Identification of Molecular Targets and Underlying Mechanisms of Xiaoji Recipe against Pancreatic Cancer Based on Network Pharmacology

Cunbing Xia,1,2 Dexuan Chen,1,2 Gaoyuan Wang,1,2 Haijian Sun,1,2 Jingran Lin,1,2 Chen Chen,1,2 Tong Shen,1,2 Hui Cheng,1,2 Chao Pan,1,2 Dong Xu,3 Hongbao Yang,4 Yongkang Zhu1,2 and Hong Zhu1,2

1Department of General Surgery, Affiliated Hospital of Nanjing University of Chinese Medicine, Jiangsu Province Hospital of Chinese Medicine, Nanjing, Jiangsu 210029, China
2The Inheriting Studio of National Famous Old Chinese Medicine Experts-Zhu Yongkang (National Traditional Chinese Medicine Science and Education 2022 No. 75), Nanjing, Jiangsu 210029, China
3Pancreas Center, The First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu 210029, China
4Center for New Drug Safety Evaluation and Research, Institute of Pharmaceutical Science, China Pharmaceutical University, Nanjing, Jiangsu 211198, China

Correspondence should be addressed to Yongkang Zhu; zhuyk8888@126.com and Hong Zhu; njtc1998@163.com

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Traditional Chinese medicine (TCM) is applied in the anticancer adjuvant therapy of various malignancies and pancreatic cancer included. Xiaoji recipe consists several TCM materials with anticancer activities. In our work, we intended to analyze the molecular targets as well as the underlying mechanisms of Xiaoji recipe against pancreatic cancer. A total of 32 active components and 522 potential targets of Xiaoji recipe were selected using the TCMSP and SwissTargetPrediction databases. The potential target gene prediction in pancreatic cancer was performed using OMIM, Disgenet, and Genecards databases, and totally, 998 target genes were obtained. The component-disease network was constructed using the Cytoscape software, and 116 shared targets of pancreatic cancer and Xiaoji recipe were screened out. As shown in the protein–protein interaction (PPI) network, the top 20 hub genes such as TP53, HRAS, AKT1, VEGFA, STAT3, EGFR, and SRC were further selected by degree. GO and KEGG functional enrichment analysis revealed that Xiaoji recipe may affect pancreatic cancer progression by targeting the PI3K/AKT and MAPK signaling pathways. Moreover, we performed in vitro assays to explore the effect of Xiaoji recipe on pancreatic cancer cells. The results revealed that Xiaoji recipe suppressed the viability and migration and promoted the apoptosis of pancreatic cancer cells via the inactivation of PI3K/AKT, MAPK, and STAT3 pathways. The findings of our study suggested the potential of Xiaoji recipe in the targeting therapy of pancreatic cancer.

1. Introduction

Pancreatic cancer is a fatal malignancy and ranks the seventh leading cause of cancer-related death in both sexes, with approximately 5 million new cases and 466000 death cases in 2020 [1]. Many risk factors may contribute to the development of pancreatic cancer, including genetic background, obesity, type II diabetes, and tobacco smoking [2]. The chemotherapy with gemcitabine is regarded as the first-line treatment for pancreatic cancer, with 23.8% clinical response and a 5-year survival rate of 2% [3, 4]. However, the prognosis of pancreatic cancer patients is still unsatisfactory due to the late diagnosis, early metastasis, and limited chemotherapy effects [5]. Therefore, it is imperative to investigate potent treatment options to improve the clinical outcome of anticancer therapy in pancreatic cancer.

