Mometasone furoate inhibits the progression of head and neck squamous cell carcinoma via regulating PTPN11

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Research Article

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Abstract

Background: Mometasone Furoate (MF) is a kind of glucocorticoid with extensive pharmacological action including inhibiting tumor progression, however, the role of MF in head and neck squamous cell carcinoma (HNSCC) was still unclear. This study was aim to evaluate the inhibitory effect of MF against HNSCC and investigate the underlying mechanism.

Methods: Cell viability, colony formation, cell cycle and cell apoptosis were detected to explore the effect of MF on HNSCC cells. Xenograft study model was conducted to investigate the effect of MF on HNSCC in vivo. The core target of MF against HNSCC was identified by network pharmacology analysis, TCGA database analysis and real-time PCR. Molecular docking was performed to detect the binding energy. Protein tyrosine phosphatase non-receptor type 11 (PTPN11) overexpressed cells were constructed and then cell viability and the expression levels of proliferation- and apoptosis-related proteins were detected after treated with MF to explore the role of PTPN11 in the inhibitory of MF against HNSCC.

Results: After cells treated with MF, cell viability and the number of colonies were decreased, cell cycle was arrested and cell apoptosis was increased. Xenograft study results showed that MF could inhibit cell proliferation via promoting cell apoptosis in vivo. PTPN11 was the core target of MF against HNSCC via network pharmacology analysis, TCGA database analysis and real-time PCR. Molecular docking results revealed PTPN11 exhibited the strongest binding ability to MF. Finally, MF could attenuate the effect of increased cell viability and decreased cell apoptosis caused by PTPN11 overexpression, suggesting that MF could inhibit the progression of HNSCC by regulating PTPN11.

Conclusion: MF targeted PTPN11 to promote cycle arrest and cell apoptosis subsequently performed an effective anti-tumor activity.

Background

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common malignant tumors in the world. The incidence rate is increasing and is expected to increase by 30% by 2030[1]. The traditional treatment of HNSCC includes surgery followed by chemoradiotherapy. Despite the multimodal treatment, over half of patients experience relapse or metastasis. For patients with recurrent/metastatic HNSCC, combined chemotherapy with platinum and paclitaxel or 5-fluorouracil plus EGFR monoclonal antibody cetuximab is the standard first-line therapeutic regimen, while the consequential drug resistant is common and finally leads to the limited efficacy of treatment[2]. FDA approved immune checkpoint inhibitor pembrolizumab is the antibody to PD1 and can effectively improve the survival rate of recurrent/metastatic HNSCC patients, while only the patients who express PD-L1 could benefit from immune checkpoint inhibitor therapy. Meanwhile, the sever adverse reaction caused by immune checkpoint inhibitor therapy needs to be taken into consideration for administration[3]. To date, the median survival of recurrent/metastatic HNSCC patients is only 11.6 months. Therefore, it's still urgent to find more effective and less side effects drug treatment.
Glucocorticoids are a class of steroidal hormones that bind to glucocorticoid receptor to involve in the regulation of multiple key biological processes, including inflammation and glucose metabolism[4]. Served as classical anti-inflammation drug, recently accumulating evidence demonstrates that glucocorticoids also could treat malignant tumors. It’s well established that glucocorticoids are the cornerstone of lymphatic cancers treatment due to their verified function of arresting cell growth and promoting apoptosis[5, 6]. Most importantly, the activation of cell cycle arrest and apoptosis are the common ways for drugs to perform anti-cancer activity in multiple cancers including HNSCC[7–9]. Mometasone Furoate (MF) is a kind of glucocorticoid with extensive pharmacological action. Previous studies regarding MF mostly focused on its treatment for inflammation. In a recent study, MF inhibited the growth and induced the apoptosis of acute leukemia cells by regulating PI3K signaling pathway[10]. For the above-mentioned reasons, MF could be identified as a promising anti-cancer drug for HNSCC. Nevertheless, the inhibitory role and underlying mechanism of MF against HNSCC remain to be determined.

