Article

Discovery Medicine 2024; 36(180): 71–81
https://doi.org/10.24976/Discov.Med.202436180.6

Curcumin Inhibits Bladder Cancer by Inhibiting Invasion via AKT/MMP14 Pathway

Kai Wang[1], Wen Xiao[2,3], Qi Zeng[4,*]

[1]Department of Pharmacy, Hunan Provincial People's Hospital (The First Affiliated Hospital of Hunan Normal University), 410006 Changsha, Hunan, China
[2]Hunan Provincial Institute of Emergency Medicine, Hunan Provincial People's Hospital (The First Affiliated Hospital of Hunan Normal University), 410006 Changsha, Hunan, China
[3]College of Life Science, Hunan Normal University, 410081 Changsha, Hunan, China
[4]Department of Ultrasonic Diagnosis, The First Affiliated Hospital of Gannan Medical University, 341001 Ganzhou, Jiangxi, China
*Correspondence: gyfycszq@163.com (Qi Zeng)
Published: 20 January 2024

Background: Bladder cancer is a malignant tumor of the urinary and reproductive tract that seriously threatens human health. It is urgent to develop new drugs for bladder cancer. This study aims to explore whether curcumin could inhibit bladder cancer and the potential mechanism.

Methods: Firstly, network pharmacology was applied to explore the potential target of curcumin in bladder cancer. Among the potential target of curcumin on bladder cancer, the role of matrix metalloproteinase-14 (MMP14) was further explored by bioinformatic analysis and the expression of MMP14 was confirmed by immunohistochemistry staining. The effect of curcumin on bladder cancer was then studied using the cell counting kit-8 (CCK-8) assay, clone formation assay, apoptosis assay, and Transwell assay. Finally, AKT, MMP14, E-cadherin and N-cadherin were analyzed by Western blot assay to confirm whether curcumin could inhibit bladder cancer by inhibiting invasion via AKT/MMP14 pathway.

Results: In the present study, we found that the target of curcumin for bladder cancer includes signal transducer and activator of transcription 3 (STAT3), AKT, cyclin A2 (CCNA2), epidermal growth factor receptor (EGFR), E1A binding protein p300 (EP300) and MMP14. MMP14 was highly expressed in bladder cancer than in normal tissues and was associated with a worse prognosis ($p < 0.05$). Curcumin could inhibit the proliferation and migration of bladder cancer cells ($p < 0.05$), while promoting cell apoptosis by inhibiting the AKT/MMP14 pathway ($p < 0.05$).

Conclusion: Curcumin could inhibit bladder cancer by inhibiting invasion through the AKT/MMP14 pathway.

Keywords: bladder cancer; curcumin; AKT; MMP14

Introduction

Bladder cancer is a malignant tumor of the urinary and reproductive tract that seriously threatens human health. Its incidence rate is seventh among malignant tumors, and about 150,000 patients worldwide die from this disease every year [1,2]. The cause of bladder cancer is not clear and is related to many factors. The incidence of bladder cancer is now believed to be related to genetic polymorphism, occupation, smoking, diet, long-term use of certain drugs, infection, and other factors, etc. [3]. In the past 30 years, bladder cancer has been treated in a variety of ways. Currently, classic methods for treating bladder cancer are transurethral resection of the bladder tumor and radical cystectomy [4,5]. However, research shows that the rate of recurrence in patients with high-risk bladder cancer after transurethral cystectomy is very high five years later, which may be due to the strong invasive capacity of bladder cancer, leading to easy recurrence and metastasis in patients after surgery. The survival rate for patients with high-risk bladder cancer is low [6,7]. In addition to resection, bladder perfusion is another effective adjuvant treatment, which can reduce the rate of tumor recurrence. However, more than half of patients will have side effects such as hematuria, cystitis, and systemic symptoms, and are also prone to tumor drug resistance [8,9]. Therefore, it is very meaningful to develop new drugs for the treatment of bladder cancer.

Turmeric (*Curcuma longa*) is a dry tuber of a perennial herbaceous plant, classified as part of the ginger family. It is grown mainly in tropical and subtropical regions and has been widely used as a seasoning and treatment of food safety for various diseases [10,11]. Curcumin is an active ingredient extracted from Turmeric (*Curcuma longa*). The molecular formula of curcumin is $C_2H_{20}O_6$. As a food additive, curcumin has a long history and many studies show that it has little side effects on the human body [12–14]. More and more studies have shown that curcumin

has a strong antioxidant function, an anti-inflammatory effect, mediation of body immunity, protection of cardiovascular and other biological activities [15–17]. Curcumin has been clinically found to have good therapeutic effects on diabetes, Alzheimer's disease, and other chronic diseases [18,19]. Research also shows that curcumin has a broad spectrum of antitumor activity, including kidney cancer, prostate cancer, ovarian cancer, and other malignant tumors. Curcumin can inhibit tumor cell survival, proliferation, invasion, migration, and induce apoptosis and autophagy [20–22].

In most types of tumors, the AKT signaling pathway is activated, while in normal cells, AKT signaling is inhibited [23]. Research has shown that the AKT signaling pathway is closely related to tumor angiogenesis and invasion, and the development of new antitumor drugs targeting the inhibition of the AKT signaling pathway is currently a research hotspot [24,25]. The AKT signaling pathway in patients with bladder cancer also has abnormal activation [26]. Some studies have shown that AKT up-regulates matrix metalloproteinase-14 (MMP14) to promote the invasion of liver cancer, gastric cancer, and glioma, but the AKT regulation of MMP14 has not been reported in bladder cancer [27–29]. Curcumin has been reported in prostate cancer and melanoma, which can inhibit the expression of MMP14 and play an antitumor role. However, it is unknown whether curcumin can inhibit the expression of MMP14 in bladder cancer [30].

Therefore, this study first analyzed the potential curcumin target in bladder cancer through network pharmacology and then confirmed the expression and role of MMP14 in bladder cancer through bioinformatic analysis. Taking the bladder cancer T24 cell model as the research object, this study explored that curcumin inhibits MMP14 expression through the AKT signaling pathway to inhibit invasion and metastasis of bladder cancer, providing a scientific basis for the clinical application of curcumin and also provides a new strategy for the treatment of bladder cancer.

Material and Method

Reagent

The cell counting kit-8 (CCK-8) was purchased from Dojindo (#CK04, Kumamoto, Japan). Curcumin was obtained from Selleck (#S1848, Shanghai Lanmu Chemical Co., Ltd., Shanghai, China). The Annexin V/FITC apoptosis detection kit was purchased from Jiamay Biotech Co., Ltd. (#LHK601-050-P, Beijing, China). Anti-E-cadherin (#14472), anti-N-cadherin (#13116), anti-phospho-AKT (#4060), anti-AKT (#4685), anti- glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (#5174) and anti-rabbit immunoglobulin G (IgG), horseradish peroxidase (HRP)-linked antibody (#7074) were obtained from Cell signaling technology (Danvers, MA, USA). Anti-MMP14 (#ab51074) was obtained from Abcam (Cambridge, UK).