Traditional Chinese medicine (TCM), especially Chinese herbal medicines and acupuncture, is used to treat advanced
cancers with low-toxic effects and is reported to increase physical function, reduce symptoms, and improve the life quality of patients [6, 7]. Increasing studies have demonstrated the antitumor effects of TCM on the proliferation, metastasis, and tumorigenes in cancer development [8]. In pancreatic cancer, it has been reported that scoparone inhibits tumor progression via PI3K/Akt signaling pathway [9]. Besides, a proteoglycan extracted from *Ganoderma lucidum* can induce cancer cell apoptosis [10]. The prescription of Xiaoji recipe is mainly composed of *Curcuma zedoaria* (10 g, E Zhu), *Polygonum cuspidatum* (10 g, Huzhang), * Clematis root* (10 g, Weilingxian), *Rhizoma Paridis* (10 g, Zhon-glu), and *Eupolyphaga Steleophaga* (10 g, Tubiecheng). The main functions of Xiaoji recipe is to eliminate the heat and dampness and promote the blood circulation and can be used in the antitumor therapy for various cancers. *Curcuma zedoaria* (Zingiberaceae) is reported to inhibit the development of gastric carcinoma, breast cancer as well as liver cancer [11–14]. * Polygonum cuspidatum* is used for the therapy of multiple diseases including hypertension, diabetes, and atherosclerosis [15–17], and its extracts have been reported with anticancer effects in lung cancer, osteosarcoma, and breast cancer [18–20]. The extracts of *Rhizoma Paridis* is revealed to suppress the cancer development in non-small-cell lung carcinoma, colon cancer, and hepatocarcinoma [21–23]. *Eupolyphaga Steleophaga* is reported to be used in the treatment of fractures, falls, uterine fibroids, or menstrual problems [24], while its effects in cancer are not fully understood.

Network-based pharmacology is widely used in drug discovery by predicting potential mechanisms via exploring the targets of drugs, diseases, and their biomolecular networks [25, 26]. In TCM, a holistic perspective has long been at the heart of the herbal treatment of various diseases. TCM prescriptions have holistic theory and rich experience in multicomponent therapy, which provides a bright prospect for systematic treatment of complex diseases. Therefore, linking emerging network science with ancient TCM will provide new methods and opportunities to discover bioactive components and biomarkers, reveal mechanisms of action, and explore the scientific basis of TCM formulations based on complex biological systems [27]. Moreover, network-based pharmacology is becoming a frontier research field in current cancer drug research. For example, Huang et al. have explored the potential effect of Tao Hong Si Wu decoction for treating breast cancer according to network pharmacology and experimental [28].

Signaling pathways such as the phosphoinositide 3 kinase/AKT (PI3K/AKT) signaling pathway, signal transducer and activator of transcription 3 (STAT3) signaling pathway, and mitogen-activated protein kinases (MAPK) signaling pathway are important in the pathological process of pancreatic cancer and are frequently activated in pancreatic cancer. They are associated with poor prognosis of pancreatic cancer. Aberrant activation of these pathways are involved in cell survival, cell cycle progression, and cell apoptosis [29, 30]. Targeting these signaling pathways may be an approach to cancer treatment.

In our study, we intended to explore the potential core targets and pathways of Xiaoji recipe against pancreatic cancer based on the TCM network pharmacology approach. The findings of our study may provide clues for the targeting therapy of Xiaoji recipe in pancreatic cancer.

2. Materials and Methods

2.1. Cell Culture and Treatment. Pancreatic cancer cell lines (CFPAC (cat. no. CRL-1918; PANC1, cat. no. CRL-1469)) were provided by the ATCC (American Type Culture Collection, USA). CFPAC cell line was cultured in Iscove’s modified Dulbecco’s medium. PANC1 cell line was cultured in Dulbecco’s modified Eagle’s medium (DMEM, Cytiva, Shanghai, China). Both culture mediums were maintained in an incubator supplemented with 10% FBS (Beyotime, Shanghai, China) at 37°C and 5% CO₂. To evaluate the effects of Xiaoji recipe on cell malignant behaviors in vitro, the CFPAC and PANC1 cells were treated with 150 μg/ml or 300 μg/ml Xiaoji recipe.

2.2. Cell Viability. The viability of pancreatic cancer cells was measured using a Cell Counting Kit-8 (CCK-8; MCE, Inc., Shanghai, China). The treated CFPAC and PANC1 cells were grown into 96-well plates at 2000 cells/well and incubated for 24, 48, and 72 h, followed with addition of 10 μl CCK-8 solution, followed with incubation for another 2 h at 37°C. A microplate reader (HBS-1096A, DeTie Laboratory Equipment Co., Ltd., Nanjing, China) was used to determine the absorbance at 450 nm.

2.3. Wound Healing Assay. The treated CFPAC and PANC1 cells were plated into 6-well plates supplemented with medium and 1.5% fetal bovine serum and cultured to reach 90% confluence. Then, the plates were scratched using a 10 μl pipette tip. A microscope (Olympus, Shanghai, China) was used to photograph the wound healing distance at 0 and 48 h.

