (A) DU145 cells treated with Rh2 for 24 hours show a dose-response curve via CCK-8 assay. (B) Growth curves indicate cell proliferation over 5 days in DMSO and Rh2 groups. (C, D) EdU assay shows reduced DNA synthesis with Rh2, with immunofluorescence images (C) and EdU-positive cell counts (D). (E, F) Wound healing assay demonstrates decreased cell migration at 24 hours with Rh2, including images (E) and migration rate data (F). (G, H) Transwell invasion assay reveals significant inhibition of DU145 cell invasion by Rh2, with images of crystal violet-stained cells (G) and invasion quantification (H). (I, J) Flow cytometry with Annexin V-APC/PI staining shows apoptosis, with flow plots (I) and apoptotic cell counts (J).

Ginsenoside Rh2 Significantly Alters the Transcriptomic Profile of PCa Cells

To systematically evaluate the impact of Rh2 on the transcriptional landscape of PCa cells, RNA-seq was performed to compare DU145 cells treated with Rh2 (Group B) versus untreated controls (Group A) (n = 3 per group; Rh2 concentration: IC_{50} , 0.125 mg/mL). Volcano plot analysis revealed 686 differentially expressed genes (DEGs) after Rh2 treatment, comprising 477 upregulated and 209 downregulated genes (Figure 2A). GO enrichment analysis indicated that these DEGs were significantly associated with diverse biological processes (BP), including cellular activities, metabolic functions, and biological regulation. The DEGs were mainly linked

to organelles, membrane structures, and the extracellular region in the context of cellular components (CC). For molecular function (MF), the DEGs were enriched in categories related to binding activity, enzyme regulation, and signal transduction (Figure 2B). KEGG pathway enrichment analysis revealed that Rh2-responsive genes participate in essential signaling pathways associated with signal transduction, cell growth and death, immune system, and metabolism. This indicates that Rh2 potentially affects cancer cell behavior through various regulatory networks (Figure 2C). Furthermore, KOG functional classification indicated that the DEGs were mainly associated with transcription, signal transduction mechanisms, cell cycle control, and posttranslational modification, reinforcing the multi-targeted regulatory potential of Rh2 in PCa cells (Figure 2D). Collectively, these transcriptomic findings demonstrate that Rh2 markedly remodels the gene expression profile of DU145 PCa cells, affecting multiple key pathways and biological processes. These results suggest that Rh2 may inhibit malignant phenotypes of PCa cells in vitro through diverse and multifaceted mechanisms.