Network pharmacology is a comparatively comprehensive and systematic way to predict the potential targets of clinical drugs[11]. Molecular docking is an important method to predict the binding between targets and drugs. The combination of these two methods provides a better reference for the application of clinical drugs as well as the repurposing of precise and effective therapeutic drugs. Therefore, it’s an effective adjuvant method to favor screening the targets and underlying mechanism of MF against HNSCC.

Protein tyrosine phosphatase non-receptor type 11 (PTPN11) is a member of the protein tyrosine phosphatase (PTP) family and is the first proto-oncogene receptor tyrosine phosphatase. PTPs work in coordination with protein tyrosine kinases (PTKs) to balance the phosphorylation status of tyrosine in signaling proteins, which determines multiple cellular processes through the transduction of signaling cascades. PTPN11 is required by most receptor tyrosine kinases (RTKs) to active the downstream signaling cascade. As a result, PTPN11 plays a central role in the activation of oncogenic signaling pathways, such as PI3K/AKT[12], RAS/Raf/MAPK[13], Jak/STAT[14]. It’s widely acknowledged that PTPN11 is highly expressed in many tumors[15] and the aberrant expression is closely related to the poorer prognosis in a range of tumors[16, 17]. In HNSCC, PTPN11 is overexpressed and participated in the invasion and metastasis of cells via activating ERK1/2-Snail/Twist1 pathway[18]. Overexpressed PTPN11 could contribute to antigen processing machinery component downregulation thus led to cytotoxic T lymphocytes evasion[19]. Accordingly, PTPN11 could be regarded as an effective target for tumor therapy in HNSCC[20, 21].

In the current study, we disclosed that MF performed an effective anti-tumor activity to HNSCC in vitro and in vivo. Mechanistically, MF downregulated the expression of PTPN11 to promote cell cycle arrest and cell apoptosis subsequently suppressed HNSCC cells growth.

Materials And Methods
Cell culture

Two human HNSCC cell lines WSU-HN6 and CAL-27 cells were purchased from the American Type Culture Collection. Both cells were cultured in high-glucose DMEM (Gibco, USA) with 10 % FBS (Gibco, USA), 1 % penicillin/streptomycin solution (Solarbio, China) and maintained in 5 % CO₂ and 37 °C.

Cell counting Kit-8 assay

The cell viability was measured using CCK-8 (Beyotime, China) according to the manufacturer’s protocol. Cells were seeded into 96-well plates at a density of 4000 cells/well. After 24, 48, and 72 h, the supernatant was replaced by empty DMEM, CCK-8 solution (10:1) and incubated for 2 h at 37 °C. The optical density (OD) at 450 nm was measured by the automatic microplate reader (BioTek ELX808 American).

Cell viability was calculated by the formula: cell viability = [(experimental wells’ OD - blank wells’ OD)/(control wells’ OD - blank wells’ OD)] * 100 %

Colony formation assay

Cells were plated into 6-well plates at 400 cells/well and cultured for 7 days. The culture medium was changed every two days. At day 7, cell clone was washed with phosphate-buffered saline (PBS), fixed with formaldehyde for 15 min and stained with 0.5 % crystal violet for 20 min.

Flow cytometric analysis

Cells were seeded into 6-well plates at 3 * 10⁵ cells/well and cultured for 48 h. For analysis of cell cycle, cells were fixed in 70 % ethanol overnight at 4 °C, rinsed twice with PBS and stained with PI, buffer and RNase A at 37 °C for 30 min using the Cell Cycle and Apoptosis Analysis Kit (Beyotime, China). For analysis of apoptosis, cells were stained with Annexin V-FITC and PI for 15 min using the Annexin V-FITC Apoptosis Detection Kit (Solarbio, China).

Western blotting

The western blotting protocol was based on our earlier publication[22]. The antibodies including: ki67 (1:1000, Beyotime, China), PCNA (1:1000, Beyotime, China), Cleaved caspase-3 (1:1000, CST, USA), Bax (1:1000, Wanleibio, China), Bcl-2 (1:1000, Wanleibio, China), GAPDH (1:10000, proteintech, China), PTPN11 (1:1000, Wanleibio, China), HRP-labeled goat anti-rabbit IgG (1:1000, Beyotime, China).
Real-time PCR analysis

Total RNA was extracted by Trizol (Invitrogen, USA), then the equivalent RNA of each group was reverse transcribed into cDNA by a Prime Script™ RT reagent Kit (TaKaRa, Japan). The cDNA was amplified by real-time PCR. All data were normalized to GAPDH. The primer sequences were listed in Table 1.

