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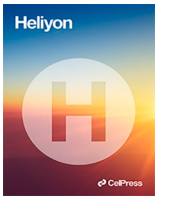

Research article

Exploring the therapeutic mechanism of curcumin in prostate cancer using network pharmacology and molecular docking

Jun Li [a,b], Xiong Wang [c], Li Xue [d,*], Qingmin He [e,**]

[a] School of Medicine, Xi'an Jiaotong University, China
[b] Department of Urology, Ankang Central Hospital, Ankang, 725000, Shaanxi Province, China
[c] Department of Pharmacology, Ankang Maternity and Child Health Care Hospital, Ankang, 725000, Shaanxi Province, China
[d] Department of Urology, The Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, 710004, Shaanxi Province, China
[e] Department of Gastroenterology, Ankang Central Hospital, Ankang, 725000, Shaanxi Province, China

A B S T R A C T

Objective: Curcumin, a phenolic compound extracted from turmeric rhizomes, exhibits antitumour effects in preclinical models of tumours. However, its mechanism of action in prostate cancer remains unclear. Exploring the molecular mechanisms of curcumin in prostate cancer based on network pharmacology and molecular docking provides a new theoretical basis for prostate cancer treatment.
Method: Using tools such as PharmMapper, SuperPred, TargetNet, and SwissTargetPrediction, we obtained information on curcumin-related targets. We comprehensively collected prostate cancer-related targets from several databases, including GeneCards, CTD, DisGeNET, OMIM, and PharmGKB. Cross-cutting drug-disease targets were then derived by screening using the Venny 2.1.0 tool. Subsequently, we used the DAVID platform to perform in-depth GO and KEGG enrichment analyses of the drug-disease-shared targets. To construct a PPI network map of the cross-targets and screen the 10 core targets, we combined the STRING database and Cytoscape 3.7.2. Molecular docking experiments were performed using AutoDockTools 1.5.7 software. Finally, we used several databases such as GEPIA, HPA, cBioPortal, and TIMER to further analyse the screened core targets in detail.
Result: We identified 307 key targets of curcumin in cancer treatment. After GO functional enrichment analysis, we obtained 1119 relevant entries, including 782 biological progression (BP) entries, 112 cellular component (CC) entries, and 225 molecular function (MF) entries. In addition, KEGG pathway enrichment analysis revealed 126 signalling pathways, which were mainly involved in the cancer pathway, such as lipid and atherosclerosis pathway, PI3K-Akt signal pathway, MAPK signal pathway, Ras signal pathways, and chemical carcinogenesis-reactive oxygen species. By applying Cytoscape 3.7.2 software, we identified SRC, PIK3R1, STAT3, AKT1, HSP90AA1, ESR1, EGFR, HSP90AB1, MAPK8, and MAPK1 as core targets. Molecular docking experiments showed that the binding energies of curcumin to these core targets were all below -1.85 kJ mol^{-1}, which fully demonstrated that curcumin could spontaneously bind to these core targets. Finally, these results were validated at multiple levels, including mRNA expression, protein expression, and immune infiltration.
Conclusion: Through in-depth network pharmacology and molecular docking studies, we have found that curcumin may have anticancer potential by upregulating the expression of PIK3R1 and STAT3, and downregulating the binding ability of molecules such as SRC, AKT1, HSP90AA1, ESR1, EGFR, HSP90AB1, MAPK8, and MAPK1. In addition, curcumin may interfere with the cyclic process of prostate cancer cells by inhibiting key signalling pathways such as the PI3K-Akt signalling pathway, MAPK signalling pathway, and Ras, thereby inhibiting their growth. This study not only reveals the potential molecular mechanism of curcumin in the treatment of prostate cancer but also provides an important theoretical basis for subsequent research.

* Corresponding author.
** Corresponding author.
E-mail addresses: Xueli1979@xjtu.edu.cn (L. Xue), 13095037353@163.com (Q. He).

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1. Introduction

Prostate cancer (PCa,Table 1) is the most common malignant tumour in the reproductive system of older men and is among the most prevalent diseases, accounting for approximately 15 % of malignant tumours, and morbidity and mortality rates have been increasing in recent years. Many early-stage patients are effectively treated with androgen deprivation therapy. However, most eventually progress from hormone-sensitive PCa to castration-resistant prostate cancer (CRPC) [1,2]. CRPC is a lethal and highly aggressive form of PCa, with more than 84 % of patients developing metastasis and a median survival of only approximately 20 months [3,4]. Although new chemotherapeutic and endocrine drugs have appeared in recent years, these drugs have several side effects, and are prone to drug resistance, which shortens the duration of drug action and hinders the treatment of PCa. Therefore, there is an urgent need to explore and develop adjuvant drugs for PCa.

Curcumin, a key phenolic compound extracted from turmeric rhizomes, exhibits remarkable anti-tumour, anti-inflammatory, anti-apoptotic, and antioxidant properties [5,6]. Previous studies have revealed that curcumin exhibits potent anti-cancer potential by modulating multiple signalling pathways. It can affect the expression of cell cycle-related genes, such as cell cycle proteins D1, PCNA, and p21, thereby effectively inhibiting the proliferation of prostate cancer cells. In addition, curcumin also up-regulates the expression of miR-34a in prostate cancer cells, while down-regulating the expression of β-catenin and c-myc, which further enhances its anti-cancer effect. Notably, inhibition of miR-34a activates the β-catenin/c-myc axis and affects the expression of cell cycle-related genes, thus weakening the antiproliferative effect of curcumin on prostate cancer cells [7]. Curcumin analogues, such as A10, B10, C10, E10, and F10, similarly exhibit growth-inhibitory effects on prostate cancer cells, and they are able to inhibit testosterone- or dihydrotestosterone-induced AR activity [8]. In addition, curcumin inhibits the JNK pathway, which plays an important role in the epigenetic regulation of prostate cancer cells, and affects the biological behaviour of these cells by inhibiting H3K4me3 [9]. In summary, curcumin exhibits multifaceted effects in prostate cancer treatment, including enhancing the sensitivity of prostate cancer to radiotherapy and chemotherapy, inhibiting cell proliferation, inducing cell death, inhibiting the expression of the PSA gene, decreasing the expression of androgen receptor, and decreasing the motility of tumour cells [10–13]. Although several studies have reported the potential benefits of curcumin in prostate cancer treatment, its specific mechanism of action needs to be comprehensively and thoroughly analysed. Therefore, in-depth studies on the targets and pathways of curcumin-related drugs for prostate cancer treatment will provide a solid theoretical basis and new ideas for the application of curcumin in the comprehensive treatment of prostate cancer.

However, the mechanism underlying the action of curcumin in PCa remains unclear. Network pharmacology is a drug research methodology based on the principles of bioinformatics and systems biology and it combines with pharmacological knowledge to provide a new perspective and tools for drug research [14–17]. Combining network pharmacology with molecular docking technology provides new ideas for drug screening and the mechanistic exploration of complex diseases. Using a molecular dynamics approach combined with kinetic modelling and molecular docking analysis, we provide insight into the dynamics of protein and ligand binding. Curcumin, as the main component of the traditional Chinese herb turmeric, has not been fully explored for its pharmacological

Table 1
Abbreviations in this article.

Abbreviation	Full name
BP	Biological Progress
CC	Celluar component
CTD	Comparative Toxicogenomics Database
DAVID	The Database for Annotation, Visualisation and Integrated Discovery
EGFR	Epidermal growth factor receptor
FYN	Tyrosine-protein kinase Fyn
GC	Gastric cancer
GEO	Gene Expression Omnibus
GO	Gene ontology
HPA	Human Protein Atlas
HRAS	GTPase HRas
HSP90AA1	Heat shock protein HSP 90-alpha
KEGG	Kyoto encyclopedia of gene and genome
LCK	Tyrosine-protein kinase Lck
MAPK1	Mitogen-activated protein kinase 1
MCC	Maximal clique centrality
MF	Molecular Function
OMIM	Online Mendelian Inheritance in Man
OS	Overall Survival
PDB	Protein Data Bank
PPI	Protein-protein interaction
PTK2	Focal adhesion kinase 1
RAC1	Ras-related C3 botulinum toxin substrate 1
RHOA	Transforming protein RhoA
ROS	Reactive oxygen species
SRC	Rabbit squamous cell carcinoma
STRING	Search Tool for the Retrieval of Interaction Gene/Proteins

properties in PCa therapy. Therefore, in a follow-up study, our primary task was to explore the association between curcumin and PCa-causing genes and predict potential target proteins of curcumin using network pharmacology and molecular docking technology. We are committed to elucidating the molecular mechanism of PCa inhibition by curcumin to provide strong theoretical support for its clinical application in PCa research.

2. Materials and methods

2.1. Database and research process

This study covers several databases (Table 2) and briefly outlines the overall research process (Fig. 1).

2.2. Prediction of curcumin and PCa targets

The canonical smiles of curcumin or the SDF files of curcumin 3D structures were used to predict the targets of curcumin in PharmMapper, SwissTargetPrediction, TargetNet, and SuperPred [18–21]. and the target names were converted to gene names in the UniProt database [22], uniformed, and the targets obtained from the four databases were combined and de-emphasised to obtain the target of curcumin action.

PCa-related targets were searched on GeneCards, CTD, DisGeNET, OMIM, and PharmGKB using "prostate cancer" as the search term [23–27].The names of the collected targets were converted to gene names using UniProt, and the targets obtained from these five platforms were collected and de-emphasised to identify the PCa disease targets.

2.3. Screening of common targets of curcumin and PCa

Venny 2.1.0 was used to take the intersection of the curcumin target and PCa disease target, to obtain the common target of disease and drug, and to draw the Venny diagram. The obtained intersection target was the potential target of curcumin for PCa.

2.4. PPI network construction and core target screening

The intersecting targets of curcumin-treated PCa were entered into the STRING database to construct a PPI network, and the tsv files were imported into Cytoscape 3.7.2 for visualisation. The "Degree" in Cytoscape 3.7.2 software was used to filter out 10 core targets (Table 3).

2.5. GO and KEGG enrichment analysis

Potential targets for the curcumin treatment of PCa were analysed in the DAVID database using GO and KEGG enrichment analyses [28]. Enrichment analysis data were analysed and sorted in Excel 2016, and the top seven items from various analyses were selected for visualisation using bioinformatics.

Table 2
Basic information of the database used for the screening of curcumin in the treatment of PCa cancer.