2.4. Flow Cytometry Analysis. The apoptosis of CFPAC and PANC1 cells after indicated treatments was assessed by flow cytometry analysis. CFPAC and PANC1 cells were harvested, washed with PBS, and resuspended in a 500 μl mixture with 5 μl Annexin and 10 μl propidium iodide (7-AAD; Multi-Sciences Biotech, Co., Ltd., Hangzhou, China). Finally, cell apoptosis rate was detected using a flow cytometer and analyzed with the FlowJo software. The apoptosis rate in each group was calculated by Q2 (late apoptosis) + Q3 (early apoptosis).

2.5. Western Blot. RIPA lysis buffer (Beyotime, Shanghai, China) was used to collect the protein in treated CFPAC and PANC1 cells. The concentration of collected proteins was evaluated using a BCA Protein Assay kit (Beyotime, Shanghai, China). Then, the proteins were separated with 10% SDS-PAGE gels and electrotransferred on the PVDF membranes. The Protein-Free Rapid Blocking Buffer (Epi-zyme, Shanghai, China) was used to block the membranes for 1 h, which were then cultured with the primary antibodies against AKT1 (1:1000, Abcam, USA), STAT3 (1:1000, Abcam, USA), EGFR (1:1000, Abcam, USA), MAPK3 (1:1000, Abcam, USA) at 4°C overnight, and
GAPDH (1:1000, Abcam, USA) served as an internal reference. Next, the membranes were washed with TBST and cultured with corresponding secondary antibodies (1:2000, Abcam, USA) for 2 h at room temperature. Finally, the protein signal was detected using a Biosharp ECL detection kit (Biosharp, Beijing, China) and analyzed with ImageJ software.

### 2.6. Exploration of Active Components and Potential Targets of Xiaoji Recipe

Xiaoji recipe is a traditional Chinese prescription composed of *Curcuma zedoaria* (E Zhu), *Polygnum cuspidatum* (Huzhang), clematis root (Weilingxian), *Rhizoma Paridis* (Zhonglou), and *Eupolyphaga Steleophaga* (Tubiechong). The active components of these TCM materials were searched on the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, http://lsp.nwu.edu.cn/tcmsp.php) [31] under the condition of oral bioavailability ($\text{OB} \geq 30\%$) and drug likeness (DL) $\geq 0.18$ and TCMID database (http://119.3.41.228:8000/tcmid/) [32]. The component structures obtained from the PubChem (https://pubchem.ncbi.nlm.nih.gov/) [33] and TCMSP databases were input into the SwissTargetPrediction database (http://www.swisstargetprediction.ch/) [34] for the prediction of the underlying target genes of main components in Xiaoji recipe.

### 2.7. Exploration of Potential Targets of Pancreatic Cancer

The potential target genes of pancreatic cancer were searched in the Disgenet (https://www.disgenet.org/) [35], OMIM (https://omim.org/) [36], and Genecards (https://www.genecards.org/) [37] databases using the "pancreatic ductal adenocarcinoma" as the keyword.

### 2.8. The Screening of Component-Disease Targets and Network Construction

The 522 targets of Xiaoji recipe and 998 targets of pancreatic cancer were imported into the Venny2.1 software, and the obtained Venn diagram showed 116 shared targets of the pancreatic cancer and the components of Xiaoji recipe. The drug-component-target-disease network was constructed using the Cytoscape 3.8.2 software and analyzed with the Network Analyzer function. The hub genes were analyzed and selected based on the topological