Xenograft study models

All animal experiments were approved by the Institutional Animal Care and Use Committee of the Peking University Health Science Center (No. LA2022230), followed the Committee of Peking University Health Science Center's Animals Usage Guideline and performed using the approved protocols of the Animal Ethical and Welfare Committee. Healthy male BALB/c nu mice (4 - 5 weeks) were injected with 5 * 10^6 cells in 100 μl of PBS subcutaneously (two groups were injected with WSU-HN6 cells, and the other two groups were injected with CAL-27 cells). When the tumor volume was about 100 mm^3, the mice were given 0 mg/kg or 15 mg/kg MF twice per week by the oral administration. Tumor size were measured every 3 days and estimated by the formula: V = length * width^2/2. After 4 weeks, mice were euthanized and the tumors were completely removed to conduct HE, immunohistochemistry (IHC) and western blotting.

HE and IHC staining

For HE staining, the slides were deparaffinized, rehydrated and stained with hematoxylin and eosin. For IHC staining, the slides were deparaffinized, rehydrated, antigen recovered, blocked and incubated with anti-ki67 (1:100, Beyotime, China) or anti-Cleaved caspase-3 (1:100, CST, USA).

Bioinformatics analysis

Pharmmapper (http://www.lilab-ecust.cn/pharmmapper/) was employed to screen the potential targets of MF. HNSCC-related genes were obtained from GeneCards (https://www.genecards.org/) and Comparative Toxicogenomics Database (CTD, http://ctdbase.org/). Taking the intersection of MF potential targets and HNSCC-related genes to set as the potential targets of MF in HNSCC. Those genes were imported into STRING (https://string-db.org/) to construct the PPI network, followed by using degree centrality (DC), closeness centrality (CC), eigenvector centrality (EC), betweenness centrality (BC), local average connectivity (LAC), and network centrality (NC) to identify the core targets of MF against HNSCC via Cytoscape3.7.1.

To further investigate the underlying mechanisms of MF against HNSCC, the potential targets were used to perform Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways
analysis by Database for Annotation Visualization and Integrated Discovery (DAVID, https://david.ncifcrf.gov/).

To validate the results of network pharmacology analysis, The Cancer Genome Atlas (TCGA) database was employed to detect the expression of the core targets of MF against HNSCC in tumor and normal tissues.

**Molecular docking**

The structure of MF was downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) and was shown in Figure 1. The protein structures were obtained from PDB (http://www.rcsb.org/). PyMOL software was used to remove the water and small molecular ligands of the protein. AutoDockTools was employed to hydrotreat protein molecules, obtain PDBQT files and determine active pockets. Run Vina 1.1.2 program to calculate the binding energy. Finally, the optimal combination model was visualized via PyMOL. The smaller binding energy indicated the stronger binding force between MF and the target proteins. The binding energy ≤ -5.0 kcal/mol indicated they could be combined, and the binding energy ≤ -7.0 kcal/mol indicated they exhibited excellent binding strength.

**Construction of PTPN11 overexpression plasmid and cell lines**

To explore whether PTPN11 acted as the downstream target of MF, we constructed stable PTPN11 overexpressed cells. PCDH plasmid was employed as the control group. Human full-length PTPN11 cDNA was amplified and cloned in PCDH plasmid by the ClonExpress II One Step Cloning Kit (Vazyme, China) according to the manufacturer’s protocol. Primer was shown in Table 2. The plasmid with correct sequence was transfected into 293T cells together with VSVG and PAX8 plasmid. Lentivirus supernatants were harvested at 48 h and were utilized to infect cells at 80 % confluence. Puromycin was added after 48 h infection to obtain the positive PTPN11 overexpressed cells.