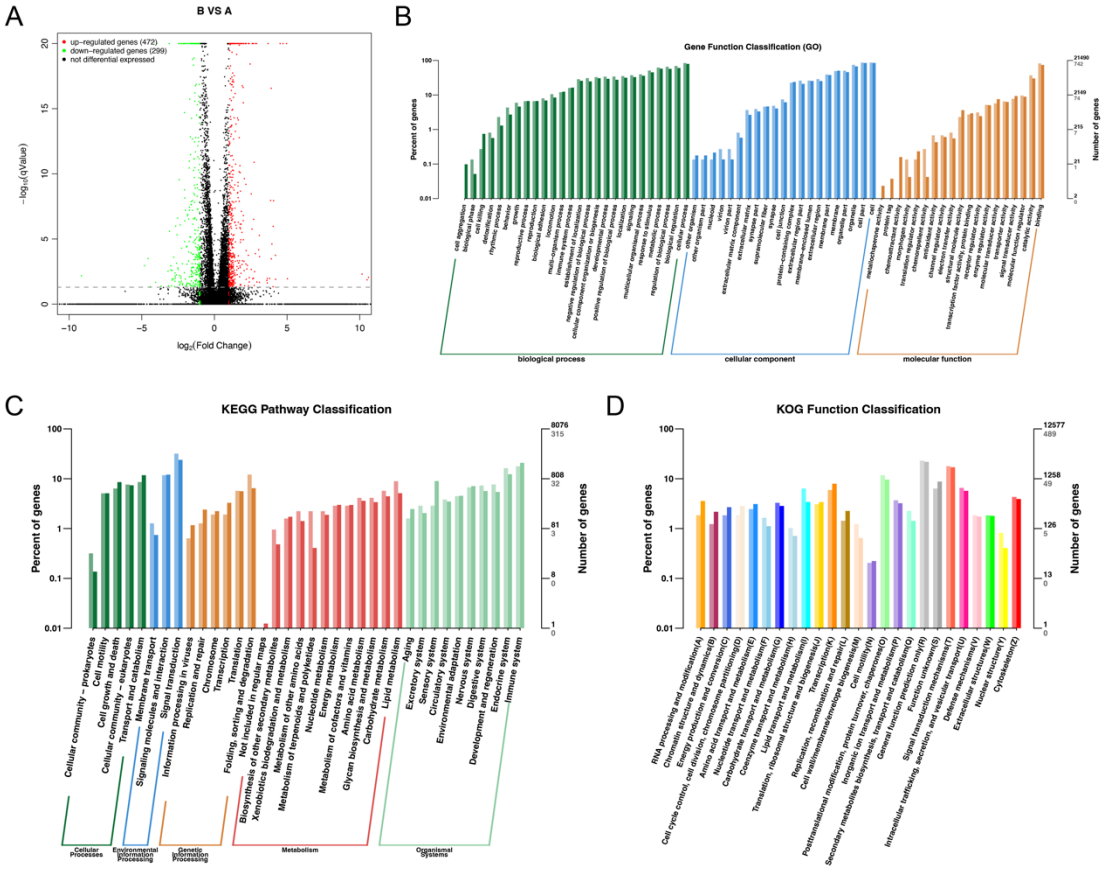

Figure 2. Transcriptomic profiling reveals gene expression changes and pathway enrichment in DU145 cells following Rh2 treatment: (A) A volcano plot displays DEGs with 477 upregulated (red) and 209 downregulated (green) genes. (B) DEGs are categorized by GO into BP, CC and MF. (C) KEGG pathway analysis shows DEGs in key signaling and metabolic pathways. (D) KOG classification sorts DEGs into functional categories like transcription, signal transduction, and posttranslational modification.

Ginsenoside Rh2 inhibit malignant phenotypes of PCa cells in vitro by downregulating MAFF expression

To validate the regulatory role of Rh2 in PCa, we integrated transcriptomic analysis with functional assays. The CCK-8 assay determined the IC_{50} of Rh2 in DU145 cells to be 0.125 mg/mL, at which no non-specific cytotoxicity was detected in subsequent mechanistic assays. At this concentration, qRT-PCR revealed that Rh2 significantly downregulated multiple differentially expressed genes, including EXOC7, MAFF, ZNF517, ETS1, CTSH, and CCDC12, with MAFF showing the most pronounced reduction, suggesting a central role in the antitumor effects of Rh2 (Figure 3A). Consistent with these findings, Western blot analysis demonstrated that Rh2 markedly decreased MAFF protein expression, while concomitantly suppressing the phosphorylation of p38, ERK, and JNK, without altering their total protein levels (Figure 3B). To further assess the functional role of MAFF, we generated a siMAFF knockdown model in DU145 cells. qRT-PCR and Western blot confirmed a significant reduction of MAFF at both mRNA and protein levels in the siMAFF group compared with controls (Figure 3C,D). Functionally, CCK-8 and EdU assays demonstrated that MAFF knockdown significantly suppressed cell proliferation (Figure 3E,F). In addition, wound healing and Transwell assays revealed a marked reduction in migration and invasion capacity following MAFF silencing (Figure 3G,H). MAFF knockdown did not affect cell viability within 24 h (Figure 3I), ruling out cytotoxicity as a confounding factor. Flow cytometry analysis further indicated that MAFF knockdown significantly increased the proportion of apoptotic cells (Figure 3I). Mechanistically, Western blot analysis showed that MAFF depletion substantially reduced the phosphorylation levels of p38, ERK, and JNK, while total protein levels remained unchanged, suggesting that MAFF is required for sustained activation of the MAPK signaling cascade (Figure 3J). Collectively, these results demonstrate that Rh2 suppresses PCa progression by downregulating MAFF.