Name	URL
PharmMapper	http://lilab-ecust.cn/pharmmapper/
PubChem	https://pubchem.ncbi.nlm.nih.gov/
SuperPred	https://prediction.charite.de/
Targetnet	http://targetnet.scbdd.com/
SwissTargetPrediction	http://www.swisstargetprediction.ch/
CTD	https://ctdbase.org/
PHarmGKB	https://www.pharmgkb.org/
OMIM	https://www.omim.org/
GeneCards	https://www.genecards.org/
DisGeNET	https://www.disgenet.org/
GEPIA	http://gepia.cancer-pku.cn/
HPA	https://www.proteinatlas.org/
CBioPortal	https://www.cbioportal.org/
TIMER	https://cistrome.shinyapps.io/timer/
Uniprot	https://www.uniprot.org/
STRING	https://cn.string-db.org/
RCSB PDB	https://www.rcsb.org/
Bioinformatics	http://www.bioinformatics.com.cn/
DAVID	https://david.ncifcrf.gov/
Venny2.1.0	https://bioinfogp.cnb.csic.es/tools/venny/index.html
KEGG Mapper	https://www.kegg.jp/kegg/mapper/

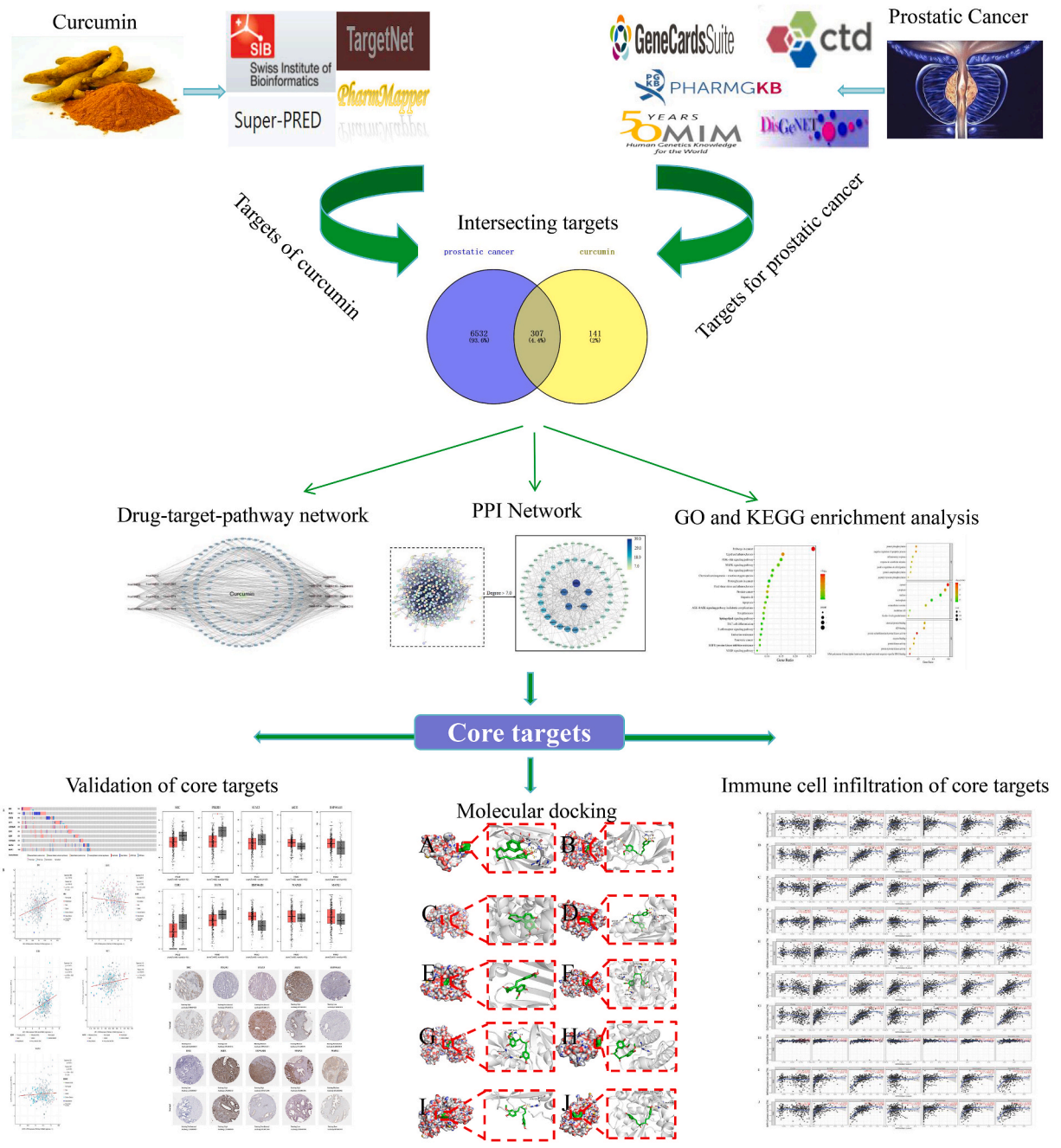


Fig. 1. This study is a detailed flowchart of web-based pharmacology research.

2.6. Construction of drug-target-pathway network

Using Cytoscape 3.7.2 software, we successfully constructed a drug-target-pathway network diagram by integrating curcumin, potential targets of action for the treatment of PCa, and the KEGG pathway. In this network, the nodes represent curcumin, genes, or pathways, respectively, while the connecting lines symbolise the interactions between biomolecules.

2.7. Molecular docking and molecular dynamics

First, we obtained a 3D SDF format file of curcumin from PubChem and converted it to mol2 format using OpenBabel-3.1.1 software for further processing. Immediately afterward, we downloaded the PDB files of the core target proteins from the PDB database and

Table 3
10 Hub genes identified using "Degree" in the Cytohubba plugin.

Rank	Gene symbol	Full name	Degree
1	SRC	SRC proto-oncogene, non-receptor tyrosine kinase	39
2	PIK3R1	phosphoinositide-3-kinase regulatory subunit 1	32
3	STAT3	signal transducer and activator of transcription 3	32
4	AKT1	AKT serine/threonine kinase 1	32
5	HSP90AA1	heat shock protein 90 alpha family class A member 1	31
6	ESR1	estrogen receptor 1	27
7	EGFR	epidermal growth factor receptor	25
8	HSP90AB1	heat shock protein 90 alpha family class B member 1	25
9	MAPK8	mitogen-activated protein kinase 8	24
10	MAPK1	mitogen-activated protein kinase 1	24

operated on the proteins in PyMOL for dehydration and ligand removal to ensure that the protein structures were suitable for molecular docking. Subsequently, we hydrogenated the proteins using AutoDockTools 1.5.7 and exported them as PDBQT format files to prepare for docking. After that, we restarted AutoDockTools 1.5.7 and imported the prepared PDBQT files of the receptors and ligands. When constructing the docking box, we centred it on the receptor protein, adjusted its size to completely cover the receptor protein, and ensured that the ligand was located outside the docking box(Table 4).When performing molecular docking in AutoDockTools 1.5.7, we focused on binding energy as a key metric that reflects the likelihood of binding between the receptor and ligand. A lower binding energy indicates a higher affinity between the receptor and ligand, as well as a more stable conformation of the receptor and ligand. After completing the docking, we collect the binding energy data of molecular docking and visualise the docking results in PyMOL for in-depth analysis and interpretation. Next, for molecular dynamics simulations, we separated the docked proteins from the small molecule ligands. Using the Antechamber tool in AmberTools software, we generated small molecule force field files and converted them to Gromacs force field files using the Acpype software tool. During the simulation, the GAFF force field was used for small molecules, whereas the AMBER14SB force field and TIP3P water model were used for proteins. By merging the files of the proteins and small-molecule ligands, we constructed a simulated system for the complex.

Molecular dynamics (MD) simulations were performed using the Gromacs 2022 program, and the simulation conditions were set to constant temperature, pressure, and periodic boundary conditions. During the MD simulations, all constraints involving hydrogen bonding were applied using the LINCS algorithm, with the integration step set to 2 fs. Electrostatic interactions were calculated using the Particle-mesh Ewald (PME) method with the cutoff value set to 1.2 nm. The cutoff value for non-bonding interactions was set at 10 Å and updated every 10 steps. To control the simulation conditions, we used the V-rescale temperature coupling method to maintain the simulation temperature at 298 K while controlling the pressure to 1 bar using the Berendsen method. We performed 100 ps NVT and NPT equilibrium simulations and 100 ns MD simulations for the complex system at 298 K, with conformations saved every 10 ps. The NVT and NPT equilibrium simulations were carried out using the VMD method, after which we performed an in-depth analysis of the simulation trajectories using VMD and PyMol, and an MMPBSA analysis of the free energy of binding between the protein and the small-molecule ligand using the g_mmpbsa program. These analyses provide an in-depth understanding of the mechanism of interaction between curcumin and core target proteins.

2.8. External verification of core objectives

The gene expression level of the core target. GEPIA validated the mRNA expression levels and pathological staging of the core target. |log2FC| cutoff:1; p value cutoff:0.01.

The protein expression level of the core target. To investigate the expression of core targets in PCa tissues in detail, we analysed the data on these core targets in the Human Protein Atlas database. Furthermore, we compared the protein expression levels of the core targets in PCa tissues with those in normal prostate tissues to reveal differences and connections.

Genetic alterations in core targets. cBioPortal selected a PCa dataset (TCGA, Firehose Legacy) containing 500 samples for analysis. Information on the genetic changes in the core targets was obtained.

Table 4
Detailed information of protein targets in PDB database and grid docking parameters in molecular docking.

Targets	PDB ID	Method	Resolution	R-Value Free	Center_X	Center_Y	Center_Z	Size_x	Size_y	Size_z
SRC	1FMK	X-RAY DIFFRACTION	1.50 Å	0.264	−11.9	19.4	27.3	17.4	18.7	12.5
PIK3R1	2XS6	X-RAY DIFFRACTION	2.09 Å	0.240	−23.5	16.5	14.3	10	10	10
STAT3	6NJS	X-RAY DIFFRACTION	2.70 Å	0.256	13.5	54.1	0.1	20	30.7	22
AKT1	1UNQ	X-RAY DIFFRACTION	0.98 Å	0.179	15.2	24.4	16.3	15.5	18.7	19.8
HSP90AA1	1BYQ	X-RAY DIFFRACTION	1.50 Å	0.247	40.3	−47	65.3	16.9	17	20.6
ESR1	1L2I	X-RAY DIFFRACTION	1.95 Å	0.243	−2.7	−8.8	22.3	16.7	14.6	20.4
EGFR	1XKK	X-RAY DIFFRACTION	2.40 Å	0.255	18.9	35.3	37.6	22.4	18.6	25.2
HSP90AB1	3NMQ	X-RAY DIFFRACTION	2.20 Å	0.267	2.3	10.4	27.1	19.6	20.7	17.9
MAPK8	2XRW	X-RAY DIFFRACTION	1.33 Å	0.187	12.1	7.5	18.6	17.3	18.3	19.3
MAPK1	6G54	X-RAY DIFFRACTION	2.05 Å	0.221	68.4	15.2	10.7	17.2	18.2	22.2

Immune cell infiltration of core targets. To gain insight into the intrinsic mechanisms of the PCa immune microenvironment, we entered the core target data into the TIMER database and investigated the potential association between core targets and immune permeability.

3. Results

3.1. Targets of curcumin and PCa

Using several tools, such as PharmMapper, SwissTargetPrediction, TargetNet, and SuperPred, we successfully identified 448 targets for curcumin action. The results of the Venny diagram analysis revealed that after comparing these 448 drug targets with 6839 disease targets, we identified 307 common targets that provide potential therapeutic directions for curcumin treatment of PCa (Fig. 2).