Curcumin Target Prediction

Use the PubChem database to obtain the molecular structure of curcumin, predict the potential target protein of curcumin based on the Swiss Target Reduction platform, and use the UniProt database (http://www.uniprot.org) to standardize the name of the target protein as the target gene for curcumin. Bladder cancer disease target: Take bladder cancer as the keyword, search the GeneCards database (http://www.genecards.org/) and select with Relevance score $\geq$10 as the condition to obtain the target of bladder cancer disease.

Construction of a Curcumin-Bladder Cancer Network

Venn analysis was carried out on the obtained curcumin drug targets and bladder cancer disease targets. Common targets of both are potential curcumin targets in the treatment of bladder cancer. The intersection targets of curcumin and drug diseases were imported into Cytoscape software (version 3.9, Oracle Company, CA, USA) for visualization, and the curcumin-bladder cancer network diagram was established.

Gene Ontology (GO) Analysis

Using Gene Ontology (GO) annotations from the R software package org. Hs. eg db (version 3.1.0, University of Auckland, Auckland City, New Zealand) as a background, map genes to the background set, and perform enrichment analysis using the R software package clusterProfiler (version 3.14.3, University of Auckland, Auckland City, New Zealand) to obtain the results of gene set enrichment. Set the minimum gene set at 5, the maximum gene set at 5000, a p-value of <0.05 and an false discovery rate (FDR) of <0.25 were considered statistically significant.

Kyoto Encyclopedia of Genes and Genomes (KEGG) Analysis

Using the Kyoto Encyclopedia of Genes and Genomes (KEGG) rest application programming interface (API) (https://www.kegg.jp/kegg/rest/keggapi.html) to obtain the latest annotation of the KEGG Pathway gene as background. Then map the genes to the background set and use the R software package clusterProfiler for enrichment analysis to obtain the results of enrichment of the gene set. Set the minimum gene set at 5, the maximum gene set at 5000, a p-value of <0.05 and an FDR of <0.25 were considered statistically significant.

Protein-Protein Interaction (PPI) Networks Analysis

Importing drug disease intersection targets into the STRING platform (http://string-db.org) to construct a protein interaction protein-protein interaction (PPI) network. Based on Cytoscape software (version 3.9, Oracle Com-

pany, CA, USA) optimization and topology analysis, targets with average values above the mean degree, centrality betweenness and centrality proximity were selected as key core targets.

Molecular Docking

Obtain 2D or 3D structures of compounds and proteins from the PBD website (http://www.rcsb.org), use AutoDock Tools (version 1.5.6, Scripps Institute, FL, USA) for dehydration, hydrogenation, and charge addition, and determine the position and size of the grid box at the protein-ligand binding site. Then, the LibDockScore score is selected to reflect the binding efficiency between the protein molecules and compounds, and the affinity between the compounds and target proteins is evaluated.

Differential Analysis of Gene Expression

Download the microarray gene expression profiling and corresponding clinical information of 431 bladder cancer tissues from the TCGA data portal website (https://xena.ucsc.edu), and download 19 adjacent tissues from the GTEx database (https://www.gtexportal.org). Download the microarray gene expression profiling and corresponding clinical information of 9 normal bladder tissues from the TCGA data portal website (https://xena.ucsc.edu). And then perform data normalization through the Toil process (28398314). Statistical analysis of gene expression levels was performed using the Welch *t*-test using packages stats [4.2.1] and cars [3.1-0] packages, and finally the data were visualized using the ggplot2 package (version [3.3.6], University of Auckland, Auckland City, New Zealand).

Prognostic Curve and Prognostic Column Chart

Based on the clinical characteristics of the patient and the expression level of MMP14, a proportional risk hypothesis test, risk score, and fitted survival Cox regression analysis were performed using the survival [3.3.1] package. The survival package and the ggplot2 package were used to visualize the results. At the same time, nomogram maps were drawn and visualized using the rms package package (version [6.3-0], University of Auckland, Auckland City, New Zealand) based on the selected clinical features (Pathological stage, histological grade).

Cell Culture

Bladder cancer T24 cells were purchased from Procell Life Science & Technology Co., Ltd. (Wuhan, Hubei, China). The result of the mycoplasma test was negative. Short tandem repeat (STR) identification did not show multiple alleles and no significant cross-contamination of cells, and matched completely with T24 cells in the database of the American type culture collection (ATCC). T24 cells were cultured in McCoy's 5A+10% fetal bovine serum (FBS)+1% penicillin/streptomycin (P/S) medium at 37 ℃ in a 5% CO_2 cell incubator. Take logarithmic growth phase cells for subsequent experiments. McCoy's 5A medium (#16600082), FBS (#10099141C), P/S (#15140148) were purchased from Invitrogen (Carlsbad, CA, USA).

Cell Counting Kit-8 (CCK-8)

T24 cells were seeded in 96 well plates with 5000 cells per well. 24 h later, curcumin of different concentrations was added (0, 10 μM, 20 μM). The culture ended at 24 hours, 48 hours, and 72 hours after the addition of the drug. The 10 uL CCK-8 reagent was added. The OD450 value was detected in the microplate reader and cell proliferation was calculated.

Cell Cloning Experiment

T24 cells were inoculated into 6-well plates with 500 cells in each well. After the cells adhered to the wall, curcumin of different concentrations was added (0, 10 μM, 20 μM). After 24 hours of dosing, they were replaced with fresh medium. After 14 days of continuous culture, the culture was terminated. Cells were fixed with anhydrous ethanol, stained with the Giemsa stain kit (#C0133, Beyotime, Hangzhou, China), and the number of clones was counted.

Apoptosis Analysis

T24 cells were seeded in 6-well plates with 1×10^6 cells per well. After the cells adhered to the wall, different concentrations of curcumin were added. Cells, including cell supernatant, were collected 24 hours after the addition of the drug. According to the instructions of the Annexin V/FITC apoptosis detection kit, stained with 5 μL of Annexin V labeled with fluorescein isothiocyanate and 5 μL of PI for 15 min in the dark. The apoptosis ratio was then analyzed using Cytoflex analysis software (version 2.4, Beckman Coulter life science, Bria, CA, USA) on Beckman Coulter.

Transwell Assay

T24 cells were seeded in 6-well plates with 1×10^6 cells per well. After the cells adhered to the wall, different concentrations of curcumin were added. Cells were collected 24 hours after drug addition. After digestion with 0.25% trypsin digestion (#15050065, Gibco™, Grand Island, NY, USA) digestion, a single cell suspension was prepared with serum-free medium and the cell concentration was adjusted to 1×10^5/mL. Inoculate 200 uL of cell suspension in the upper chamber, and add 500 uL of culture medium containing FBS to the lower chamber for 48 hours. Take out the chamber, wash with phosphate buffer saline (PBS) (#10010002, Invitrogen, CA, USA) for 2–3 times, fix with absolute ethanol at room temperature for 10 minutes, dye with 0.1% crystal violet (diluted with PBS) at room temperature for 10 minutes, and then wash with clean water. The pictures were then randomly selected under a

microscope (#CKX31, Olympus, Tokyo, Japan). The number of migrated cells was counted using the direct counting method, as the number of inoculated cells was consistent. The experiments were repeated in triplicate.