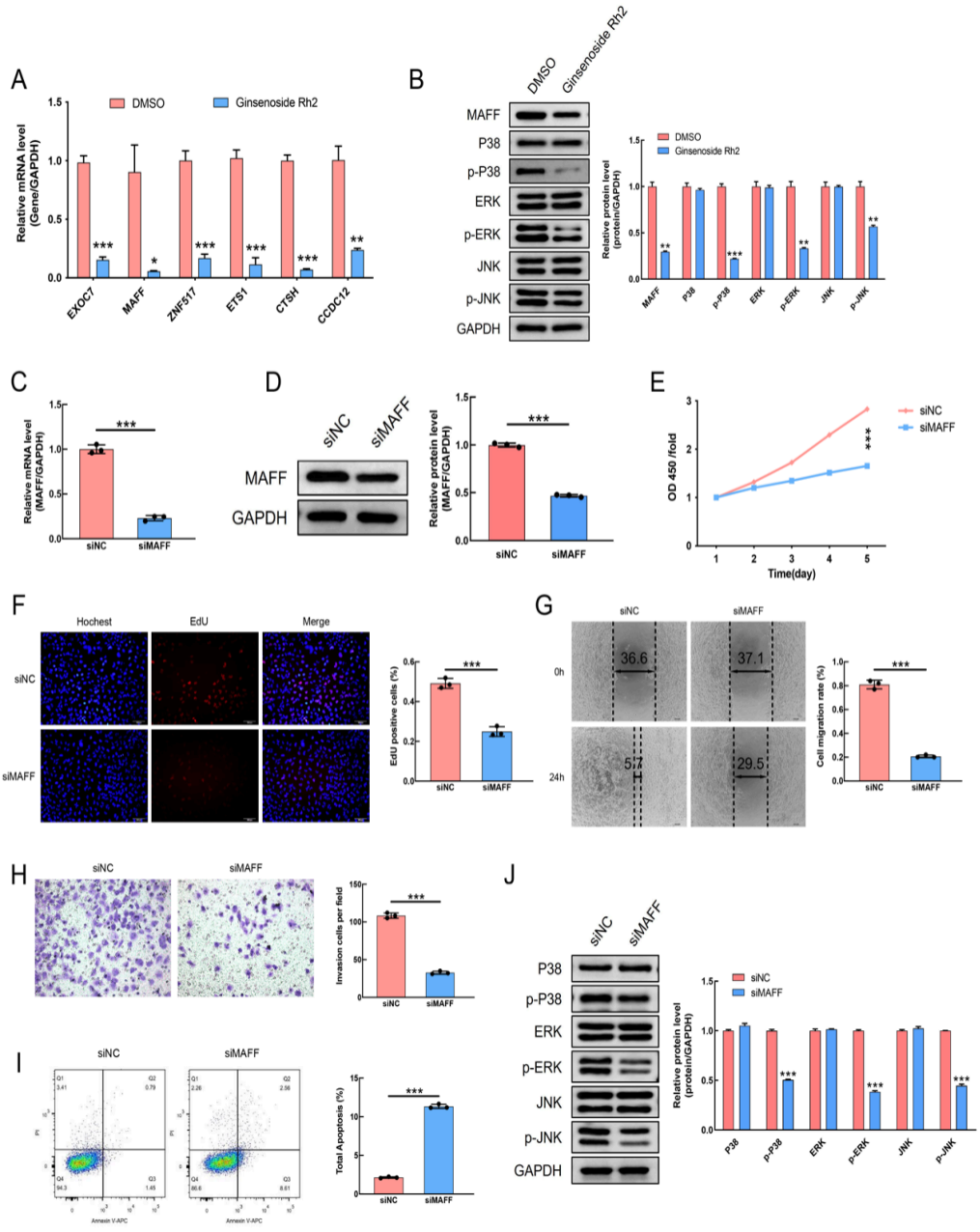

Figure 3. Ginsenoside Rh2 suppresses prostate cancer progression by downregulating MAFF and inhibiting MAPK pathway activation: (A) qRT-PCR analysis showing that Rh2 treatment (0.125 mg/mL) significantly reduced the mRNA expression of several differentially expressed genes, with MAFF exhibiting the most pronounced decrease. (B) Western blot analysis demonstrating that Rh2 downregulated MAFF protein levels and reduced the phosphorylation of p38, ERK, and JNK, while total protein levels remained unchanged. (C, D) qRT-PCR and Western blot confirming efficient knockdown of MAFF expression in DU145 cells by siRNA. (E, F) CCK-8 and EdU assays showing that MAFF knockdown markedly suppressed DU145 cell proliferation. (G, H) Wound healing and Transwell assays indicating that MAFF silencing significantly inhibited DU145 cell migration and invasion. (I) Flow cytometry analysis revealing that MAFF knockdown significantly increased apoptosis compared with control cells. (J) Western blot analysis showing that MAFF knockdown decreased phosphorylation of p38, ERK, and JNK, suggesting impaired MAPK pathway activation.