3.2. PPI network construction and core target screening

A total of 307 common targets were imported into the STRING database and analysed to obtain the PPI network data. The TSV file of the PPI data was imported into Cytoscape 3.7.2 software, and the isolated targets were deleted and visualised. The "Network analyser" tool in the software can be used to analyse the degree value of the target points, and the average value of "Degree>7.0″ was selected to visualise the PPI network (Fig. 3). 80 nodes and 380 edge nodes were found in the PPI network, and the average value of "Degree>7.0″ was selected to visualise the PPI network. There were 80 nodes and 380 edge nodes in the PPI network. The larger the shape and darker the colour of the point, the larger the degree value of the node. Using the "Cytohubba" plug-in in Cytoscape 3.7.2 software, the top 10 core targets were filtered out by this calculation method: SRC, PIK3R1, STAT3, AKT1, HSP90AA1, ESR1, EGFR, HSP90AB1, MAPK8, and MAPK1.

3.3. GO and KEGG enrichment analysis

After GO functional enrichment analysis, we obtained 1119 items, including 782 items in the Biological Progress (BP) domain, 112 items in the Cellular Composition (CC) domain, and 225 items in the Molecular Function (MF) domain (Fig. 4A). The potential targets of curcumin in the treatment of PCa include protein phosphorylation, negative regulation of apoptosis, inflammatory response, response to xenobiotic stimuli, positive regulation of cell migration, protein autophosphorylation, and peptidyl-tyrosine phosphorylation. The main CC plays a role in the cytosol, cytoplasm, nucleus, nucleoplasm, extracellular exosomes, membrane raft, and Ficolin-1-rich granule lumen. The MF involved included identical protein binding, ATP binding, protein/serine/threonine/tyrosine kinase activity, enzyme binding, protein kinase activity, protein tyrosine kinase activity, RNA polymerase II transcription factor activity, and ligand-activated sequence-specific DNA binding.

KEGG pathway enrichment analysis identified 126 signalling pathways, of which the top 20 were visualised (Fig. 4B). It is mainly involved in pathways related to cancer, lipids, and atherosclerosis, such as PI3K-Akt, MAPK, Ras signalling pathway, chemical carcinogenesis-reactive oxygen species, proteoglycans in cancer, fluid shear stress, atherosclerosis, prostate cancer, Hepatitis B, apoptosis, AGE-RAGE signalling pathway in diabetic complications, Toxoplasmosis, Sphingolipid signalling pathway, Th17 cell differentiation, T cell receptor signalling pathway, endocrine resistance, pancreatic cancer, EGFR tyrosine kinase inhibitor resistance, and VEGF signalling pathway.

In the KEGG enrichment analysis, we found that the "pathway in cancer, lipids and atherosclerosis" showed a high trend of enrichment in both tumour and non-tumour tissues, indicating that these pathways are not specific to a particular disease type. the PI3K-Akt signalling pathway is an important pathway closely related to disease onset and progression. Therefore, we selected "PI3K-Akt signalling pathway" for further mapping (Fig. 5). The red markers in the figure indicate potential targets of curcumin intervention, shedding light on the potential mechanism of curcumin in the treatment of diseases.

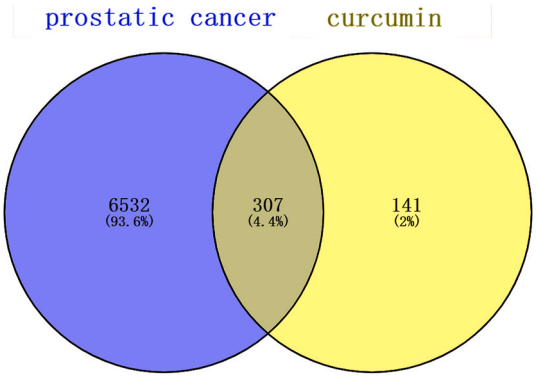


Fig. 2. Targets relevant to the treatment of PCa.

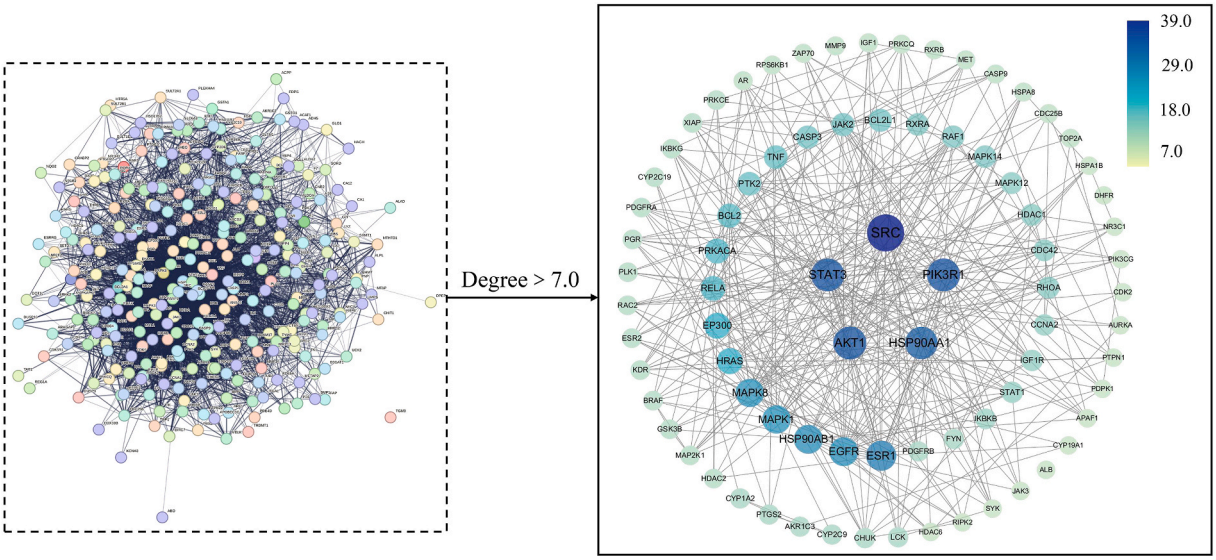


Fig. 3. PPI network of potential targets for curcumin in the treatment of PCa.

3.4. Drug-target-pathway network

The first 20 KEGG pathways were introduced into Cytoscape3.7.2, and a drug-target-pathway network diagram was constructed for the treatment of PCa with curcumin (Fig. 6). In the graph, the blue circles symbolise targets, orange diamonds denote pathways, and green triangles indicate curcumin.

3.5. Molecular docking validation of curcumin and core targets

Molecular docking results showed that the binding energy of curcumin to the target protein was less than −1.85 kcal/mol (Table 5). Curcumin binds tightly to the amino acid residues through hydrogen bonds. Taken together, these molecular docking results are plausible and authentic. Curcumin has a strong binding ability to core target proteins, and a potential role in the treatment of PCa through core target proteins. The molecular docking results were visualised (Fig. 7).

The root mean square deviation (RMSD) was calculated to study the stability of the complexes of small molecule-binding proteins. The (RMSD), as a key parameter that measures the sum of all atomic deviations between the conformation and the target conformation at a certain moment and is of great significance in assessing the stability of the system. By observing the graphs, we found that the RMSD values of the protein gradually leveled off during the simulation, which fully proved the stability of the protein structure. The RMSD of the small molecule-protein complex STAT3 shows more drastic fluctuations, which indicates that the complex is not stable enough. The RMSDs of the other small-molecule-protein complexes gradually stabilised during the simulation, indicating that the other groups of complexes remained stable.

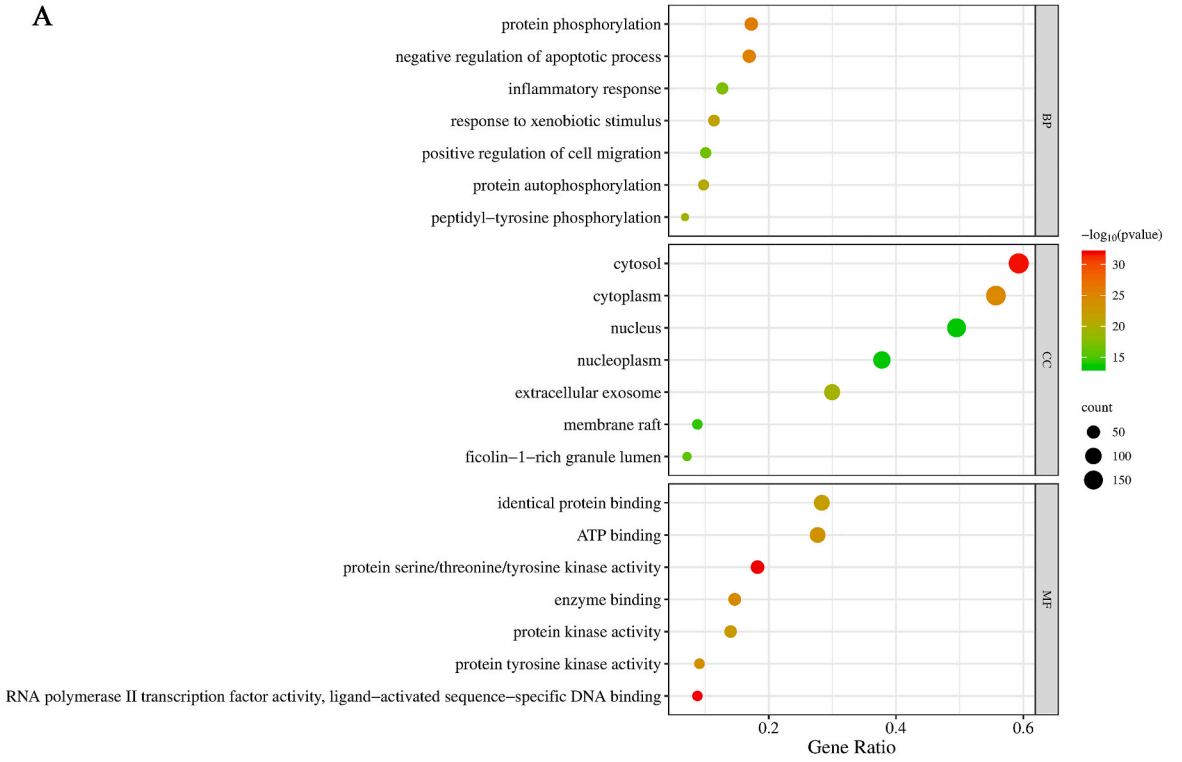
To explore the binding state of small molecules to proteins, we calculated the buried surface area of the small molecules within the proteins (Buried SASA). This index can intuitively reflect the size of the interface between small molecules and protein binding, and by deeply analysing the change in the buried area, we can effectively understand the binding state between small molecules and proteins. As shown in the figure, the buried SASA of the small molecule-protein complex STAT3 fluctuates around 5 nm^2 during 0–40 ns, indicating that the small molecule is in a bound state with the protein during 0–40 ns. The occurrence of 0 during 40–100 ns indicates that, during this period, the small molecule detaches from the original binding site, and the contact area with the protein drops to 0. The buried SASA of the other small-molecule proteins remained stable, suggesting that these protein groups were in a stable state of binding with the small molecule(Fig. 8).