Western Blot

T24 cells were seeded in 6-well plates with 1×10^6 cells per well. After the cells adhered to the wall, different concentrations of curcumin were added. Cells were collected 24 hours after the drug was added to extract the total protein. The bicinchoninic acid (BCA) protein concentration measurement method was used to determine protein concentration, with a sample of 10 ug per well. After electrophoresis, membrane transfer, and incubation of primary antibodies at a dilution of 1:1000 overnight at 4 ℃ and secondary antibodies at a dilution of 1:2000 for 2 h at room temperature, Electrochemiluminescence (ECL) luminescence was used for development and Image J was used for greyscale analysis (version 1.52e, National Institutes of Health, Bethesda, MD, USA).

Hematoxylin-Eosin (HE) Staining

Collect tissues from surgical patients, prepare them into paraffin sections, and use xylene and different concentrations of ethanol for the dewaxing treatment. Paraffin slices were stained with hematoxylin for 1 minute, rinsed with water, differentiated with 1% hydrochloric acid alcohol for 15 seconds, rinsed with water, followed by a solution of 1% ammonia water that returned to blue for 1 minute, rinsed with water for 15 seconds, and stained with eosin dye for 30 seconds. Rinse with water. The paraffin sections were sequentially treated with different concentrations of ethanol and xylene and then sealed with neutral gum for microscopic observation. All pathological samples obtained were informed to each patient and signed an informed consent form, which was also approved by Hunan Provincial People's Hospital ethics committee (Ethical Application Number: 2021-12).

Immunohistochemistry (IHC) Staining

The tissues of surgical patients were collected, prepared in paraffin sections, dried, dewaxed, hydrated, antigen repaired, sealed, incubated with anti-MMP14 (at a dilution of 1:500) and HRP-linked antibody (at a dilution of 1:1000) and then stained with 3,3'-diaminobenzidine (DAB) (#G1212, Servicebio Co., Ltd., Wuhan, China) and hematoxylin. After the tissue dehydration process and transparent paraffin wax, neutral gum sealing was used and observed under the microscope (Olympus, Tokyo, Japan).

Statistical Analysis

Statistical Product and Service Solutions (SPSS) software (version 22.0, SPSS Inc., Chicago, IL, USA) was used for statistical analysis, and the measurement data were expressed as mean plus or minus the standard deviation. Differences between the two groups were analyzed using *t*-tests, while differences between multiple groups were analyzed using one-way analysis of variance (ANOVA) and a Bonferroni *post hoc* test. A bilateral p less than 0.05 was considered statistically significant and all graphs were completed using GraphPad Prism software (version 9.0, GraphPad Software, Inc., La Jolla, CA, USA).

Result

Potential Inhibitory Effect of Curcumin on Bladder Cancer

The network pharmacology method was applied to analyze the curcumin target and related genes for bladder cancer, and we obtained 34 potential curcumin targets for bladder cancer. These include signal transducer and activator of transcription 3 (STAT3), AKT, cyclin A2 (CCNA2), epidermal growth factor receptor (EGFR), E1A binding protein p300 (EP300) and matrix metalloproteinase-14 (MMP14) (Fig. 1A,B and **Supplementary Table 1**). Analysis of these genes showed that the biological process (BP) mainly focused on the negative regulation of cell death and protein physiology (Fig. 1C). The display of the cellular component (CC) focuses mainly on the serine/threonine protein kinase complex and the nuclear part (Fig. 1D). The display of molecular function (MF) is mainly focused on catalytic activity (Fig. 1E). The KEGG display is focused primarily on cancer pathways (Fig. 1F).

Subsequently, we further conducted a protein interaction analysis of PPI and found that the main targets of action were STAT3, AKT, CCNA2, EGFR, EP300, and MMP14 (Fig. 2A,B). Furthermore, we performed a molecular coupling verification and found that curcumin has possible binding sites with STAT3, AKT, CCNA2, EGFR, EP300, and MMP14 (Fig. 2C–H). These results suggest that curcumin may play a role in inhibiting bladder cancer by acting on these action targets.

MMP14 Plays an Important Role in Promoting the Occurrence and Development of Bladder Cancer.

We explored the expression of the *STAT3*, *AKT*, *CCNA2*, *EGFR*, *EP300*, and *MMP14* genes in normal and tumor tissues in the TCGA database and found that *CCNA2 and MMP14* were significantly overexpressed in tumor tissues (Fig. 3A–F) ($p < 0.001$). Then we verified the relationship between *CCNA2* and *MMP14* and the prognosis and found that patients with high expression of *MMP14* had significantly worse prognosis. However, *CCNA2* expression does not have a significant correlation with the prognosis of bladder cancer patients (Fig. 3G and **Supplementary Fig. 1**. And it was found that *MMP14* has a significant prognostic significance for patients (Fig. 3H). We collected samples from 17 patients with pathologically diagnosed with bladder cancer by hematoxylin-eosin (HE) staining (Fig. 3I,J). Subsequently, these slices were also de-