**Statistical analysis**

GraphPad Prism 8.4.3 software (San Diego, USA) was employed for the statistical analysis. All experiments were repeated three times to ensure the validity of the data. All Error bars were expressed as the mean ± standard deviation (SD, n = 3). Data among more than two groups were tested for homogeneity followed by one-way ANOVA analysis. P < 0.05 was considered significant statistically.

**Results**

**MF inhibited proliferation of HNSCC cells.**
To determine the cell cytotoxicity of MF, we first detected the half-maximal inhibitory concentration (IC$_{50}$) of HNSCC cells treated with MF, which was decreased as time increased (Figure 2A, B). According to the IC$_{50}$, we chose 0, 10, 20, and 50 μM for the further experiments. Then cell viability was detected after cells treated with different doses of MF and incubated different times (24, 48, and 72 h). Cell viability was gradually decreased with the dose and time increased (Figure 2C, D), indicating that MF could inhibit cell proliferation in a time- and dose-dependent manner. Similar results were observed in the colony formation assay. The number of colonies in MF treated groups were decreased along with increasing concentrations of MF (Figure 2E). Accordingly, these data recapitulated the proliferation inhibitory of MF in HNSCC cells.

**MF regulated cell cycle and induced apoptosis in vitro.**

Since glucocorticoids suppressed lymphoid progression via regulating cell cycle and apoptosis, we detected the cell cycle and apoptosis of cells treated by MF to further get insight into the proliferation inhibitory of MF. Compared with the control group, cells treated with MF performed obviously S phase arrest (Figure 3A-D). Meanwhile, cell apoptosis rate was significantly elevated along with the increased concentration of MF (Figure 3E-H). This notion was further supported by the observation that the protein expression levels of cell proliferation markers ki67, PCNA and anti-apoptotic protein Bcl-2 were decreased and the apoptosis-related proteins Bax and Cleaved caspase-3 were upregulated concomitant with the elevated concentration of MF (Figure 3I, J). Collectively, these results demonstrated that MF inhibited cell proliferation via inducing cell cycle arrest and cell apoptosis.

**MF suppressed tumor growth in vivo.**

We then examined whether MF could inhibit cell proliferation in vivo. Nude mice were injected with HNSCC cells and then oral administration of MF in concentration of 15 mg/kg when the tumor volume was about 100 mm$^3$ (the tenth day after cell injection). Mice given equal volume DMSO (0 mg/kg) was employed as control group. Compared with the control group, tumor volume in the MF treated group was remarkably decreased after 19 days tumor inoculation, indicating that MF suppressed HNSCC cells growth in vivo (Figure 4A, B). There was no organ toxicity in MF treated group relative to control group (Figure 4C). In addition, the IHC staining results showed that ki67 was decreased and Cleaved caspase-3 was increased in the MF treated group compared with the control group (Figure 4D, E). Agreed with the IHC data, western blotting revealed the downregulated protein expression levels of ki67, PCNA, Bcl-2 and the upregulated protein expression levels of Bax and Cleaved caspase-3 after mice treated with MF (Figure 4F, G).

**PTPN11 was the core target of MF against HNSCC**
We then performed network pharmacology analysis to identify the potential targets of MF against HNSCC. Total 168 genes set as the potential targets of MF against HNSCC were obtained from the intersection of 225 genes of MF targets from pharmmapper, 4748 genes and 16986 genes associated with HNSCC from GeneCards and CTD, respectively (Figure 5A). The GO analysis results showed that the 168 genes were mainly associated with the response to steroid hormone, nuclear receptor activity (Figure 5B). KEGG analysis revealed that proteoglycans in cancer was the most significantly enrichment signaling pathway of the potential targets of MF against HNSCC (Figure 5C, Table 3). 168 genes were imported into STRING database and then the primary PPI network with 394 edges and 134 nodes (namely targets) was obtained. 134 targets were performed by two-step topology analysis and finally 10 targets were identified (Figure 5D). To further narrow the potential functional targets, we then took the intersection of 10 targets and 26 genes enriched in proteoglycans in cancer. As a result, 8 genes EGFR, ESR1, GRB2, IGF1, IGF1R, MAPK1, PTPN11, SRC were identified (Figure 5E). ESR1 and IGF1 were ruled out due to the non-significantly and downregulated mRNA expression level, respectively, in HNSCC compared with normal tissues by analyzing the TCGA database (Figure 6A). We then detected the mRNA expression levels of 6 genes after cells treated with MF. Interestingly, only the expression level of PTPN11 notably decreased in a MF dose-dependent manner (Figure 6B). Molecular docking revealed PTPN11 showed the particularly strongest combination with MF and the binding energy was -8.3 kcal/mol (Figure 6C). Taken together, these data strongly indicated MF was involved in multiple tumors associated signaling pathways and the most important thing was that MF maybe exert anti-tumor activity by targeting PTPN11.