3.6. External validation of core targets

The mRNA expression levels of core targets. There were differences in the core target expression between PCa and normal prostate tissues. The expression level of PIK3R1 in cancer tissues was significantly lower than that in normal tissues (Fig. 9).

Protein expression levels of core targets. We performed an in-depth analysis of immunohistochemical staining images from the HPA database to observe the expression of core target proteins in PCa. We found that the expression levels of SRC, AKT1, HSP90AA1, ESR1, HSP90AB1, MAPK8, and MAPK1 were elevated in PCa tissues compared to those in normal prostate tissues, while EGFR was highly expressed in both PCa and prostate tissues. In addition, PIK3R1 and STAT3 were weakly expressed in the PCa tissues (Fig. 10).

Genetic alterations in core targets. The study found that 254 of 500 patients (51 %) had mutations in these targets (Fig. 11A) and that



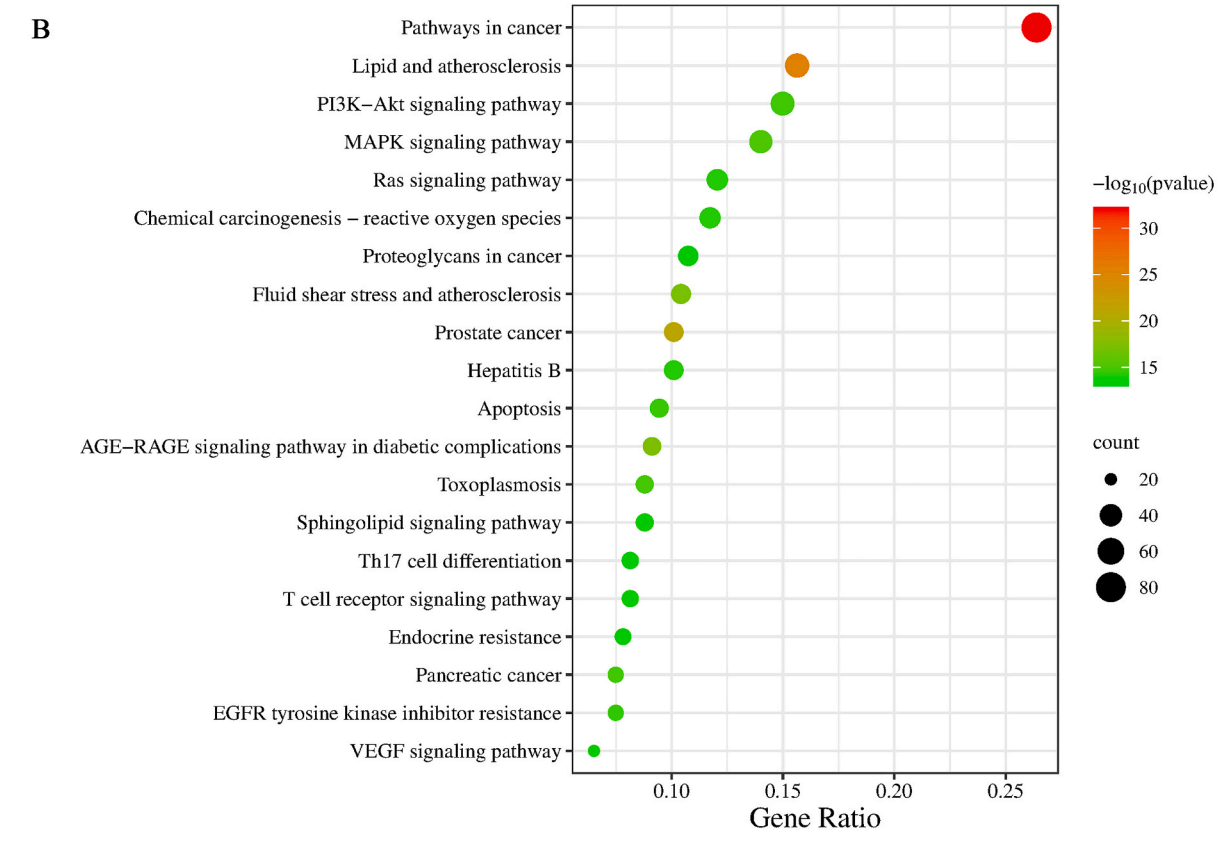


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Fig. 4. The bubble diagram of enrichment analysis (A. GO functional enrichment analysis of curcumin in prostate cancer. B. KEGG pathway enrichment analysis of curcumin in prostate cancer).

protein expression of the core targets was positively correlated with mRNA levels (Fig. 11B).

Immune cell infiltration of core targets. Analysis of the relationship between core targets and immune cell infiltration (Fig. 12). The results showed that the SRC expression was positively correlated with the infiltration of B cells (cor = 0.233), CD8+T cells (cor = 0.274), CD4+T cells (cor = 0.167), macrophages (cor = 0.261), neutrophils (cor = 0.298), and dendritic cells (cor = 0.225), while it was negatively correlated with purity (cor = −0.115). PIK3R1 expression was positively correlated with the infiltration of B cells (cor = 0.525), CD8+T cells (cor = 0.626), CD4+T cells (cor = 0.308), macrophages (cor = 0.649), neutrophils (cor = 0.461), and dendritic cells (cor = 0.477), while it was negatively correlated with purity (cor = −0.289). STAT3 expression positively correlated with the infiltration of B cells (cor = 0.449), CD8+T cells (cor = 0.672), CD4+T cells (cor = 0.255), macrophages (cor = 0.561), neutrophils (cor = 0.566), and dendritic cells (cor = 0.559). while it wa STAT3 was negatively correlated with purity (cor = −0.242).

AKT1 expression positively correlated with B cells (cor = 0.156), CD8+T cells (cor = 0.35), macrophages (cor = 0.173), neutrophils (cor = 0.165), dendritic cells (cor = 0.19), and purity (cor = 0.082), while it was negatively correlated with CD4+T cell infiltration (cor = −0.027). The expression of HSP90AA1 was positively correlated with B cell infiltration (cor = 0.288), CD8+T cells (cor = 0.496), macrophages (cor = 0.276), neutrophils (cor = 0.252), and dendritic cells (cor = 0.302), while it was negatively correlated with the infiltration of CD4+T cells (cor = −0.057). No correlation was observed between HSP90AA1 expression and purity (cor = 0). ESR1 expression positively correlated with the infiltration of B cells (cor = 0.373), CD8+T cells (cor = 0.325), CD4+T cells (cor = 0.354), macrophages (cor = 0.444), neutrophils (cor = 0.448), and dendritic cells (cor = 0.513), while it was negatively correlated with purity (cor = −0.371). EGFR expression was positively correlated with the infiltration of fine B cells (cor = 0.43), CD8+T cells (cor = 0.678), CD4+T cells (cor = 0.179), macrophages (cor = 0.592), neutrophils (cor = 0.497), and dendritic cells (cor = 0.503), whereas the expression of EGFR was negatively correlated with purity (cor = −0.199). HSP90AB1 expression was positively correlated with B cells (cor = 0.236), CD8+T cells (cor = 0.317), macrophages (cor = 0.152), neutrophils (cor = 0.097), dendritic cells (cor =

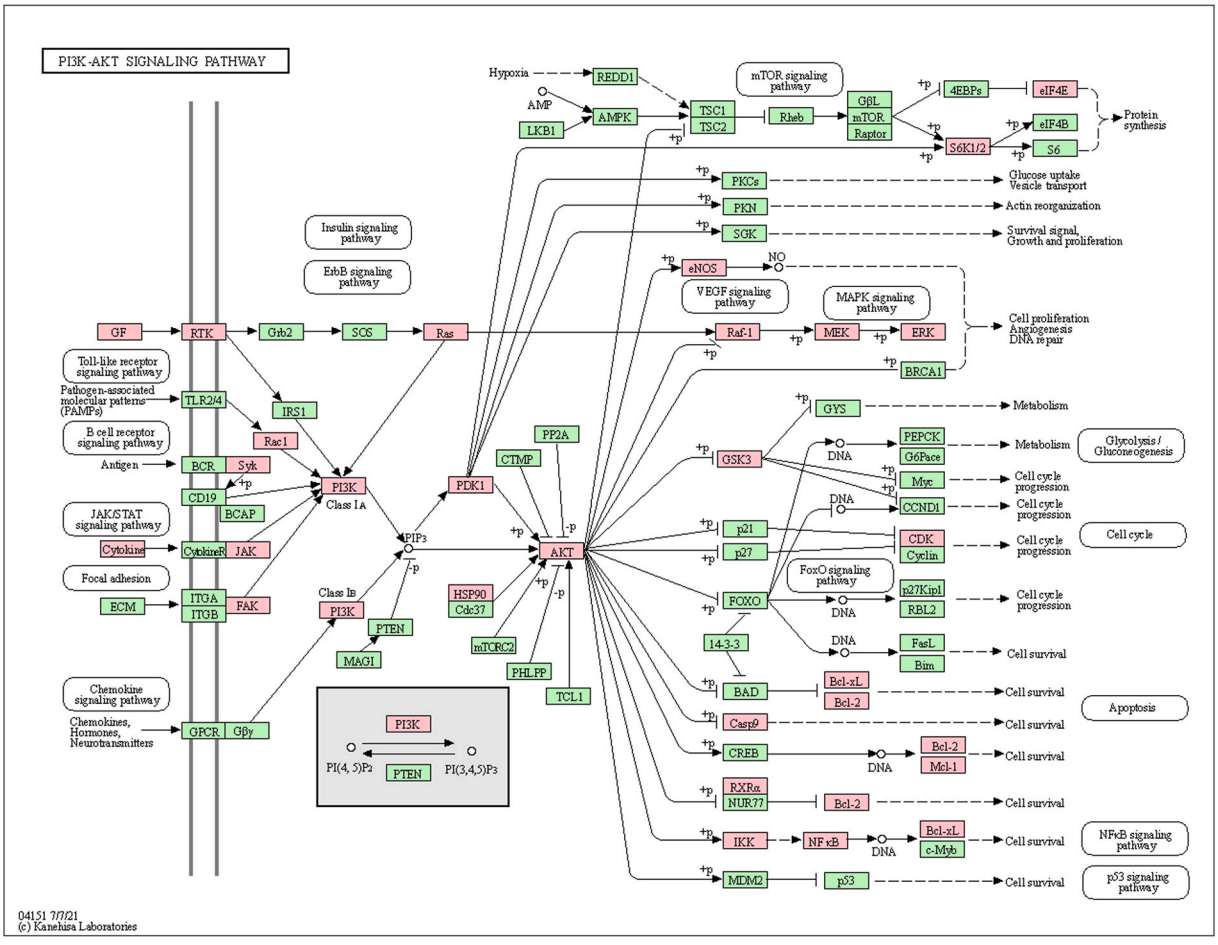


Fig. 5. PI3K-Akt signaling pathway (red marks represent potential targets of curcumin intervention)