### Table 1: Relation between potential targets and active components in Xiaoji recipe.

| Component name                                      | Degree | Betweenness centrality | Closeness centrality | Eccentricity |
|-----------------------------------------------------|--------|------------------------|----------------------|--------------|
| Bisdemethoxycurcumin                                | 36     | 0.049102808            | 0.422096317          | 4            |
| Luteolin                                            | 31     | 0.024267623            | 0.408219178          | 4            |
| Quercetin                                           | 30     | 0.021502194            | 0.403794038          | 4            |
| Flavone                                             | 30     | 0.029632826            | 0.412742382          | 4            |
| Beta-ecdysone                                       | 27     | 0.02982601             | 0.40599455           | 4            |
| Physovenine                                         | 22     | 0.016298752            | 0.38501292           | 4            |
| (4aS,6aR,6bR,8aR,10R,12aR,14bS)-10-hydroxy-2,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,7,8a,10,11,12,13,14b-tetradecahydricene-4a-carboxylic acid | 18     | 0.011697317            | 0.37913486          | 4            |
| Pennogenin                                          | 18     | 0.008901981            | 0.37531486           | 5            |
| ClematosideA′_qt                                     | 17     | 0.010539948            | 0.37721519           | 4            |
| Picralinal                                           | 16     | 0.007242384            | 0.37343358           | 4            |
| Rhein                                               | 16     | 0.009358031            | 0.37157107           | 5            |
| Pennogenin VI                                       | 16     | 0.006015765            | 0.37157107           | 5            |
| Pennogenin VII                                       | 16     | 0.006015765            | 0.37157107           | 5            |
| 6,8-Dihydroxy-7-methoxyxanthone                     | 14     | 0.005718922            | 0.36253041           | 4            |
| Physciondiglucoside                                  | 11     | 0.005127525            | 0.36253041           | 4            |
| Diosgenin                                           | 10     | 0.002912114            | 0.35731419           | 5            |
| Beta-sitosterol                                      | 9      | 0.011919371            | 0.35560859           | 4            |
| Polysaccharide                                      | 9      | 0.003448702            | 0.34731934           | 5            |
| Hederagenin                                         | 8      | 0.004162798            | 0.35731419           | 4            |
| Stigmasterol                                         | 6      | 0.00185844             | 0.34731934           | 5            |
| Wenjine                                              | 5      | 0.001187609            | 0.32603938           | 5            |
| Cholesterol                                          | 5      | 0.005333285            | 0.34411085           | 4            |
| Embinin                                             | 4      | 0.001738369            | 0.33786848           | 5            |
| Dioscin I                                           | 4      | 3.06E-04               | 0.33333333           | 5            |
| Dioscin II                                          | 4      | 3.06E-04               | 0.33333333           | 5            |
| Torachrysone-8-O-beta-D-(6′-oxayl)-glucoside         | 3      | 2.64E-04               | 0.33483146           | 5            |
| Heptyl phthalate                                     | 3      | 3.77E-04               | 0.33634115           | 4            |
| Pariphyllin                                          | 3      | 1.43E-04               | 0.32891832           | 5            |
analysis, and the degree value indicates the relation between the components and targets (Table 1).

2.9. Protein–Protein Interaction (PPI) Network Construction. The interaction of the 116 component-disease targets was explored on the STRING platform (https://cn.string-db.org/) [38] under “Homo sapiens.” The protein was presented as nodes, and the association between proteins was shown as edges in the PPI network, and the size and shade of color represented the value of degree. Furthermore, based on the topological analysis, the results from the STRING database were analyzed with the Cytoscape 3.8.2 software using the Network Analyzer function, and the core targets