**MF exerted anti-tumor activity by targeting PTPN11**

To further investigate whether the inhibition of PTPN11 was required for the anti-tumor efficacy of MF, we constructed the stable overexpression PTPN11 cells. The efficiency of overexpression was confirmed in mRNA and protein level (Figure 7A-C). Interestingly, we observed that cells treated with MF revealed a significantly decreased expression level of PTPN11 but there was still a relative higher expression of PTPN11 compared with the control group cells (Figure 7A-C), indicating that MF couldn’t completely inhibit the overexpression of PTPN11. After cells treated with MF, cell viability of the cells transfected with PCDH plasmid was slightly better than that of PTPN11 overexpressed (Figure 7D), indicating that MF could inhibit cell proliferation after PTPN11 overexpressed. Similarity results were observed in the western blotting (Figure 7E, F), after MF treated, protein expression levels of ki67, PCNA, Bcl-2 in cells transfected with PCDH plasmid were slightly higher than that of PTPN11 overexpressed cells. Taken together, MF could target PTPN11 to exert anti-tumor activity.

**Discussion**

Served as the classical anti-inflammation drug, accumulating evidence has demonstrated that glucocorticoids also could treat malignant tumors such as lymphoid malignancies[23], prostate cancer[24], rectal cancer[25] and breast cancer[26]. Inflammation was closely related with the
development of most type of cancers. Cancer-extrinsic inflammation triggered by elements such as viral infections and autoimmune disease has been reported as the induced factors of tumor initiation and progression. Cancer-intrinsic inflammation is involved in the recruitment and activation of inflammatory cells exerting the immunosuppressive tumor microenvironment and eventually accelerates malignant progression. Therefore, the anti-inflammation drugs targeting the inflammatory tumor microenvironment has been identified as the pivotal determinant for the conventional chemotherapy and immunotherapy efficacy[27]. Besides, it has been reported that the overexpressed of inflammation-related factors was related to the drug resistant. Increased circulating cytokines interleukin-6 expression was associated with the acquired resistance to dasatinib[28], which was further confirmed by a clinical phase II clinical trial in HNSCC[29]. Furthermore, anti-inflammatory drugs could attenuate the toxicity of chemotherapeutic agents[30]. For instance, combined with docetaxel and celecoxib to treat patients with metastatic prostate cancer could reduce hematologic toxicity[31]. And emerging studies reported that local application of MF remarkably reduced acute radiation dermatitis after radiotherapy of HNSCC[32] and breast cancer[33]. Based on aforementioned intimate relationship between inflammation and tumor, anti-inflammation drugs seem to be a powerful method to enhance the tumor therapeutic efficiency in HNSCC. MF is a traditionally glucocorticoid with effective anti-inflammatory capacity, however, previous studies regarding MF mostly focused on its treatment for inflammation and researchers has just kick started the engine to delineate the role of MF on cancer. Since glucocorticoids could promote cell apoptosis via binging with glucocorticoid receptors in lymphoid cells, most extensive application of glucocorticoids in cancer is to treat lymphoid malignancies[23, 34]. Consistent with that, it was reported MF could inhibit the growth of acute leukemia cells through promoting cell apoptosis[10]. While there was still no exploration of the pharmacological effect of MF on solid tumor. Here, we found MF could inhibit the HNSCC cell proliferation both in vitro and in vivo. Mechanistically, the protein expression level of anti-apoptotic molecule Bcl-2 was significantly decreased and that of the pro-apoptotic Bax was increased after cell treated with MF, which were consistent with the previous study that the downregulation of Bcl-2 was essential for glucocorticoid induced cell apoptosis[34]. Meanwhile, the decreased Bcl-2 subsequently releases cytochrome C into the cytoplasm and then promotes the activation of Cleaved caspase-3, which also is a sign of cell apoptosis. Our results also revealed that the protein expression level of Cleaved caspase-3 was remarkably increased after MF treated. These results supported the notion that MF could exert anti-tumor effect via promoting cell apoptosis.