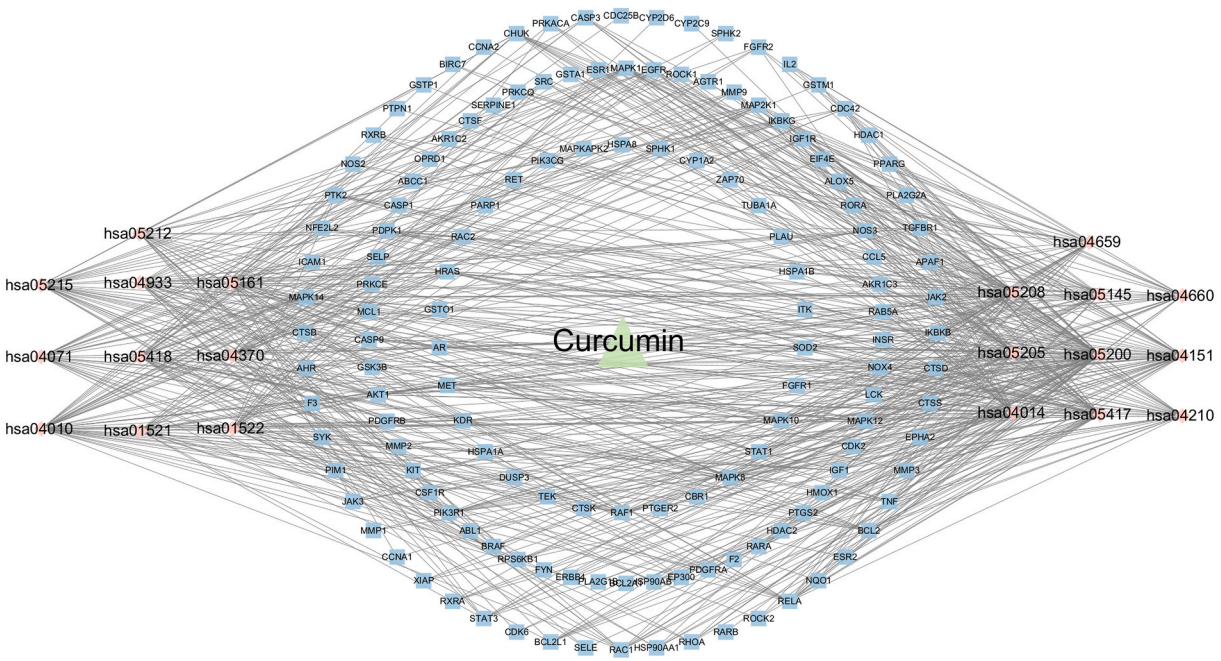


Fig. 6. Drug-target-pathway network diagram. The blue circle represents the target, the orange diamond is the path, and the green triangle is curcumin.

Table 5
Basic information on the molecular docking of curcumin and target proteins.

Molecular name	Targets	PDB ID	Residue involved in H bonding	H-bond length (Å)	Binding energy(kcal/Mol)
Curcumin	AKT1	1UNQ	ARG-23; ILE-19; TYR-18; GLU-1	2.1; 1.8; 2.0; 2.9,1.7	−5.327
Curcumin	EGFR	1XKK	MET-793; LEU-788	2.2; 2.6	−5.736
Curcumin	ESR1	1L2I	–	–	−4.541
Curcumin	HSP90AA1	1BYQ	LYS-58; PHE-138	2.5,1.9; 2.2,1.9	−5.743
Curcumin	HSP90AB1	3NMQ	–	–	−7.118
Curcumin	MAPK1	6G54	LYS-54; MET-108; LYS-114; GLU-33	2.2; 2.1; 2.6; 2.8,1.7	−5.855
Curcumin	MAPK8	2XRW	ASN-114; MET-111	2.0; 1.8	−6.804
Curcumin	PIK3R1	2XS6	HIS-217	1.8	−1.85
Curcumin	SRC	1FMK	ARG-155	2.2	−5.025
Curcumin	STAT3	6NJS	GLN-644	2.6	−6.441

0.14), and purity (cor = 0.149), while it was negatively correlated with CD4+T cell infiltration (cor = −0.121).

MAPK8 expression was positively correlated with B cell infiltration (cor = 0.418), CD8+T cells (cor = 0.559), CD4+T cells (cor = 0.011), macrophages (cor = 0.321), neutrophils (cor = 0.378), and dendritic cells (cor = 0.35), while it was negatively correlated with purity (cor = −0.001). MAPK1 expression positively correlated with the infiltration of B cells (cor = 0.445), CD8+T cells (cor = 0.665), CD4+T cells (cor = 0.049), macrophages (cor = 0.449), neutrophils (cor = 0.369), and dendritic cells (cor = 0.423), while it was negatively correlated with purity (cor = −0.102). The expression of SRC, PIK3R1, STAT3, ESR1, EGFR, MAPK8, and MAPK1 was positively correlated with the infiltration of B cells, CD8+T cells, CD4+T cells, macrophages, neutrophils, and dendritic cells, and negatively correlated with purity. The expression of AKT1 and HSP90AB1 was positively correlated with B cells, CD8+T cells, macrophages, neutrophils, dendritic cells, and purity, and negatively correlated with CD4+T cell infiltration.

4. Discussion

At the time of diagnosis, most patients with prostate cancer have already developed metastases, and the treatment of advanced PCa not only utilises substantial medical resources but also causes great physical pain and financial burden on patients. In addition to the existing standard treatments, researchers are actively exploring new treatments. Curcumin has demonstrated pharmacological effects in preclinical models of various tumours, such as pancreatic, PCa, breast, and lung cancers [29]. Some experiments have effectively enhanced the oral bioavailability of curcumin by optimising the delivery method using innovative technologies such as nanosuspensions, micelles, nanoparticles, and nanoemulsions. This, in turn, significantly strengthened its efficacy in vivo, allowing curcumin to show great potential as an antitumour drug [30]. However, the mechanism of TCM for treating diseases is multi-targeted and

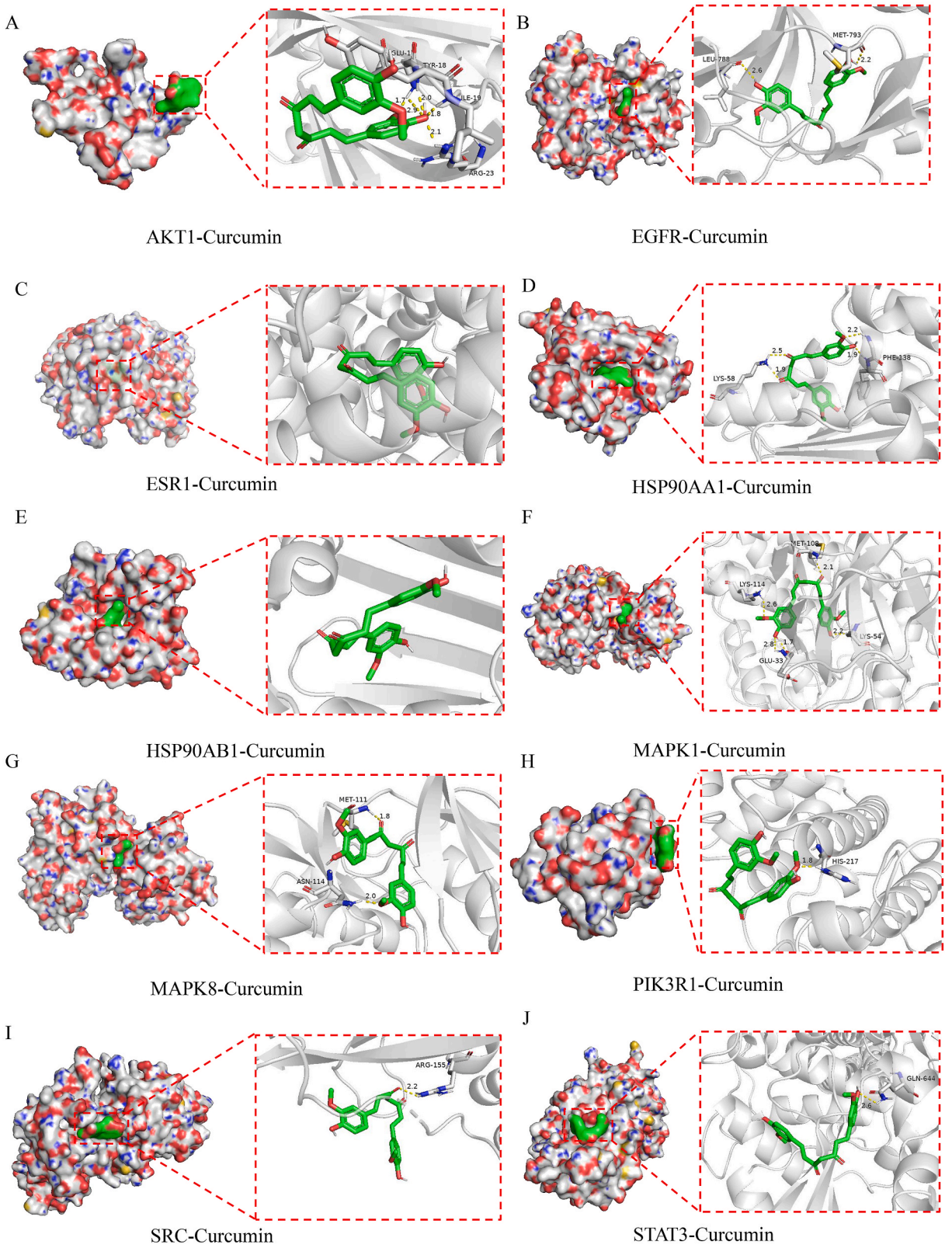


Fig. 7. Molecular docking mode of curcumin with core target protein. (A. Curcumin-AKT1,B. Curcumin-EGFR, C. Curcumin-ESR1, D. Curcumin-HSP90AA1, E.Curcumin-HSP90AB1,F.Curcumin-MAPK1,G.Curcumin-MAPK8,H.Curcumin-PIK3R1, I. Curcumin-SRC, J. Curcumin-STAT3).