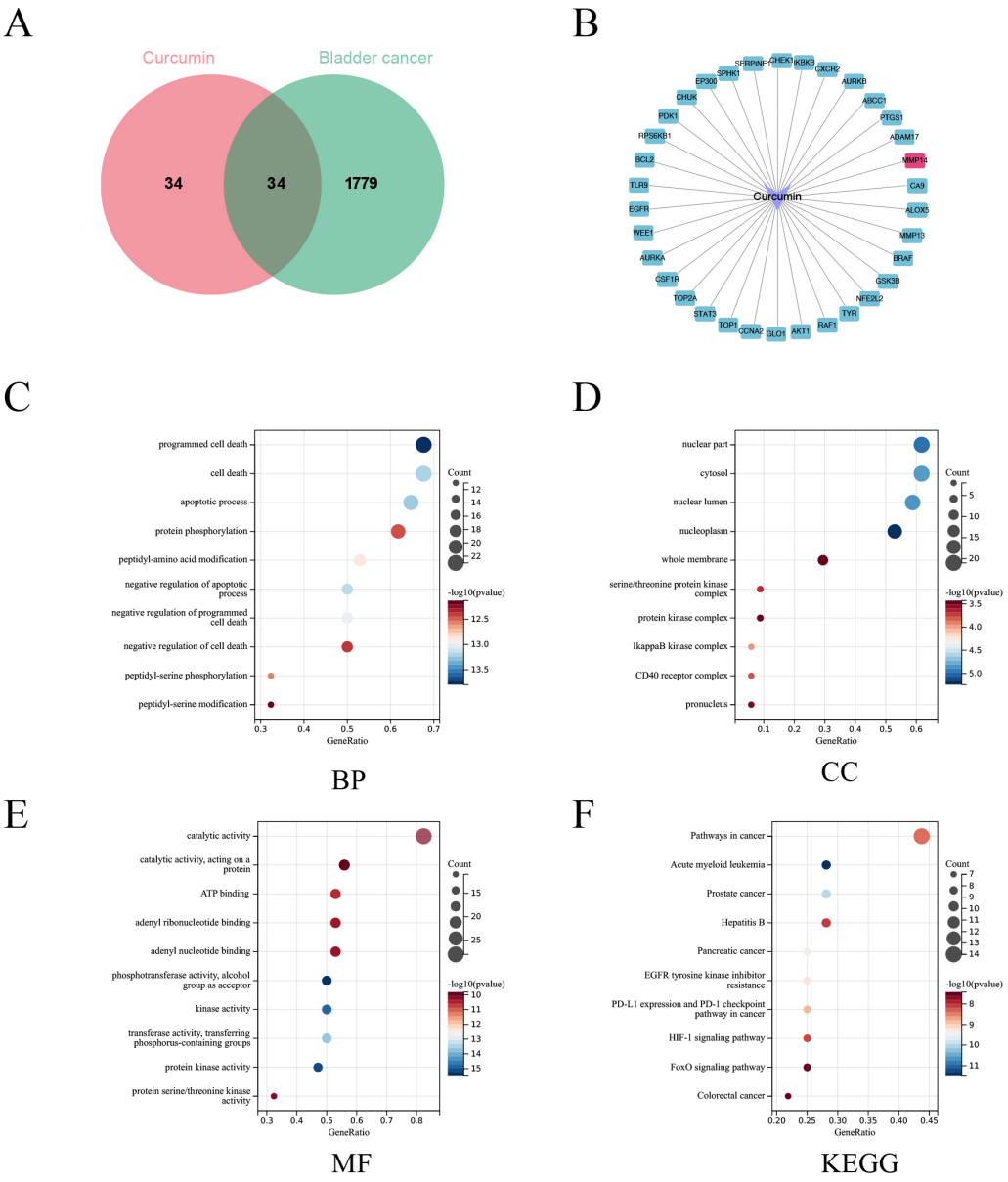

Fig. 1. Potential inhibitory effect of curcumin on bladder cancer. (A) The Venn of the target of curcumin-bladder cancer. There were 34 potential curcumin targets for bladder cancer. (B) The 34 curcumin targets in the treatment of the bladder cancer network. (C) The biological process (BP) analysis of 34 target genes. (D) The analysis of the cellular component (CC) of 34 target genes. (E) The molecular function (MF) analysis of 34 target genes. (F) The Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of 34 target genes.

tected for MMP14 expression using immunohistochemical methods, and it was found that MMP14 was mainly expressed in the cytoplasm, with a positive expression ratio of 13/17 (Fig. 3I,J). The Tumor Node Metastasis (TNM) stage of bladder cancer was according to the 8th edition of American Joint Committee on Cancer (AJCC) Cancer Staging Manual. Information on the clinical characteristics is shown in **Supplementary Table 2**. In future work, we will expand the sample to verify the relationship between the expression level of MMP14 in bladder cancer patients and the prognosis.

Curcumin Inhibits Bladder Cancer Proliferation and Invasion and Promotes the Apoptosis of Bladder Cancer Cells

When T24 cells were treated with curcumin, the CCK-8 results showed that curcumin inhibited T24 cell proliferation activity in a dose-dependent and time-dependent manner (Fig. 4A–C) ($p < 0.05$). Then, we chose the concentration below half-maximal inhibitory concentration (IC50) with proliferation inhibition to observe the inhibitory effect of curcumin on bladder cancer. We treated T24 cells with two concentrations of 10 and 20 µM for 24 h, and the re-

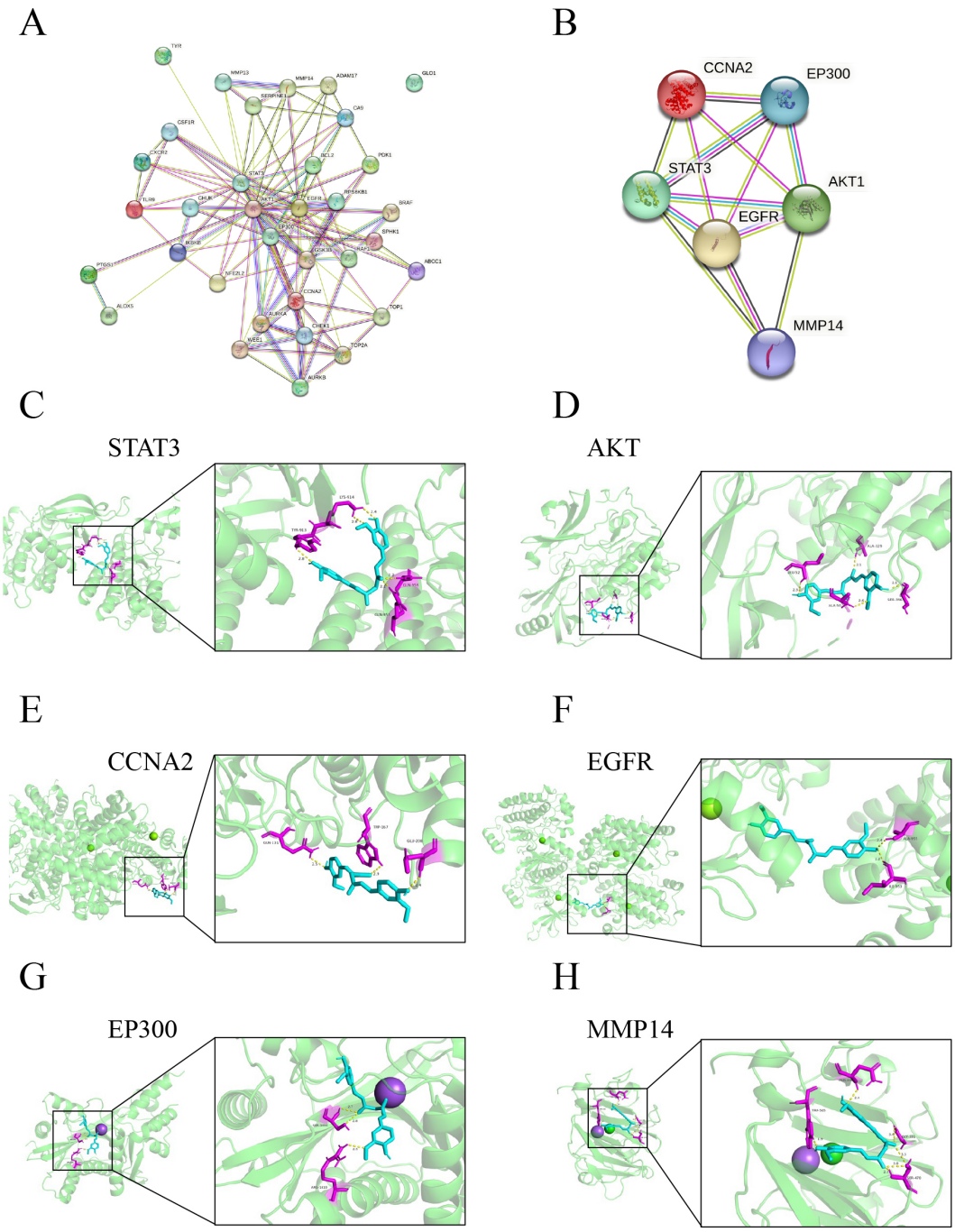

Fig. 2. The core target of curcumin in bladder cancer. (A) The protein-protein interaction (PPI) of curcumin on bladder cancer. (B) The PPI of the top 6 core targets. (C–H) The molecular docking of curcumin with STAT3, AKT, CCNA2, EGFR, EP300, and MMP14.

sults of the cloning experiment also showed that curcumin could inhibit the cloning capacity of T24 cells (Fig. 4D) ($p < 0.01$). The flow cytometric results showed that curcumin could promote T24 cell apoptosis (Fig. 4E) ($p < 0.05$). The results of the Transwell experiment showed that curcumin could inhibit T24 cell migration ability (Fig. 4F) ($p < 0.01$).