### Table 2: Active ingredients of Xiaoji recipe.

| Mol ID     | Molecule name                                                                 | OB (%) | DL   | Chinese medicinal materials                  |
|------------|-------------------------------------------------------------------------------|--------|------|-----------------------------------------------|
| MOL000296  | Hederagenin                                                                   | 36.91  | 0.75 | Curcuma zedoaria                              |
| MOL000906  | Wenjine                                                                        | 47.93  | 0.27 | Curcuma zedoaria                              |
| MOL000940  | Bisdemethoxycurcumin                                                          | 77.38  | 0.26 | Curcuma zedoaria                              |
| MOL013281  | 6,8-Dihydroxy-7-methoxyxanthone                                                | 35.83  | 0.21 | Polygonum cuspidatum                          |
| MOL013287  | Physsovenine                                                                   | 106.21 | 0.19 | Polygonum cuspidatum                          |
| MOL013288  | Picralinal                                                                     | 58.01  | 0.75 | Polygonum cuspidatum                          |
| MOL002259  | Physciondiglucoside                                                           | 41.65  | 0.63 | Polygonum cuspidatum                          |
| MOL002268  | Rhein                                                                          | 47.07  | 0.28 | Polygonum cuspidatum                          |
| MOL002280  | Torachrysone-8-O-beta-D-(6′-oxayl)-glucoside                                  | 43.02  | 0.74 | Polygonum cuspidatum                          |
| MOL00358   | Beta-sitosterol                                                                | 36.91  | 0.75 | Polygonum cuspidatum, Clematis root, Eupolyphaga Steleophaga |
| MOL00492   | (+)-catechin                                                                   | 54.83  | 0.24 | Polygonum cuspidatum                          |
| MOL000006  | Luteolin                                                                       | 36.16  | 0.25 | Polygonum cuspidatum                          |
| MOL00098   | Quercetin                                                                      | 46.43  | 0.28 | Polygonum cuspidatum                          |
| MOL001663  | (4αS,6αR,6αS,6bR,8aR,10R,12αR,14bS)-10-hydroxy-2,2,6a,6b,9,9,12α-heptamethyl-1,3,4,5,6,6a,7,8,8a,10,11,12,13,14b-tetradecahydropicene-4α-carboxylic acid | 32.03  | 0.76 | Clematis root                                  |
| MOL00372   | (6Z,10E,14E,18E)-2,6,10,15,19,23-hexamethyldiacetacosa-2,6,10,14,18,22-hexaene | 33.55  | 0.42 | Clematis root                                  |
| MOL00449   | Stigmasterol                                                                   | 43.83  | 0.76 | Clematis root                                  |
| MOL00594   | ClematosideA_qu                                                                 | 37.51  | 0.76 | Clematis root                                  |
| MOL00598   | Embinin                                                                       | 33.91  | 0.73 | Clematis root                                  |
| MOL005603  | Heptyl phthalate                                                               | 42.26  | 0.31 | Clematis root                                  |

### Table 3: Active ingredients and their potential targets in Xiaoji recipe.

| Name                        | Ingredients (n) | Predicted targets (n) |
|-----------------------------|-----------------|-----------------------|
| Curcuma zedoaria (Ezhu)     | 3               | 159                   |
| Polygonum cuspidatum (Huzhang) | 10             | 313                   |
| Clematis root (Weilingxian) | 7               | 107                   |
| Rhizoma Paridis (Zhonglou)  | 10              | 271                   |
| Eupolyphaga Steleophaga (Tubiechong) | 4     | 44                    |
were selected under the condition of degree value over the average. The top 30 targets were selected and exported the bar graph using the R 4.0.5 software. Based on the clustering analysis, the results from the STRING database were imported into the Cytoscape 3.8.2 software and analyzed using the Molecular Complex Detection (MCODE) plugin. Three gene clusters were obtained, and 2 core genes (HSP90AA1, CDK6) were selected.

2.10. GO and KEGG Functional Enrichment Analysis. Gene Ontology (GO) analysis including the biological process (BP), molecular function (MF), and cellular components (CC) and the biological pathway (KEGG) enrichment analysis of 116 disease-component targets were performed using the R software with Bioconductor package under \( p < 0.05 \). The results were exported with the bar graphs and pathway maps.

2.11. Statistical Analysis. All experiments were completed three times independently. The results were analyzed using GraphPad 8 software and presented as the mean ± SD. Student’s \( t \)-test was used to compare the difference between two groups, and one-way ANOVA was used for multiple group comparisons. \( p < 0.05 \) indicates statistical significance.

3. Results

3.1. Active Ingredients and Their Potential Targets in Xiaoji Recipe. We found 32 potential active ingredients of Xiaoji recipe Curcuma zedoaria (E Zhu), Polygonum cuspidatum (Huzhang), clematis root (Weilingxian), Rhizoma Paridis (Zhonglou) and Eupolyphaga Steleophaga (Tubiechong) based on the TCMSP database, under \( OB \geq 30 \% \) and \( DL \geq 0.18 \) and TCMID database (Table 2). There were 3 ingredients from Curcuma zedoaria, 10 ingredients from Polygonum cuspidatum, 7 ingredients from clematis root, 10 ingredients from Rhizoma Paridis, and 4 ingredients from Eupolyphaga Steleophaga (Table 3). Curcuma zedoaria, Polygonum cuspidatum, and clematis root share the same ingredient beta-sitosterol. Based on the SwissTargetPrediction platform, we performed the prediction of the potential targets of active components in Curcuma zedoaria (E Zhu), Polygonum cuspidatum (Huzhang), clematis root (Weilingxian), Rhizoma Paridis (Zhonglou), and Eupolyphaga Steleophaga. Totally, 522 target genes of these active ingredients of Xiaoji recipe were screened out, and there were 159 targets for Curcuma zedoaria, 313 targets for Polygonum cuspidatum, 116 targets for clematis root, 271 targets for Rhizoma Paridis, and 44 targets for Eupolyphaga Steleophaga (Table 3).