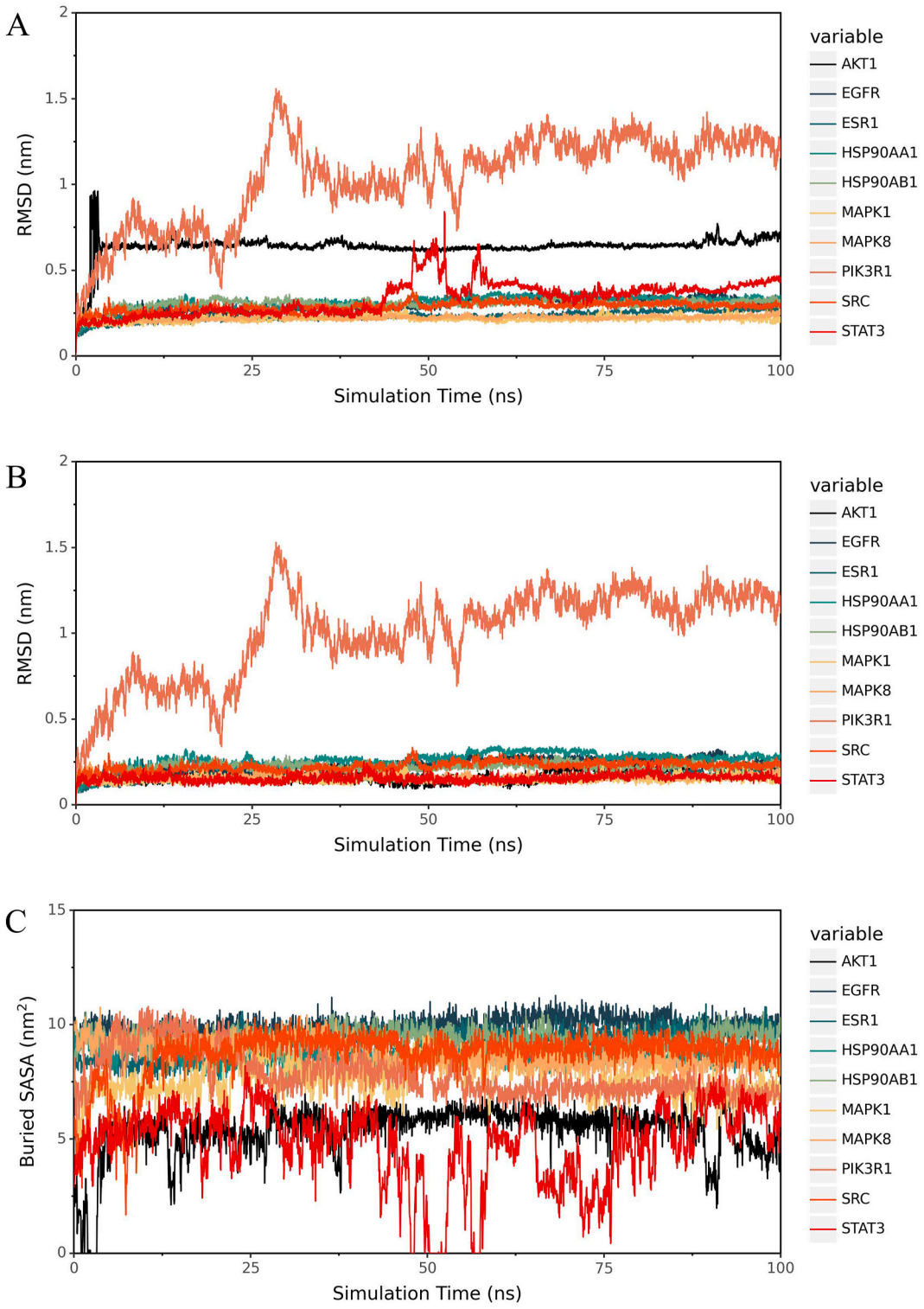


Fig. 8. A.RMSD of small molecule-protein complexes. B.RMSD of proteins. C.Encapsulation area of small molecules in proteins.

involves multi-pathway. Therefore, in order to deeply explore the target and pathway of action of curcumin in PCa, the application of big data is especially necessary, which will provide a strong theoretical basis for clinical application.

According to the GO enrichment results, curcumin mainly acts in the cytoplasm, nucleus, nuclear cytoplasm, exocrine space,

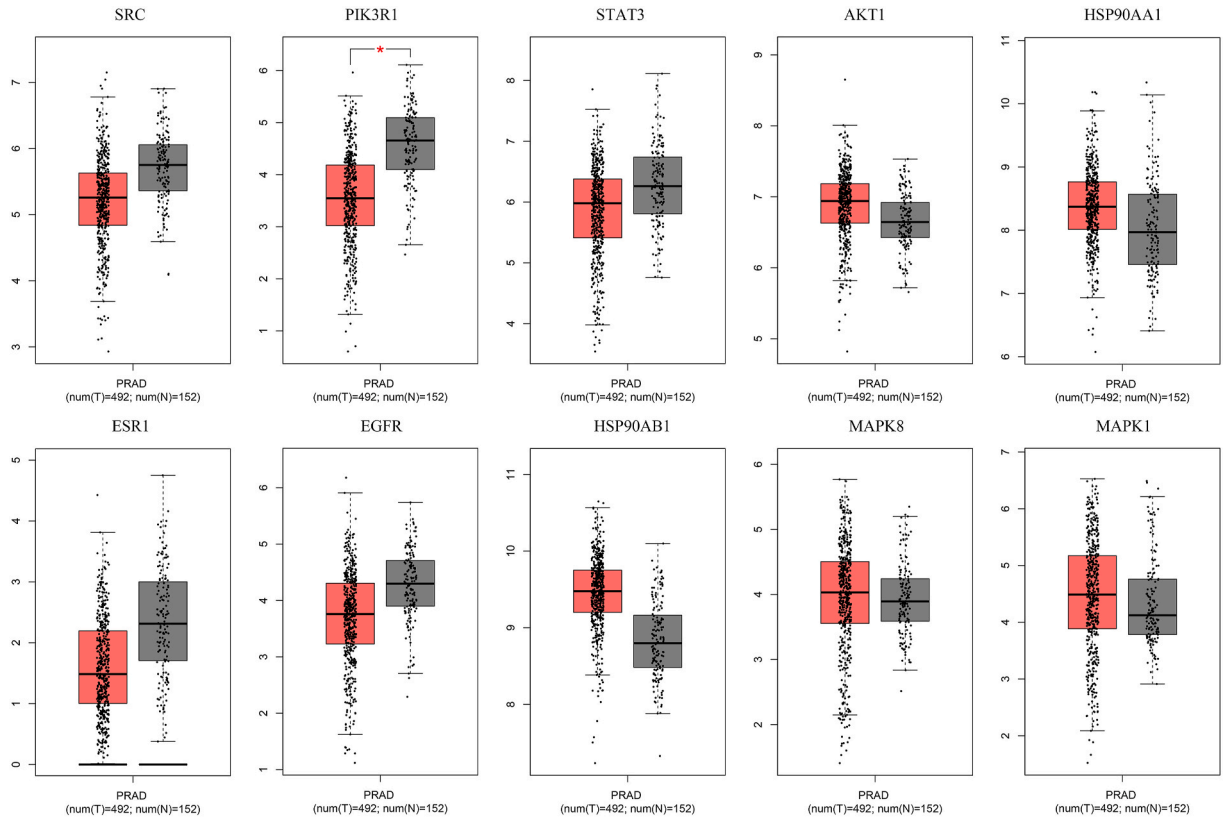


Fig. 9. Expression of Hub gene in GEPIA database. Box plot of Hub gene mRNA expression levels in the GEPIA database. Red represents tumor tissue, while gray represents normal tissue.

membrane raft, and ficolin-1-rich granular cavity. The molecular functions involved in bladder cancer include protein binding, ATP binding, protein serine/threonine/tyrosine kinase activity, enzyme binding, protein kinase activity, protein tyrosine kinase activity, RNA polymerase II transcription factor activity, and ligand-activated sequence-specific DNA binding. Curcumin has also been found to play a role in various biological processes, such as protein phosphorylation, negative regulation of apoptosis, inflammatory response, response to foreign body stimulation, positive regulation of cell migration, protein autophosphorylation, and peptide tyrosine phosphorylation.

KEGG enrichment analysis revealed that PCa development is regulated by multiple pathways and targets, which is an extremely complex cascade process. Drugs act on different targets and molecular pathways that affect the viability or apoptosis of cancer cells. We found that curcumin works through the "PI3K-Akt, MAPK, and Ras signalling pathways" in the treatment of bladder cancer, whereas other nonspecific pathways are common in many tumours. Interestingly, we also found that the "Prostate cancer" pathway is significantly enriched in PCa, which is closely related to PCa. The PI3K-Akt signalling pathway is activated by various cellular stimuli or toxic insults and regulates core cellular functions, such as transcription, translation, proliferation, growth, and survival. Overactivation of the PI3K/AKT signalling pathway promotes the malignant transformation of tumour cells by regulating cell proliferation, apoptosis, migration, invasion, angiogenesis, immune escape, and drug resistance [31–33]. This study revealed that activation of the PI3K pathway is closely linked to the development of resistant PCa, providing new insights into the pathogenesis of the disease [34]. Inhibition of the PI3K/AKT signalling pathway inhibits the growth of PCa cells. In vitro studies have shown that PI3K interacts with AR, and the combination of AR deprivation therapy and inhibition of the PI3K pathway is more effective in treating PCa [35]. Studies have shown that curcumin inhibits cancer cell survival by modulating the PI3K/Akt/mTOR pathway, and treatment of ADPC (LNCaP) cells results in apoptosis and cell cycle arrest due to downregulation of this pathway. Other studies have found that curcumin also induces apoptosis in AIPC (DU145 and PC-3) cells, increases mitochondrial outer membrane permeability by downregulating the phosphorylation status of PI3K and Akt, and inhibits the downregulation of the MDM2 oncogene via the PI3K/mTOR/ETS2 pathway, exhibiting sensitisation to chemotherapy and radiotherapy [36–38]. These findings indicate the mechanism of action of curcumin in inhibiting prostate cancer cell survival and inducing apoptosis, which is expected to ultimately improve the therapeutic outcome of prostate cancer. Therefore, blocking the PI3K-Akt signalling pathway could be a potential direction for the treatment of PCa.

The MAPK signalling pathway is involved in a variety of biological functions, including proliferation, transformation, and apoptosis. Activation of the MAPK pathway increases resistance to enzalutamide and decreases drug effectiveness, suggesting that the prognosis of these patients may be poor [39]. It was also found that the down-regulation of the MAPK pathway restored resistance to