Curcumin Plays an Antitumor Role by Inhibiting the AKT Signaling Pathway and Down-Regulating MMP14

Taking into account the important regulatory effect of the AKT signaling pathway on MMP14 reported in the previous literature [25], and the above results indicate that AKT is included in the main curcumin target, we then used Western blot to detect the impact of curcumin on phospho-AKT (p-AKT)/AKT, E-cadherin, N-cadherin and

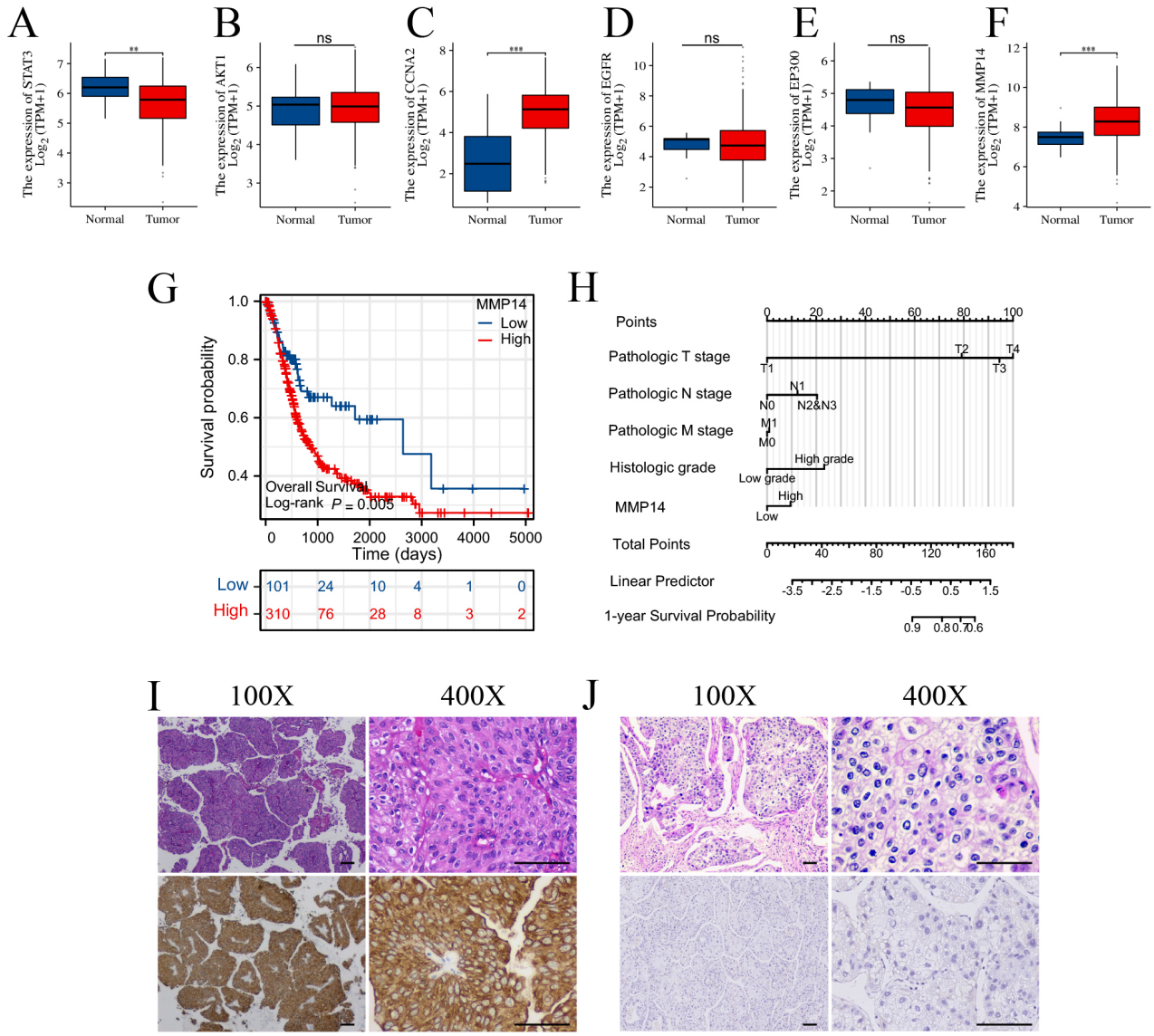

Fig. 3. MMP14 is highly expressed in bladder cancer. (A–F) The expression of the *signal transducer and activator of transcription 3* (*STAT3*), *AKT*, *cyclin A2* (*CCNA2*), *epidermal growth factor receptor* (*EGFR*), *E1A binding protein p300* (*EP300*), and *matrix metalloproteinase-14* (*MMP14*) genes in normal and tumor tissues from the TCGA database. (G) Survival curve of bladder cancer patients with high and low expression of *MMP14*. (H) Prognostic column chart of bladder cancer with *MMP14*. (I) Hematoxylin-eosin (HE) staining and MMP14 positive staining of bladder cancer (scale bar: 100 μm). (J) HE staining and MMP14 negative staining of bladder cancer (scale bar: 100 μm). Note: ns, $p > 0.05$. **$p < 0.01$, ***$p < 0.001$.

MMP14. As expected, curcumin can inhibit the activity of the AKT signaling pathway, while up-regulating E-cadherin and down-regulating the expression of N-cadherin and MMP14 (Fig. 5) ($p < 0.05$). These results support the notion that curcumin plays an antitumor role by inhibiting the AKT signaling pathway and downregulating MMP14.

Discussion

Bladder cancer is one of the malignant tumors that endangers human health. Now, studies have found that curcumin can inhibit bladder cancer in a variety of ways [1–4]. This study analyzed the potential target of curcumin on bladder cancer through network pharmacology, further analyzed the possible mechanism of curcumin against bladder cancer and provided new ideas for future research. We found that curcumin may play an anti-bladder cancer role by targeting 34 genes, which are mainly enriched in protein photosynthesis, apoptotic process, cancer pathways, etc., and may play a role in regulating catalytic activity. Furthermore, we also found that the core target of action is EGFR, AKT1, STAT3, EP300, CCNA2, and MMP14. Possible target sites of action were identified by molecular docking.