For better understanding the inhibitory effect of MF in HNSCC, we then conducted network pharmacology and molecular docking, which would be effectively aid methods to provide the more comprehensive perspective of MF against HNSCC. KEGG analysis demonstrated that the potential target genes of MF against HNSCC were mainly associated with proteoglycans in cancer signaling pathway, which was related to various biological process of cancer[35]. By a series of bioinformatics analysis, PTPN11 seemed to be the most important target of MF against HNSCC. Our experimental results confirmed that MF decreased the mRNA expression level of PTPN11 in a dose-dependent manner. It is widely known that sustained activation of PTPN11 was responsible for the occurrence, development and prognosis in multiple cancers. Allosteric inhibition of PTPN11 via SHP099 suppressed the RTKs-driven human cancer
cells through inhibiting RAS-ERK signaling pathway[36]. RAS was overexpressed in HNSCC cells and was responsible for the tumor growth[37]. Therefore, we supposed that MF exerted the excellent anti-tumor capacity via targeting PTPN11. To verify that, we constructed PTPN11 overexpressed cells to explore whether MF could perform anti-tumor capacity by suppressing PTPN11. More interestingly, we observed that MF indeed could decrease the mRNA and protein expression levels of PTPN11 as well as inhibit the cell proliferation and induce cell apoptosis after PTPN11 overexpressed, however, MF couldn’t completely eliminate the exogenous overexpression of PTPN11 as well as the promoting cell growth caused by PTPN11 overexpressed. We predicted that there were 8 genes of MF against HNSCC via network pharmacology analysis, thus MF also has the potential to bind with the other targets, resulting in the competitive combination between the targets and MF, which maybe account for the reason why MF couldn’t inhibit PTPN11 completely. Further studies are needed to identify this issue. In addition, among the 8 targets, EGFR is upregulated in 80% – 90% HNSCC patients and the overexpression of EGFR is associated with the poor prognosis[38]. EGFR monoclonal antibody cetuximab combined with chemotherapy or radiotherapy was approved by FDA as the first-line therapy of HNSCC patients, while the acquisition EGRF resistant was a frequent occurrence finally leading to the failure of therapy. What’s more, PTPN11 inhibitor could overcome EGFR resistant in NSCLC[39]. Thus, we reasonably assumed that the single usage of MF could target PTPN11 to produce a marked anti-tumor effect and the combination usage of MF and PTPN11 inhibitor could conquer the drug resistant to achieve better therapeutic effect of HNSCC.

Overall, we proved that MF could exert an excellent anti-tumor effect through regulating PTPN11, which provides a theoretic basis for the clinical application, however, the detailed molecular mechanism still needs to further exploration for the clinical drug delivery.

Conclusion

We preliminarily revealed that MF played a vital role in suppressing the proliferation of HNSCC via regulating the expression level of PTPN11, which provided a new perspective of MF in the treatment of HNSCC.

Declarations

Ethical Approval

The ethics approval statements of all animal experiments were approved by the Institutional Animal Care and Use Committee of the Peking University Health Science Center and followed the Committee of Peking University Health Science Center’s Animals Usage Guideline.

Consent for publication

All authors approved the publication of the manuscript.
Competing interests

The authors report no conflicts of interest in this paper.