MAFF promotes malignant progression of PCa cells by activating the MAPK pathway

To further determine whether MAFF influences the biological behavior of PCa cells through the MAPK-ERK signaling pathway, we established a MAFF overexpression model and performed functional assays in the presence or absence of MAPK pathway inhibition. qRT-PCR and Western blot analyses confirmed that MAFF expression was markedly increased in the overexpression group, however, treatment with the p38 MAPK-spe-cific inhibitor BIRB796 partially reduced its expression levels (Figure 4A-B). Functional studies demonstrated that MAFF overexpression significantly enhanced cell proliferation, as evidenced by CCK-8 and EdU assays, while BIRB796 treatment attenuated this effect (Figure 4C-D). Flow cytometric analysis showed that MAFF overexpression suppressed apoptosis, an effect that was partially reversed upon BIRB796 treatment (Figure4E). In migration and invasion assays, wound healing and Transwell analyses revealed that MAFF overexpres-sion significantly promoted the motility and invasiveness of DU145 cells, whereas inhibition of p38 MAPK markedly reduced these oncogenic phenotypes (Figure 4F-G).

Mechanistically, Western blotting indicated that MAFF overexpression strongly activated the MAPK pathway, as demonstrated by elevated phosphorylation of p38, ERK, and JNK, whereas BIRB796 treatment effectively suppressed these phosphorylation events (Figure 4H).

Taken together, these results suggest that MAFF promotes PCa cell oncogenic phenotypes through MAPK pathway activation, with p38 MAPK inhibition by BIRB796 partially reversing these effects, while the roles of ERK and JNK remain to be further clarified.