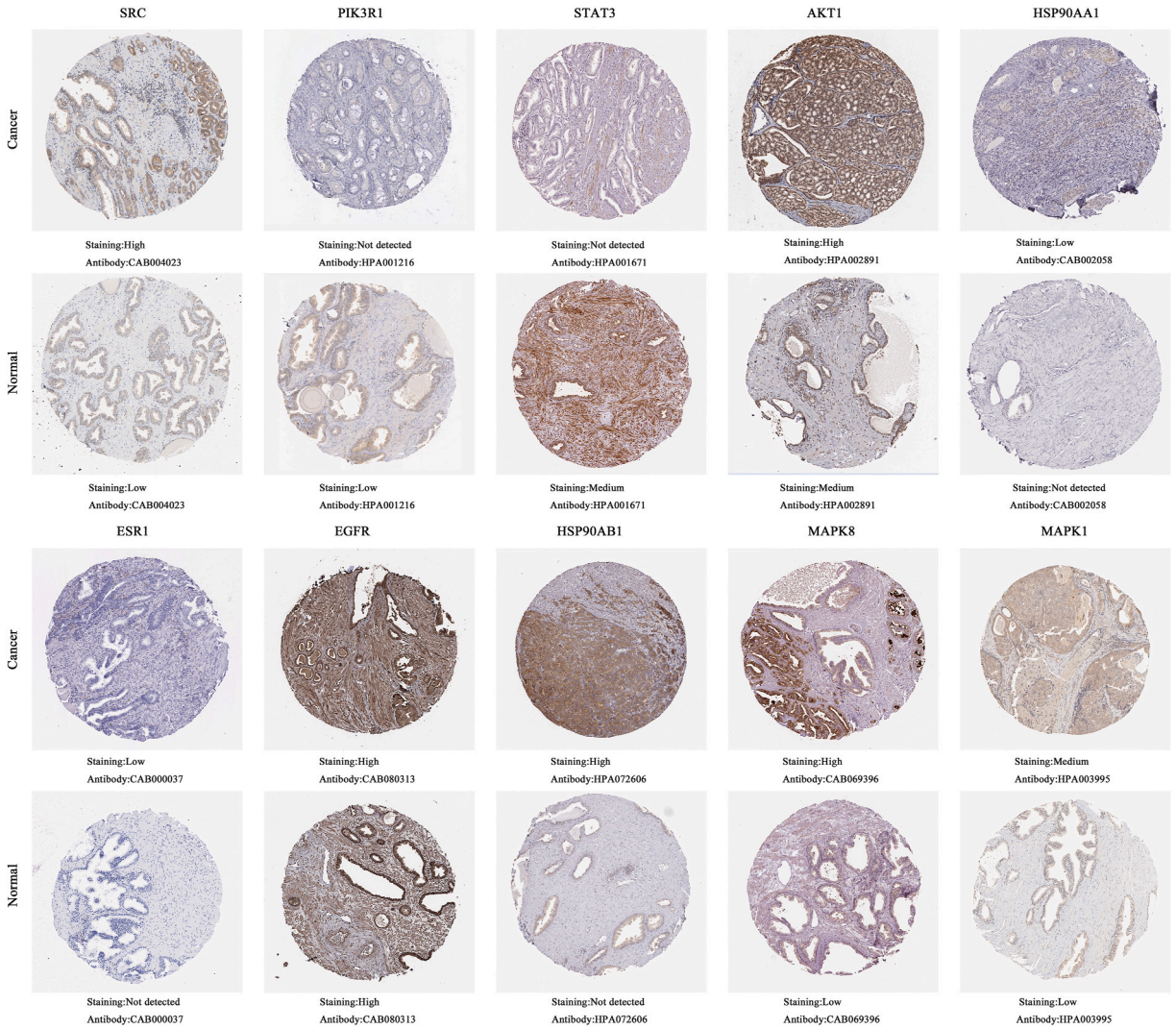
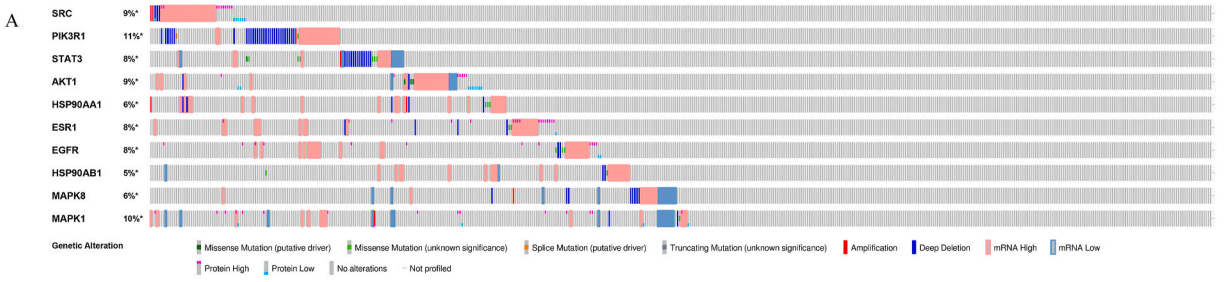


Fig. 10. Immunohistochemical image of HUB gene protein expression levels in the HPA database.

enzalutamide, thereby improving its efficacy against CRPC [40]. Inhibits tumour cell migration by inhibiting MAPK signalling and ERM protein activation [41]. MAPK inhibitors have also been found to prevent the progression of desmoplasia-resistant PCa [42]. Curcumin has been shown to stimulate apoptosis and inhibit prostate cancer proliferation in vitro and in vivo by modulating various cellular mediators such as NF-κB, epidermal growth factor receptor (EGFR), and MAPK [43]. Another study indicated that curcumin was able to activate protein kinase D1 (PKD1), thereby attenuating oncogenic signals from MAPK and β-catenin, and effectively inhibiting prostate cancer development [44]. In summary, inhibition of the MAPK pathway can inhibit PCa cell growth, prevent epithelial-mesenchymal transition, prevent tumour migration, and improve drug tolerance, especially in treatment-resistant PCa, and can improve drug sensitivity, and delay distant metastasis, thereby increasing the likelihood of survival.

The main function of Ras is to assemble intracellular signalling complexes and activate downstream cellular signalling pathways. Therefore, Ras proteins coordinate various cellular responses, including proliferation, differentiation, apoptosis, aging, and metabolism. Research has shown that Ras gene expression is significantly downregulated in PCa [45]. It was also found that triggering the RAS signalling pathway leads to a more aggressive phenotype in PCa cells. In addition, the RAS signalling pathway activates NF-κB [46], playing an important role in PCa development. It also inhibits RAS signalling, blocks the malignant transformation of prostate cells, inhibits the growth and movement of cancer cells, and prevents angiogenesis [46]. Some studies have also shown that the Wnt and RAS signalling pathways play important roles in promoting the progression of PCa tumours, especially in the formation of osteoblasts/osteoclasts and the activation of EMT/proliferation [47]. By inhibiting the Ras signalling pathway, bone metastasis and the related complications of PCa can be effectively controlled. The inhibition of K562 cell proliferation by curcumin may be associated with the downregulation of p210bcr/abl, which in turn may lead to the retardation of the Ras signalling pathway [48]. In addition, curcumin was found to inhibit LPS-induced MMP-2 activity by blocking Ras/MEK1/2 and NF-κB signalling, which indirectly implies



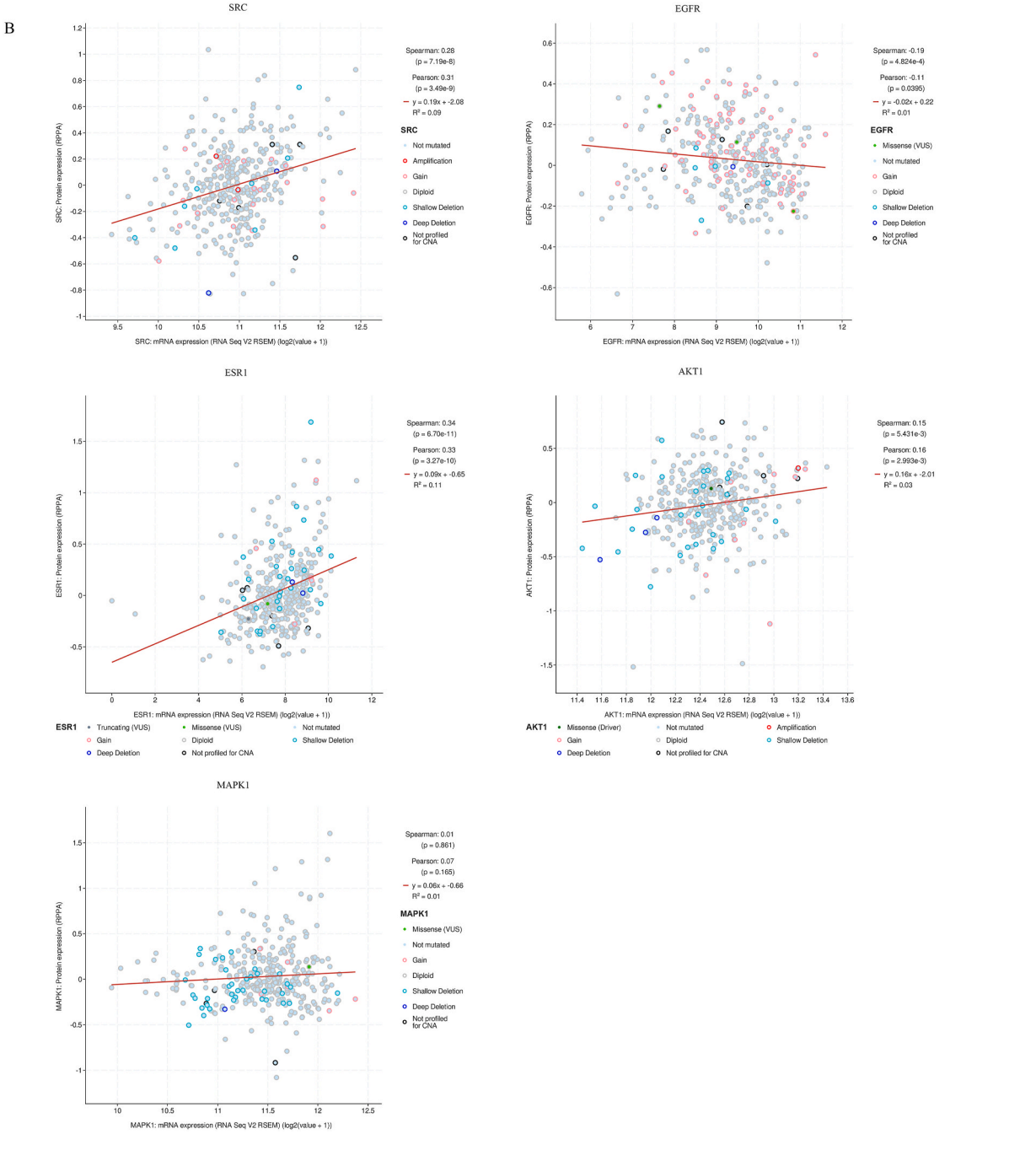


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Fig. 11. Genetic information of HUB gene. (A. Data shows that 254 out of 500 patients (51 %) have genetic mutations at these targets. B. This graph shows the correlation between the mRNA and protein levels of SRC, EGFR, ESR1, AKT1, and MAPK1).

that curcumin may affect prostate cancer cell growth through the Ras pathway [49]. Therefore, inhibiting the Ras pathway has become a potential target for treating PCa, especially in the field of bone metastasis.