Authors' contributions

Lin Qiu and Qian Gao contribute equally to this work and share first authorship. Lin Qiu, Qian Gao and Anqi Tao performed experiments; Lin Qiu and Qian Gao analyzed the data; Lin Qiu, Qian Gao, Jiuhui Jiang and Cuiying Li wrote the manuscript. All authors reviewed the manuscript.

Corresponding author

Correspondence to Jiuhui Jiang or Cuiying Li.

Funding

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Availability of data and materials

The data supporting this study are available from the corresponding author according to reasonable requirements.

References


Tables

Table 1. The real-time PCR primers.
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<tr>
<th>Gene</th>
<th>Primer sequence</th>
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<tr>
<td>EGFR</td>
<td>F GGTGAGTGCGCTTGTCTGGAA</td>
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<tr>
<td>EGFR</td>
<td>R CCTTACGCCCTTCACTGTGT</td>
</tr>
<tr>
<td>GBR2</td>
<td>F AAGCTACTGCAGACGACGAG</td>
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<td>GBR2</td>
<td>R CTTGGCTCTGGGGATTTTG</td>
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<tr>
<td>IGF1R</td>
<td>F AGGCTGGGGCTTTGTTTAC</td>
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<td>IGF1R</td>
<td>R CCTCTCTCGAGTTGCCTTG</td>
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<tr>
<td>SRC</td>
<td>F TTCTGCTTTGACTGGCTGT</td>
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<td>SRC</td>
<td>R TGAGGATGGTCAGGGT</td>
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<tr>
<td>PTPN11</td>
<td>F CGTCATGCCTGTAGGAACG</td>
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<td>R TCTCTCCGTATTCCCCTTGG</td>
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<tr>
<td>MAPK1</td>
<td>F TCCTTTGAGCCGTTGGAGG</td>
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<td>MAPK1</td>
<td>R AGTACATACTGCAGGTC</td>
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Table 2. Oligonucleotides sequence used for PTPN11 overexpressed.

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<th>Primer</th>
<th>Oligonucleotides sequence</th>
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<td>PTPN11-OE-F</td>
<td>GGGGGAGGAGGGGATCCCGGAATGACATCGCGGAGATGGT</td>
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<td>PTPN11-OE-R</td>
<td>GATCCTTCGCGCCGCGGATCCTCATCTGAACTTTTCTGC</td>
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Table 3. The top ten KEGG pathways.
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<th>ID</th>
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<th>p.adjust</th>
<th>qvalue</th>
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<tr>
<td>hsa05205</td>
<td>Proteoglycans in cancer</td>
<td>26/150</td>
<td>4.1534E-15</td>
<td>1.109E-12</td>
<td>5.2901E-13</td>
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<td>hsa01522</td>
<td>Endocrine resistance</td>
<td>18/150</td>
<td>1.4301E-13</td>
<td>1.9092E-11</td>
<td>9.1077E-12</td>
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<td>hsa05215</td>
<td>Prostate cancer</td>
<td>17/150</td>
<td>1.5834E-12</td>
<td>1.4092E-10</td>
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<td>hsa01521</td>
<td>EGFR tyrosine kinase inhibitor resistance</td>
<td>15/150</td>
<td>1.0373E-11</td>
<td>6.9241E-10</td>
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<td>hsa04068</td>
<td>FoxO signaling pathway</td>
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<td>1.3092E-09</td>
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<td>hsa04659</td>
<td>Th17 cell differentiation</td>
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<td>Prolactin signaling pathway</td>
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<td>3.5417E-09</td>
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**Figures**

![Two-dimensional](image1.png) ![Three-dimensional](image2.png)
Figure 1

The structure of MF.

Figure 2

The effect of MF on the cell proliferation of HNSCC cells in vitro.

(A, B) The IC$_{50}$ of WSU-HN6 cells (A) and CAL-27 cells (B) were detected after cells treated with 0, 10, 20, 40, 60, 80, and 100 µM MF for 24, 48, and 72 h.

(C, D) The percentage of cell viability of WSU-HN6 cells (C) and CAL-27 cells (D) after cells treated with 0, 10, 20, and 50 µM MF for 24, 48, and 72 h.
(E) The colony formation of WSU-HN6 and CAL-27 cells after cells treated with 0, 10, 20, and 50 μM MF for 7 days.