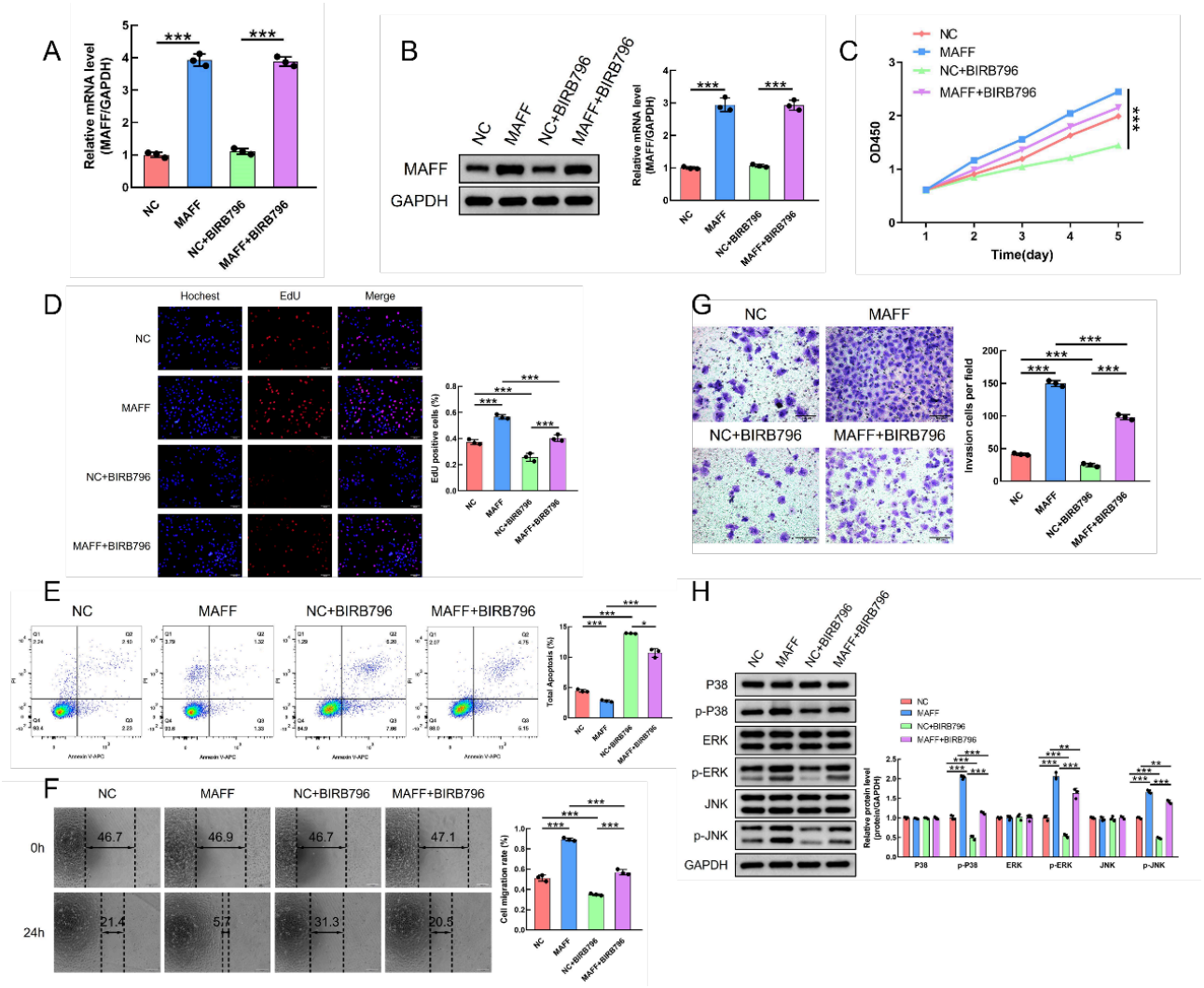

Figure 4. MAFF promotes malignant phenotypes of prostate cancer cells through MAPK signaling: (A, B) qRT-PCR (A) and Western blot (B) analysis confirmed significant upregulation of MAFF in the overexpression group, while the p38 MAPK inhibitor BIRB796 partially reduced its levels. (C, D) CCK-8 (C) and EdU (D) assays demonstrated that MAFF overexpression enhanced cell proliferation, which was attenuated by BIRB796 treatment. (E) Flow cytometry revealed that MAFF overexpression suppressed apoptosis, whereas BIRB796 partially restored apoptotic activity. (F, G) Wound healing (F) and Transwell (G) assays showed that MAFF markedly promoted migration and invasion, effects that were reversed by BIRB796. (H) Western blotting indicated that MAFF overexpression increased phosphorylation of p38, ERK, and JNK, while BIRB796 effectively inhibited MAPK pathway activation.

DISCUSSION

PCa is a common malignancy in men, noted for its heterogeneity and frequent resistance to treatment, especially in advanced or castration-resistant stages. These clinical challenges underscore the urgent necessity for developing novel therapeutic strategies that are both multi-targeted and low in toxicity [15, 16]. TCM is increasingly recognized in cancer therapy for its distinct benefits in enhancing patient quality of life, boosting

treatment effectiveness, and overcoming drug resistance. Rh2, a rare active component extracted from red ginseng, has demonstrated broad-spectrum anticancer effects in various tumor types, including the inhibition of cell proliferation, induction of apoptosis, suppression of migration, and reversal of drug resistance [17-19]. The exact mechanisms through which Rh2 influences PCa are not well understood and necessitate thorough investigation.

This study systematically assessed the antitumor effects of Rh2 on DU145 PCa cells and investigated the molecular mechanisms using transcriptome analysis. Experimental results demonstrated that Rh2 effectively suppressed PCa cell proliferation, migration, and invasion, while enhancing apoptosis. Transcriptome sequencing revealed that multiple key genes were significantly altered following Rh2 treatment, among which the transcription factor MAFF was markedly downregulated and occupied a central position in signaling-related pathways. MAFF, a member of the small Maf transcription factor family, plays roles in oxidative stress, inflammation, and tumor progression, exhibiting context-dependent and pathway-specific functions [20]. For example, in breast cancer, MAFF forms heterodimers with BACH1 to directly regulate IL11 transcription, thereby activating the STAT3 pathway and promoting tumor invasion and metastasis [20]. In colorectal cancer, MAFF is also a key regulatory factor whose aberrant expression—mediated by the ubiquitin–proteasome system—is closely associated with patient prognosis [21].

Our findings demonstrated that Rh2 treatment led to significant downregulation of MAFF expression at both mRNA and protein levels, accompanied by reduced phosphorylation of core MAPK pathway kinases, including p38, ERK, and JNK. MAFF knockdown using siRNA mirrored the effects of Rh2 on DU145 cells, leading to reduced proliferation, migration, and invasion, enhanced apoptosis, and MAPK pathway inhibition. The findings indicate that MAFF potentially acts as a putative upstream regulator of the MAPK signaling pathway in PCa, facilitating malignant progression by sustaining MAPK kinase activation with a currently uncharacterized molecular link. Notably, existing studies have reported close associations between Maf family transcription factors and the MAPK pathway. For instance, MAF regulates downstream gene expression in malignant peripheral nerve sheath tumors (MPNSTs) through the RAS/MAPK/AP-1 signaling axis [22]. As a central regulator of tumor proliferation and metastasis, the MAPK pathway plays a particularly critical role in castration resistance, maintenance of stemness, and chemotherapy resistance in PCa [23-26].