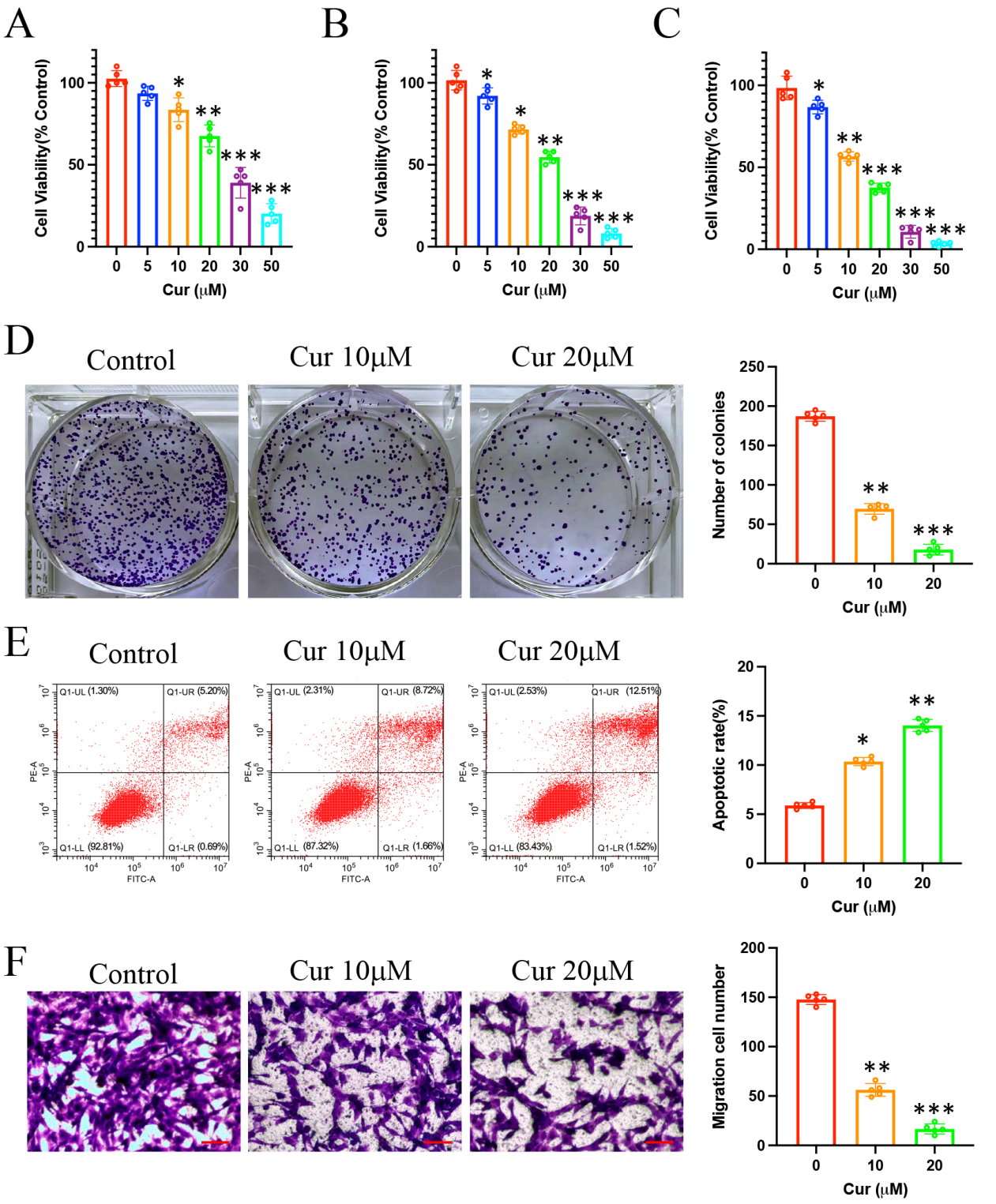

Fig. 4. Curcumin (Cur) inhibits bladder cancer and promotes apoptosis of bladder cancer cells. (A) The cell counting kit-8 (CCK-8) assay of different concentrations of curcumin treatment for 24 h. (B) The CCK-8 assay of different concentrations of curcumin treatment for 48 h. (C) The CCK-8 assay of different concentrations of curcumin treatment for 72 h. (D) The clone formation assay of curcumin treatment for T24 cells. (E) The apoptotic assay of curcumin treatment for T24 cells. (F) The migration assay of curcumin treatment for T24 cells (scale bar: 50 μm). Note: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

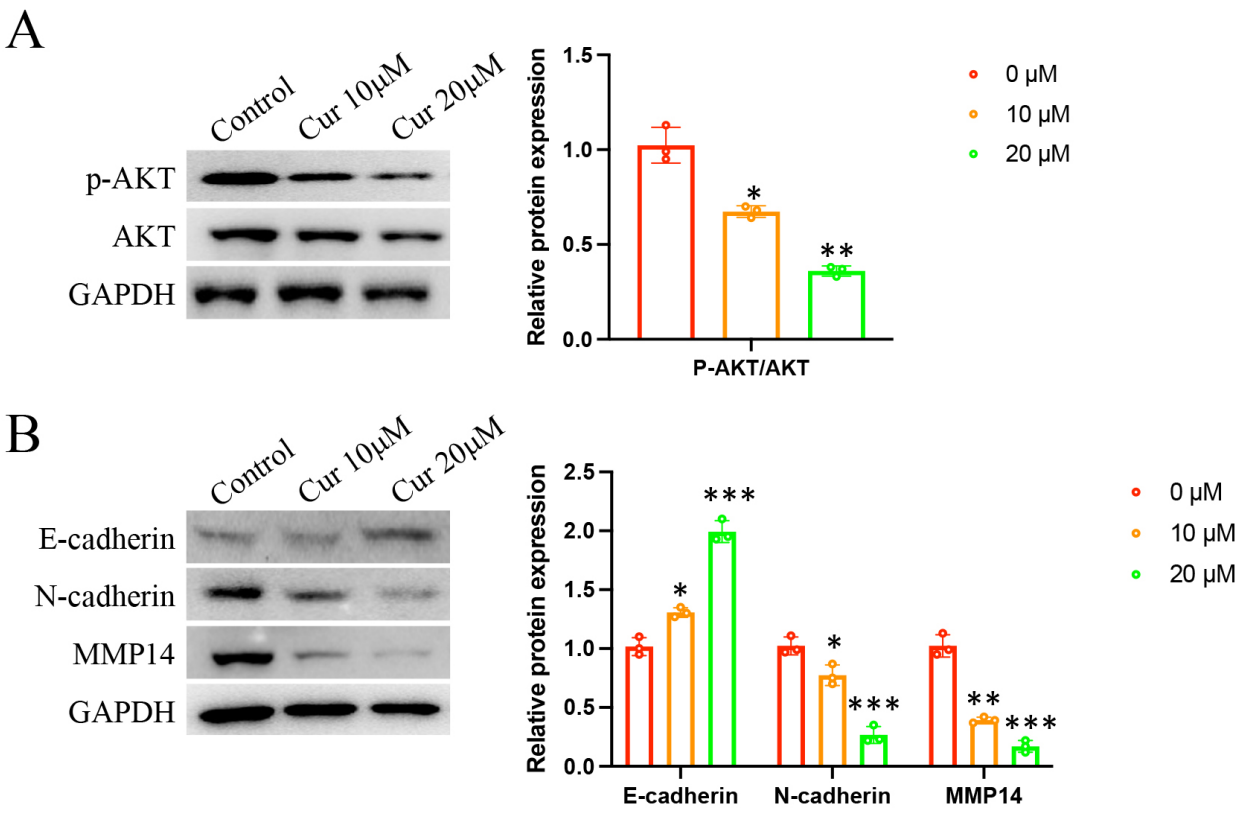

Fig. 5. Curcumin plays an antitumor role by inhibiting the AKT signaling pathway and downregulating MMP14. (A) Western blot assay for protein levels of phospho-AKT (p-AKT)/AKT. (B) Western blot assay for protein levels of E-cadherin, N-cadherin and MMP14. Note: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

Matrix metalloproteinase (MMP) is a collagen hydrolase that plays a role in many biological processes, such as tissue remodeling and growth, wound repair, tissue defense mechanisms, and immune responses [31,32]. Induction of MMP activity contributes to the disintegration of intercellular connections and degradation of the extracellular matrix, thus overcoming the physical limitations of cell movement and participating in tumor invasion [33,34]. MMP14 is an important member of MMPs. More and more evidence shows that MMP14 is involved in malignancy and a poor prognosis of bladder cancer [35,36]. We identified MMP14 in bladder cancer through bioinformatic analysis and found that MMP14 is overexpressed in bladder cancer and associated with a poor prognosis, which can be used as a prognostic marker for bladder cancer patients.