MF induced cell cycle arrest and cell apoptosis of HNSCC cells in vitro.
(A-D) Distribution (A, C) and statistics analysis (B, D) of WSU-HN6 and CAL-27 cell cycle, respectively, for cells treated with 0, 10, 20, and 50 μM MF for 24 h.

(E-H) Representative flow cytometry plots (E, G) and statistics analysis of the cell apoptosis rate (F, H) of WSU-HN6 and CAL-27 cells, respectively, for cells treated with 0, 10, 20, and 50 μM MF for 24 h.

(I) The expression levels of proliferation- and apoptosis-related proteins of WSU-HN6 and CAL-27 cells for cells treated with 0, 10, 20, and 50 μM MF.

(J) The quantitative analysis results of (I).

*, #, & P < 0.05, **, ##, && P < 0.01, ### P < 0.001, n = 3. * was G1 phase, apoptosis or the corresponding protein expression levels of cells treated with 10, 20, and 50 μM MF (MF treated groups) compared with cells treated with 0 μM MF (control group), # was S phase in MF treated groups compared with control group; & was G2/M phase in MF treated groups compared with control group.
Figure 4

MF inhibited HNSCC progression in vivo.

(A) Representative images of tumor-bearing mice and tumor samples.
(B) Tumor volume of the mice treated with 15 mg/kg MF compared with the mice treated with 0 mg/kg MF.

(C, D) HE staining images of heart, liver, spleen, lung, kidney (C) and tumor tissues (D) of the mice treated with 0 mg/kg, 15 mg/kg MF.

(E) IHC staining images of tumor tissues of the mice treated with 0 mg/kg, 15 mg/kg MF.

(F) The expression levels of proliferation- and apoptosis-related proteins of the mice treated with 0 mg/kg, 15 mg/kg MF.

(G) The quantitative analysis results of (F).

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, $n = 3$. * was mice treated with 15 mg/kg MF compared with mice treated with 0 mg/kg MF.
Figure 5

Network pharmacology analysis of the MF targets against HNSCC.

(A) Venn diagram of MF targets and HNSCC-related genes.

(B) The top ten GO enrichment chart.
Figure 6

PTPN11 was the core target of MF against HNSCC

(A) The expression of the eight targets in TCGA database. The blue boxes represented normal tissue and the red boxes represented HNSCC tumor tissue.

(B) The mRNA expression levels of the six targets of MF against HNSCC after cells treated with 0, 10, 20, and 50 μM MF for 24 h.

(C) Molecular docking of the six targets of MF against HNSCC. The affinity represented the binding energy.
$* P < 0.05$, $** P < 0.01$, $*** P < 0.001$, $n = 3$. * was HNSCC tumor tissues compared with normal tissue or cells treated with 10, 20, and 50 $\mu$M MF compared with cells treated with 0 $\mu$M MF.

**Figure 7**

MF inhibited the HNSCC progression induced by PTPN11 overexpression.

(A, B) The mRNA (A) and protein (B) expression levels of PTPN11 of the PTPN11 overexpressed cells treated with MF.

(C) The quantitative analysis results of (B).
(D) The percentage of cell viability of the PTPN11 overexpressed cells treated with MF.

(E) The expression levels of proliferation- and apoptosis-related proteins of the PTPN11 overexpressed cells treated with MF.

(F) The quantitative analysis results of (E).

*, #, & $P< 0.05$, **, ##, && $P< 0.01$, $$$, &&& $P< 0.001$, n = 3. * was PCDH+, MF+ group or PTPN11+, MF- group compared with PCDH+, MF- group (A, C). # was PTPN11+, MF+ group compared with PTPN11+, MF- group (A, C). & was PTPN11+, MF+ group compared with PCDH+, MF+ group (A, C). * was PCDH+, MF+ group compared with PCDH+, MF- group (D, F). # was PTPN11+, MF+ group compared with PCDH+, MF+ group (D, F).