Through network pharmacology analysis, the top 10 core targets were selected as follows: SRC, PIK3R1, STAT3, AKT1, HSP90AA1, ESR1, EGFR, HSP90AB1, MAPK8, and MAPK1. We found that these 10 core targets were closely related to tumours. SRC plays an important role in regulating a variety of biologically active signalling pathways, including key processes such as gene transcription, immune response, cell adhesion, cell cycle progression, apoptosis, migration, and transformation. SRC inhibitors exhibit higher potency in inducing apoptosis in PCa cells than drugs such as ositinib, dasatinib, and cisplatin [50]. Curcumin was found to inhibit tumour cell growth by inhibiting the phosphorylation of Src and stat3 through PRL-3 down-regulation [51]. Furthermore, it was also found to modulate PKCα- and Src-regulated phosphorylation, and to metabolise 5α-dihydrotestosterone (DHT) and 3α-androstane-5αby acting on the UDP-glucuronosyltransferase (UGT) 2B15 distributed in the human prostate. 17β-diol metabolites support the androgen receptor-specific effects on the prostate [52]. This suggests that curcumin can activate SRC-related targets, providing a new direction for prostate cancer treatment. PIK3R1 is considered a tumour suppressor associated with increased cell proliferation and invasion, and decreased cell apoptosis [53,54]. Curcumin effectively inhibits NF-κB activity by blocking PIK3R1 in the NF-κB cascade pathway of CD4-T cell receptor signalling, thereby triggering cellular G2/M phase blockade and apoptotic processes [55]. It also mediates apoptosis of PCa cells by inducing the expression of PIK3R1 [56]. The protein encoded by the STAT3 gene plays a key role in cell biology as a transcriptional activator that is essential for the response of cytokines and growth factors, which in turn play an integral role in numerous cellular processes such as cell growth and apoptosis. In vitro experiments have demonstrated that curcumin synergises with CDK4/6 inhibitors to activate the STAT3 pathway, thereby enhancing the anticancer efficacy in prostate cancer (PCa) [57]. Research has shown that the activation of STAT3 inhibits the development of mPCa [58]. AKT1 encodes a serine/threonine protein kinase that plays an important role in regulating cellular functions as a key component of the PI3K/AKT signalling pathway. Abnormal AKT activation can lead to tumour development. Studies have shown that the inhibition of AKT1 expression significantly increases the sensitivity of refractory PCa cells to abirone [59]. The results of in vitro experiments showed that curcumin analogues (HMBME) effectively inhibited the proliferation of human and mouse PCA cells by acting on the Akt/NF-κB signalling pathway [60]. Curcumin enhances the sensitivity of prostate cancer cells to TRAIL by inhibiting Akt-regulated NF-κB and NF-κB-dependent anti-apoptotic factors, such as Bcl-2, Bcl-xL, and XIAP [61]. As a highly conserved molecular chaperone protein, HSP90 plays a pivotal role in signal transduction, protein folding, protein degradation, and morphological evolution. There are two main HSP90 proteins in the cytoplasm: HSP90AA1 (MIM 140571), which is inducible, and HSP90AB1, which is constitutive. Induction of HSP90AA1 expression inhibits apoptosis in PCa cells [43]. It was also found that high expression of HSP90AB1 and HSP90AAl was associated with poor prognosis of PCa [44]. Curcumin binds to HSP90AA1 and inhibits the growth of hepatocellular carcinoma/-glioblastoma and neuroblastoma cells [62]. This finding indirectly suggests that curcumin may inhibit the growth of prostate cancer cells by binding to HSP90AA1/HSP90AB1; however, further experimental validation is needed to confirm this conclusion.

Recent studies have shown that ESR1 can serve as a prognostic marker for PCa. This demonstrates that ESR1 plays a key role in cancer initiation and progression, and high expression and variation of ESR1 indicate a high risk of PCa [63,64]. Curcumin regulates miRNA expression in human pancreatic cancer cells, as evidenced by the upregulation of miRNA-22 and the downregulation of miRNA-199a, which in turn inhibits the expression of oestrogen receptor 1 (ESR1) [65]. These findings imply that curcumin may play a role in prostate cancer cells by affecting ESR1. However, further experimental validation is needed to confirm this hypothesis. EGFR is a member of the epidermal growth factor receptor family that regulates the PI3K-AKT-mTOR signalling pathway for cell survival and the RAS-RAF-MEK-ERK signalling pathway for cell proliferation. Mutations and expression of EGFR are associated with various types of cancers. Research has found that the downregulation of EGFR can significantly inhibit the proliferation, cell cycle, and migration of PCa cells in vitro and in vivo, and promote cell apoptosis [66]. It has been shown that using EGFR peptide-modified nanoparticles (NPs) as carriers to synergistically deliver doxorubicin (DTX) and pH-sensitive curcumin (CUR) prodrugs enables precision targeted therapy for prostate cancer.

MAPK8 and MAPK1 belong to the same MAP kinase family, and as intersections of biochemical signals, they play key roles in various processes, such as cell proliferation, differentiation, transcriptional regulation, and development. Recent studies have shown that MAPK8 inhibition significantly increased the sensitivity of PCa cells to drug therapy [67]. A previous study revealed that reducing MAPK1 expression effectively inhibited the tumourigenicity and metastaticity of PCa cell lines in vitro and in vivo. Conversely, when PCa cells overexpressed MAPK1, their tumourigenicity and metastaticity were enhanced [68]. As verified by in vitro and in vivo experiments, curcumin promoted apoptosis and inhibited the proliferation of prostate cancer cells by modulating various cellular mediators, including MAPK, thus revealing its remarkable anticancer mechanism.

Advancements in modern medicine have failed to prevent the occurrence of PCa metastasis. Therefore, it is necessary to understand the genetic structure and signalling pathways of metastatic PCa to identify novel treatment options. PCa progresses to CRP several years after castration, relying on androgen signalling. However, the frequent occurrence of ARs (mutations or amplifications) is a distinct feature of CRPC. The expression of AR cofactors, chromatin modifiers, and transcriptional co-activators changes during this process, necessitating in-depth and precise analyses using RNA sequencing [69]. Therefore, in order to improve the quality of life and overall survival of patients with advanced PCa, we need to discover gene targets and signalling pathways (e.g., PI3K-Akt, MAPK, Ras) that are independent of androgen signalling for the treatment of CRP. According to the results of molecular docking, curcumin binds to core target proteins with binding energies of less than −1.85 kcal/mol, demonstrating its strong binding ability to core target proteins.

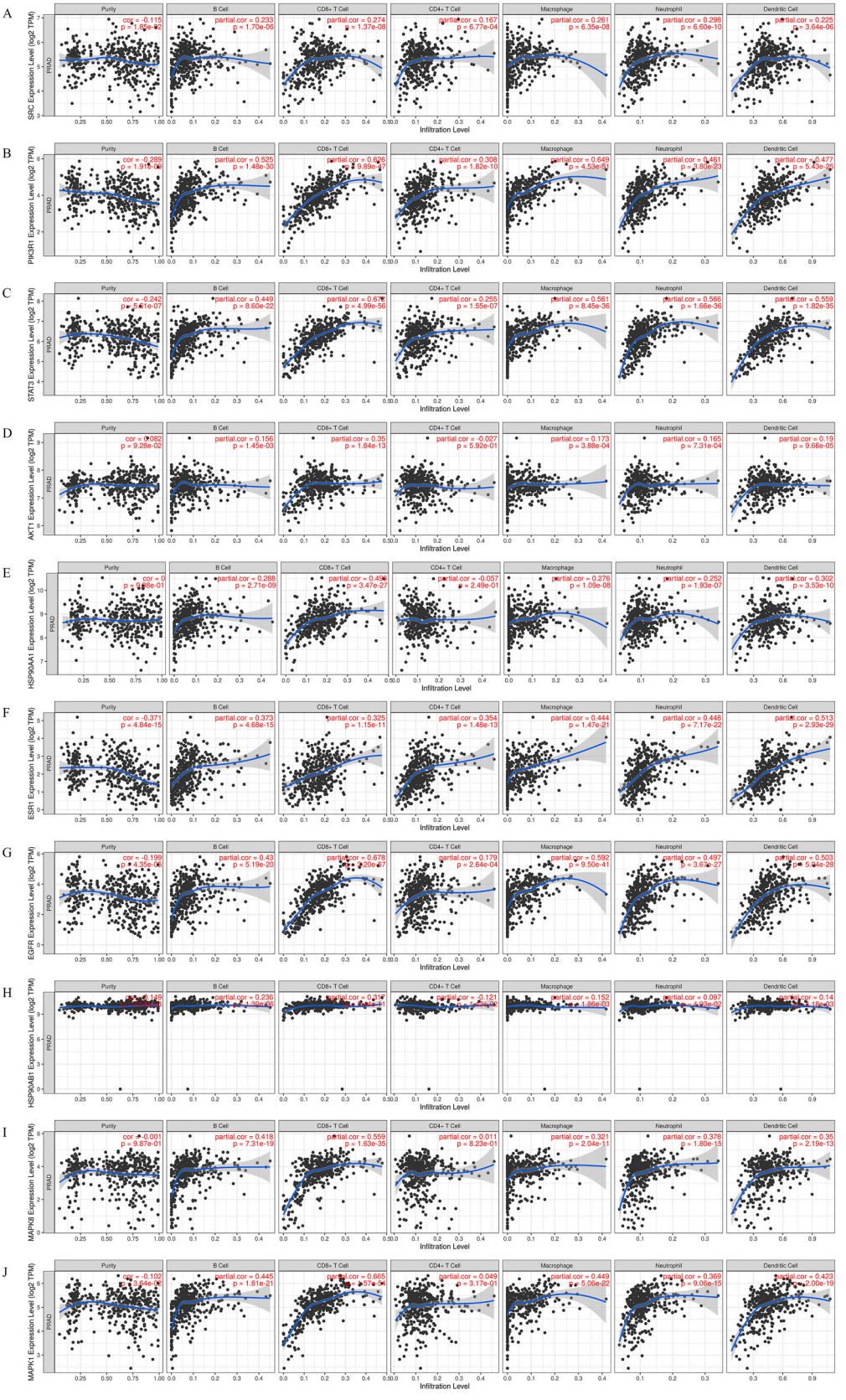

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Fig. 12. The relationship between the core targets of differential expression and immune cell infiltration. (A. SRC, B. PIK3R1, C. STAT3, D. AKT1, E. HSP90AA1, F. ESR1, G. EGFR, H. HSP90AB1, I. MAPK8, J. MAPK1).

The stability of small molecules binding to proteins was analysed by molecular dynamics simulation, and the proteins that stably bind to small molecules, such as AKT1, EGFR, ESR1, HSP90AA1, HSP90AB1, MAPK1, MAPK8, PIK3R1, SRC, were screened out. These proteins have the potential to serve as target proteins for small molecules. The binding of small molecules to STAT3 is weaker compared with that of other proteins, and curcumin may exert a therapeutic effect on PC through its core target protein. The reliability of curcumin as a core target for PC treatment was verified using relevant databases through network pharmacology screening.

5. Conclusion

In the present study, we systematically elucidated the possible mechanisms of action of curcumin in prostate cancer treatment by integrating network pharmacology and molecular docking. Curcumin acts on multiple targets and pathways involved in the treatment of prostate cancer. The results showed that curcumin may exert anticancer effects through the upregulation of PIK3R1, STAT3, and the down-regulation of SRC, AKT1, HSP90AA1, ESR1, EGFR, HSP90AB1, MAPK8, and MAPK1. Curcumin may interfere with the proliferation and apoptosis of prostate tumour cells by inhibiting signalling pathways such as the PI3K-Akt, MAPK, and Ras pathways. Given that this study was conducted using relevant databases and the conclusions lacked experimental support, subsequent biological experiments, and evidence-based drug validation are required to ensure the reliability of the findings. In addition, we explored the molecular mechanism of action of curcumin in treating prostate cancer, providing a theoretical basis for the future development and clinical application of this traditional Chinese medicine.

Data availability statement

All data in this paper can be collated from the open-source website provided by us and analysed by relevant software.Data related to our study have not been deposited in publicly available repositories. Readers may obtain original data from the corresponding author upon reasonable request.

Ethical and informed consent

Not applicable.

CRediT authorship contribution statement

Jun Li: Writing – original draft. **Xiong Wang:** Writing – review & editing. **Li Xue:** Writing – review & editing. **Qingmin He:** Writing – review & editing.

Declaration of competing interest

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

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