Curcumin can reduce the expression of Survivin and Bid2 and other genes in bladder cancer cells and induce upregulation of the p53 and Bax genes related to apoptosis, thus causing apoptosis in bladder cancer cells [37]. In the rat model, curcumin can negatively regulate vascular endothelial growth factor (VEGF) expression to prevent the proliferation of bladder cancer cells [38]. Curcumin can also up-regulate *miR-203* in bladder cancer cells to inhibit growth and induce apoptosis of bladder cancer cells [39]. Furthermore, curcumin can also be used together with other antitumor drugs to improve the killing effect of cisplatin on tumor cells and the inhibitory effect of epirubicin on bladder tumor cells [37,40]. Through network pharmacology and bioinformatic methods, we have identified that MMP14 may be an important curcumin target protein in bladder cancer, and MMP14 plays an important role in the malignant progression of bladder cancer. Then we also verified in bladder cancer T24 cells that curcumin can inhibit bladder cancer cell proliferation, induce cell apoptosis, inhibit cell invasion and significantly inhibit the level of phospho-AKT (p-AKT), simultaneously regulate the expression of EMT related proteins, promote the expression of E-cadherin, and inhibit the expression of N-cadherin and MMP14.

Conclusion

In summary, this study shows that curcumin can inhibit the invasion and metastasis of bladder cancer. Curcumin has been preliminarily found to inhibit bladder cancer, related to inhibition of the AKT/MMP14 signaling pathway. It provides a scientific basis for curcumin in the treatment of bladder cancer and also provides a new idea for the treatment of bladder cancer.

Availability of Data and Materials

The data sets used or analyzed during the current study are available from the corresponding author upon reasonable request.

Author Contributions

KW designed the research study. KW, WX, and QZ performed the research. KW and QZ collected and analyzed the data. KW and WX have been involved in drafting the manuscript and all authors have been involved in revising it critically for important intellectual content. All authors give final approval of the version to be published. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

This is a non-interventional study. The patients' information and pathological samples collected were approved by Hunan Provincial People's Hospital ethics committee (Ethical Application Number: 2021-12). Their privacy was protected without adding additional risks and financial burdens. Each patient has signed a written informed consent before the study.

Acknowledgment

Not applicable.

Funding

This work was financially supported by the following grant: The Hunan Provincial Natural Science Foundation of China (grant no. 2021JJ70096).

Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.24976/Discov.Med.202436180.6.

References

[1] Lenis AT, Lec PM, Chamie K, Mshs MD. Bladder Cancer: A Review. JAMA. 2020; 324: 1980–1991.

[2] Compérat E, Amin MB, Cathomas R, Choudhury A, De Santis M, Kamat A, *et al*. Current best practice for bladder cancer: a narrative review of diagnostics and treatments. Lancet. 2022; 400: 1712–1721.

[3] Tran L, Xiao JF, Agarwal N, Duex JE, Theodorescu D. Advances in bladder cancer biology and therapy. Nature Reviews. Cancer. 2021; 21: 104–121.

[4] Coen JJ, Zhang P, Saylor PJ, Lee CT, Wu CL, Parker W, *et al*. Bladder Preservation With Twice-a-Day Radiation Plus Fluorouracil/Cisplatin or Once Daily Radiation Plus Gemcitabine for Muscle-Invasive Bladder Cancer: NRG/RTOG 0712-A Randomized Phase II Trial. Journal of Clinical Oncology. 2019; 37: 44–51.

[5] Russell CM, Lebastchi AH, Borza T, Spratt DE, Morgan TM. The Role of Transurethral Resection in Trimodal Therapy for Muscle-Invasive Bladder Cancer. Bladder Cancer. 2016; 2: 381–394.

[6] Piliszek R, Brożyna AA, Rudnicki WR. Computational Analysis Identifies Novel Biomarkers for High-Risk Bladder Cancer Patients. International Journal of Molecular Sciences. 2022; 23: 7057.

[7] Chamie K, Litwin MS, Bassett JC, Daskivich TJ, Lai J, Hanley JM, *et al*. Recurrence of high-risk bladder cancer: a population-based analysis. Cancer. 2013; 119: 3219–3227.

[8] Zhang J, Li M, Chen Z, OuYang J, Ling Z. Efficacy of Bladder Intravesical Chemotherapy with Three Drugs for Preventing Non-Muscle-Invasive Bladder Cancer Recurrence. Journal of Healthcare Engineering. 2021; 2021: 2360717.

[9] Chen H, Wang M, Weng T, Wei Y, Yang L, Ren K, *et al*. Prognostic Analysis of Diagnostic Ureteroscopic Biopsy for Intravesical Recurrence of Upper Urinary Tract Urothelial Carcinoma. Urologia Internationalis. 2022; 106: 186–194.

[10] Vaughn AR, Branum A, Sivamani RK. Effects of Turmeric (Curcuma longa) on Skin Health: A Systematic Review of the Clinical Evidence. Phytotherapy Research. 2016; 30: 1243–1264.

[11] Wang Z, Jones G, Winzenberg T, Cai G, Laslett LL, Aitken D, *et al*. Effectiveness of *Curcuma longa* Extract for the Treatment of Symptoms and Effusion-Synovitis of Knee Osteoarthritis: A Randomized Trial. Annals of Internal Medicine. 2020; 173: 861–869.

[12] Akaberi M, Sahebkar A, Emami SA. Turmeric and Curcumin: From Traditional to Modern Medicine. Advances in Experimental Medicine and Biology. 2021; 1291: 15–39.

[13] Man S, Yao J, Lv P, Liu Y, Yang L, Ma L. Curcumin-enhanced antitumor effects of sorafenib via regulating the metabolism and tumor microenvironment. Food & Function. 2020; 11: 6422–6432.

[14] Yousefsani BS, Dadmehr M, Shirani K, Jamshidi A, Sathyapalan T, Sahebkar A. Health Benefits of Turmeric and Curcumin Against Food Contaminants. Advances in Experimental Medicine and Biology. 2021; 1328: 171–197.

[15] Kocaadam B, Şanlier N. Curcumin, an active component of turmeric (Curcuma longa), and its effects on health. Critical Reviews in Food Science and Nutrition. 2017; 57: 2889–2895.