3.2. Construction of the Target Gene Network of Pancreatic Cancer and Xiaoji Recipe. We searched the potential targets in the OMIM, Disgenet, and Genecards using the keywords “pancreatic ductal adenocarcinoma,” and totally, 998 potential targets of PDAC were obtained. Venny 2.1 software was used to select the shared targets for PDAC and Xiaoji recipe, and the results showed that there were 116 shared targets in the intersection area (Figure 1(a)). Then, the 32 active
Figure 2: Continued.
The remaining 28 active components were marked in red in Table 2. The main active biomolecules were analyzed using the Network Analyzer function. As shown in Figure 1(b), the red button represented the disease, the blue bubbles represented the 116 compound-disease targets, the purple rectangle represented the Chinese medical materials, and the green triangles represented the 28 active ingredients in Xiaoji recipe. The top five core ingredients were bisdeme-thoxycurcumin, luteolin, quercetin, flavone, and beta-ecdysone, as shown in Table 1.

3.3. Construction of the PPI Network and Analysis of Targets. The PPI network was constructed based on the STRING database and Cytoscape software to investigate the underlying interaction among the 116 targets. Based on the topology analysis, the 116 compound-disease targets were input in the STRING platform for the protein-protein interaction network construction. There were 116 nodes and 2210 edges (Figure 2(a)). Further, the interaction data from the STRING database were imported into the Cytoscape software for the interaction network. The degree was determined by the size and color shade of the nodes (Figure 2(b)). TP53, HRAS, VEGFA, AKT1, STAT3, EGFR, and SRC were the core target genes.

The hub genes in the network were identified by the top 30 targets ranked by degree on the PPI network, which was performed using the R 4.0.5 software as shown in Figure 3(a). Moreover, based on the clustering analysis and analysis using Cytoscape software, three gene clusters were obtained with two core target genes, HSP90AA1 and CDK6, which were potentially involved in the development of PDAC (Figure 3(b)).

3.4. GO and KEGG Pathway Enrichment Analysis. Based on the GO enrichment analysis, the biological process, cellular component, and molecular function of the 116 component-disease targets were analyzed using R software. The results revealed that the target genes were enriched in 2144 BP, 50 CC expression process, and 132 MF-related process. The biological functions of targets mainly included the peptidyl-serine modification and phosphorylation, positive modulation of protein serine/threonine kinase activity and MAP kinase activity, and gland development (Figure 4). The molecular functions mainly included the protein tyrosine kinase activity, phosphatase binding, insulin receptor substrate binding, and
growth factor binding (Figure 5). The cellular component mainly included the transferase complex, protein kinase complex, serine/threonine protein kinase complex, membrane raft, and transcription regulator complex (Figure 6).

A total of 159 KEGG signaling pathways of the 116 shared target genes were obtained using the R software. The top 20 significant signaling pathways were shown in histograms. The results revealed that these targets were closely
associated with the PI3K-AKT pathway, EGFR tyrosine kinase inhibitor resistance, endocrine resistance and MAPK signaling, and pancreatic cancer, which may improve the understanding of the molecular mechanism associated with pancreatic cancer progression (Figure 7). Moreover, the target genes involved in the PI3K-AKT and MAPK signalings in pancreatic cancer were shown in Figures 8(a) and 8(b).