Our findings suggest that Rh2 suppresses malignant phenotypes in PCa cells by downregulating MAFF, which in turn reduces MAPK pathway activation. Consistently, functional assays confirmed that MAFF overexpression

significantly enhanced oncogenic phenotypes, while pharmacological inhibition of p38 MAPK with BIRB796 partially reversed these effects. Together, these data support a model in which MAFF acts as a putative upstream regulator sustaining MAPK pathway activation, thereby driving PCa progression. The Rh2–MAFF–MAPK axis not only enhances our understanding of Rh2′s anticancer mechanisms but also highlights MAFF as a potential therapeutic target in PCa.

While these results provide important insights, several limitations need to be addressed. First, our experiments were limited to DU145 cells; validation in other PCa cell lines (LNCaP, PC-3) is needed for generalizability. Second, although our results establish MAFF as a key activator of MAPK signaling, the precise molecular mechanisms—such as whether MAFF directly regulates transcriptional targets that feed into MAPK activation—require further investigation. Approaches such as dual-luciferase reporter assays, ChIP-qPCR, and rescue experiments could provide more mechanistic insight. Finally, in vivo studies and clinical sample analyses are needed to evaluate the impact of Rh2-mediated MAFF suppression on tumor progression and patient prognosis. Additionally, only a p38 MAPK inhibitor was used in this study; future work will employ ERK and JNK-specific inhibitors to fully dissect their roles in the MAFF-MAPK axis. Furthermore, our study only shows a correlation between MAFF and MAPK phosphorylation; future ChIP-seq/luciferase assays will clarify their direct link and intermediate proteins. These mechanistic findings may also provide molecular insights for future interdisciplinary studies exploring the integration of bioactive natural compounds into biomedical materials or related therapeutic systems.

In summary, this study identifies a novel molecular mechanism by which Rh2 inhibits may inhibit malignant phenotypes of PCa cells in vitro through the downregulation of MAFF and subsequent suppression of MAPK signaling. The Rh2–MAFF–MAPK regulatory axis offers new molecular insight and positions MAFF as potential molecular target for TCM-based interventions in prostate cancer.

CONCLUSION

This study clarifies that ginsenoside Rh2 inhibits the malignant phenotypes of prostate cancer (PCa) cells in vitro by targeting the MAFF-MAPK signaling axis. Rh2 downregulates MAFF expression, which in turn suppresses the phosphorylation of p38, ERK and JNK in the MAPK pathway, thereby inhibiting PCa cell proliferation, migration and invasion, and promoting apoptosis; MAFF overexpression-mediated oncogenic effects can be partially reversed by the p38 MAPK inhibitor BIRB796, confirming MAFF as a key mediator in regulating PCa cell malignant behaviors via the MAPK pathway. Our findings reveal a novel molecular mechanism for

Rh2′s anti-malignant activity in PCa cells and identify MAFF as a potential therapeutic target for PCa research, providing experimental evidence for the application of Rh2 in PCa intervention. Limitations of this study include the sole use of the DU145 cell line and restriction to in vitro assays; future validation in multiple PCa cell lines, in vivo animal models and clinical samples is needed to further confirm the clinical relevance and translational potential of the Rh2-MAFF-MAPK axis. The authors thank the reviewers for their valuable comments on this study.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by XF and QZ. The first draft of the manuscript was written by XF, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Funding

This research received no external funding.

Abbreviations

ADT: Androgen deprivation therapy

DEGs: Differentially expressed genes

GO: Gene Ontology

IC_{50}: Half-maximal inhibitory concentration

KEGG: Kyoto Encyclopedia of Genes and Genomes

KOG: Eukaryotic Orthologous Groups

PCa: Prostate cancer

PSA: Prostate-specific antigen

qRT-PCR: Quantitative real-time PCR

Rh2: Ginsenoside Rh2

TCM: Traditional Chinese medicine

Ethics Approval

Ethical approval is not applicable for this article.

Statement of Human and Animal Rights

This article does not contain any studies with human or animal subjects. This study was based on in vitro cell experiments and publicly available transcriptomic datasets, with no involvement of human participants, human tissue samples, or animal models in any experimental procedures.

Statement of Informed Consent

There are no human subjects in this article, and informed consent as well as consent for publication are not applicable.

Availability of Data and Material

The transcriptomic datasets generated and/or analyzed during this study are available from the corresponding author upon reasonable request.

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