[16] He Y, Yue Y, Zheng X, Zhang K, Chen S, Du Z. Curcumin, inflammation, and chronic diseases: how are they linked? Molecules. 2015; 20: 9183–9213.

[17] Jabczyk M, Nowak J, Hudzik B, Zubelewicz-Szkodzińska B. Curcumin and Its Potential Impact on Microbiota. Nutrients. 2021; 13: 2004.

[18] Shafabakhsh R, Mobini M, Raygan F, Aghadavod E, Ostadmohammadi V, Amirani E, *et al*. Curcumin administration and the effects on psychological status and markers of inflammation and oxidative damage in patients with type 2 diabetes and coronary heart disease. Clinical Nutrition ESPEN. 2020; 40: 77–82.

[19] Begum AN, Jones MR, Lim GP, Morihara T, Kim P, Heath DD, *et al*. Curcumin structure-function, bioavailability, and efficacy in models of neuroinflammation and Alzheimer's disease. The Journal of Pharmacology and Experimental Therapeutics. 2008; 326: 196–208.

[20] Ibáñez Gaspar V, McCaul J, Cassidy H, Slattery C, McMorrow T. Effects of Curcumin Analogues DMC and EF24 in

Combination with the Cytokine TRAIL against Kidney Cancer. Molecules. 2021; 26: 6302.

[21] Mandalapu D, Saini KS, Gupta S, Sharma V, Yaseen Malik M, Chaturvedi S, *et al*. Synthesis and biological evaluation of some novel triazole hybrids of curcumin mimics and their selective anticancer activity against breast and prostate cancer cell lines. Bioorganic & Medicinal Chemistry Letters. 2016; 26: 4223–4232.

[22] Muhanmode Y, Wen MK, Maitinuri A, Shen G. Curcumin and resveratrol inhibit chemoresistance in cisplatin-resistant epithelial ovarian cancer cells via targeting P13K pathway. Human & Experimental Toxicology. 2022; 41: 9603271221095929.

[23] Li W, Wang Z, Xiao X, Han L, Wu Z, Ma Q, *et al*. Curcumin attenuates hyperglycemia-driven EGF-induced invasive and migratory abilities of pancreatic cancer via suppression of the ERK and AKT pathways. Oncology Reports. 2019; 41: 650–658.

[24] Zhang L, Chen C, Duanmu J, Wu Y, Tao J, Yang A, *et al*. Cryptotanshinone inhibits the growth and invasion of colon cancer by suppressing inflammation and tumor angiogenesis through modulating MMP/TIMP system, PI3K/Akt/mTOR signaling and HIF-1α nuclear translocation. International Immunopharmacology. 2018; 65: 429–437.

[25] Xing Y, Lin NU, Maurer MA, Chen H, Mahvash A, Sahin A, *et al*. Phase II trial of AKT inhibitor MK-2206 in patients with advanced breast cancer who have tumors with PIK3CA or AKT mutations, and/or PTEN loss/PTEN mutation. Breast Cancer Research. 2019; 21: 78.

[26] Che X, Zhan J, Zhao F, Zhong Z, Chen M, Han R, *et al*. Oridonin Promotes Apoptosis and Restrains the Viability and Migration of Bladder Cancer by Impeding TRPM7 Expression via the ERK and AKT Signaling Pathways. BioMed Research International. 2021; 2021: 4340950.

[27] Wang H, Cheng H, Shao Q, Dong Z, Xie Q, Zhao L, *et al*. Leptin-promoted human extravillous trophoblast invasion is MMP14 dependent and requires the cross talk between Notch1 and PI3K/Akt signaling. Biology of Reproduction. 2014; 90: 78.

[28] Regős E, Abdelfattah HH, Reszegi A, Szilák L, Werling K, Szabó G, *et al*. Syndecan-1 inhibits early stages of liver fibrogenesis by interfering with TGFβ1 action and upregulating MMP14. Matrix Biology. 2018; 68-69: 474–489.

[29] Alexiades NG, Auffinger B, Kim CK, Hasan T, Lee G, Deheeger M, *et al*. MMP14 as a novel downstream target of VEGFR2 in migratory glioma-tropic neural stem cells. Stem Cell Research. 2015; 15: 598–607.

[30] Yang J, Wang C, Zhang Z, Chen X, Jia Y, Wang B, *et al*. Curcumin inhibits the survival and metastasis of prostate cancer cells via the Notch-1 signaling pathway. APMIS. 2017; 125: 134–140.

[31] LeBert DC, Squirrell JM, Rindy J, Broadbridge E, Lui Y, Zakrzewska A, *et al*. Matrix metalloproteinase 9 modulates collagen matrices and wound repair. Development. 2015; 142: 2136–2146.

[32] de Almeida LGN, Thode H, Eslambolchi Y, Chopra S, Young D, Gill S, *et al*. Matrix Metalloproteinases: From Molecular Mechanisms to Physiology, Pathophysiology, and Pharmacology. Pharmacological Reviews. 2022; 74: 712–768.

[33] Caria CREP, Gotardo ÉMF, Santos PS, Acedo SC, de Morais TR, Ribeiro ML, *et al*. Extracellular matrix remodeling and matrix metalloproteinase inhibition in visceral adipose during weight cycling in mice. Experimental Cell Research. 2017; 359: 431–440.

[34] Gross KS, Lincoln CM, Anderson MM, Geiger GE, Frick KM. Extracellular matrix metalloproteinase-9 (MMP-9) is required in female mice for 17β-estradiol enhancement of hippocampal memory consolidation. Psychoneuroendocrinology. 2022; 141: 105773.

[35] Wang JF, Gong YQ, He YH, Ying WW, Li XS, Zhou XF, *et al*. High expression of MMP14 is associated with progression and poor short-term prognosis in muscle-invasive bladder cancer. European Review for Medical and Pharmacological Sciences. 2020; 24: 6605–6615.

[36] Wang J, Zhang N, Peng M, Hua X, Huang C, Tian Z, *et al*. p85α Inactivates MMP-2 and Suppresses Bladder Cancer Invasion by Inhibiting MMP-14 Transcription and TIMP-2 Degradation. Neoplasia. 2019; 21: 908–920.

[37] Park BH, Lim JE, Jeon HG, Seo SI, Lee HM, Choi HY, *et al*. Curcumin potentiates antitumor activity of cisplatin in bladder cancer cell lines via ROS-mediated activation of ERK1/2. Oncotarget. 2016; 7: 63870–63886.

[38] Chadalapaka G, Jutooru I, Chintharlapalli S, Papineni S, Smith R, 3rd, Li X, *et al*. Curcumin decreases specificity protein expression in bladder cancer cells. Cancer Research. 2008; 68: 5345–5354.

[39] Saini S, Arora S, Majid S, Shahryari V, Chen Y, Deng G, *et al*. Curcumin modulates microRNA-203-mediated regulation of the Src-Akt axis in bladder cancer. Cancer Prevention Research. 2011; 4: 1698–1709.

[40] Ashrafizadeh M, Yaribeygi H, Sahebkar A. Therapeutic Effects of Curcumin against Bladder Cancer: A Review of Possible Molecular Pathways. Anti-Cancer Agents in Medicinal Chemistry. 2020; 20: 667–677.