3.5. Xiaoji Recipe Inhibited the Proliferation and Migration of Pancreatic Cancer Cells. Furthermore, we explored the
Effects of Xiaoji recipe on the malignant behaviors of pancreatic cancer cells. As revealed by the CCK-8 assay, the viability of CFPAC and PANC1 cells exhibited significant reduction with Xiaoji recipe treatment in a concentration-dependent way (Figures 9(a) and 9(b)). Furthermore, we conducted wound healing assays to explore the impact of Xiaoji recipe on pancreatic cancer cell migration. The results demonstrated that the migration ability of CFPAC and
Mapk signaling pathway

Classical MAP kinase pathway

Heterotrimetric G-protein

CACN

C2a+

RasGRF

RasGRP

PKC

Rap1

RafA

RafB

Raf1

MEK1

MEK2

MP1

ERK

PTP MKP

PPP3C

c-Myc

Sapla

Elk-1

CREB

RSK2

MNK1/2

cPLA2

STMN1

Tau

IP3

cAMP

DAG

Phosphatidylinositol signaling system

NIK

IKK

NFkB

Proliferation, inflammation

anti-apoptosis

SRF

DNA

c-Fos

DNA

Proliferation,
differentiation

GF RTK GRB2 SOS Ras

NF1

Gap1

mG12

JNK and p38 MAP kinase pathway

Senium, cytotoxic drugs,
irradiation, heatshock,
reaction oxygen species,
lipopolysaccharide,
and other stress

TNF TNFR

IL1

FASL

TGFB

IL1R

FAS

TGFBR

CD14

TRADD

MYD88

CASP

TRAF2

MST1/2

GCK

DAXX

IRAK1/4

TRAF6

GADD45

TAB1

TAB2

ECSIT

Scaffold

GLK

HGK

HPK1

PAK1/2

Tpl2/Cot

MEKK1

MLK3

LZK

MUK

MLTK

ASK2

ASK1

TAK1

PP2CB

MEKK4

TAO

FLNA JIP3

ARRB CrkII

MKK4

MKK7

HSP72

GST

𝜋

Evil NFAT-2

NFAT-4

AP1

JunD

JNK

JIP1/2

PP2CA

AKT PTP

MKP

ATF-2

Elk-1

p53

MKK3

MKK6

PP5

p38

Sapla

MAX

MEF2C

HASP27

CREB

PRAK

MSK1/2

Cdc25B

NLK

LPS

DNA damage

ERK5 pathway

Serum, EGF,
reactive oxygen species

or

Srk tyrosin kinase
downstream

DNA damage

p53 downstream

pathway

DNA

Wnt signaling pathway

MAPKK MAPK Transcription
factor

Nur77

DNA cycle

Apoptosis

Cell cycle

Transcription factor

Proliferation, differentiation

Data on KEGG graph rendered by pathview

(a)

Figure 8: Continued.
PANCl was significantly suppressed by the treatment of Xiaoji recipe, and the higher the concentration, the more significant the suppression (Figures 9(c) and 9(d)).

3.6. Xiaoji Recipe Promoted the Apoptosis of Pancreatic Cancer Cells In Vitro. The effects of Xiaoji recipe on the apoptosis of pancreatic cancer cells were explored using flow cytometry analysis. As shown in Figures 10(a) and 10(b), the apoptosis rate of CFPAC and PANCl cells were continuously elevated as the concentration of Xiaoji recipe increased, which indicated that Xiaoji recipe facilitated pancreatic cancer cell apoptosis in a dose-dependent way.

3.7. Xiaoji Recipe Exerted Inhibitory Effects on the Activation of AKT, MAPK, and STAT3 Signaling Pathways. Whether Xiaoji recipe affected the activation of AKT, MAPK, and STAT3 signaling pathways was further explored. The protein expression of AKT1, STAT3, EGFR, and MAPK3 in pancreatic cancer cells was detected using Western blot. We found that the AKT1, STAT3, EGFR, and MAPK3 protein expression showed significant decrease in CFPAC and PANCl cells after Xiaoji recipe treatment in a dose-dependent manner (Figures 11(a) and 11(b)).

4. Discussion

Pancreatic cancer, as one of the most fatal malignancies, is reported with increasing incidence in recent years. The prognosis of pancreatic cancer patients is still unsatisfying due to the atypical early symptoms and distal metastasis [39]. Thus, it is imperative to explore the underlying mechanism of pancreatic cancer pathogenesis. In our work, the primary active components of Xiaoji recipe and the potential targets and mechanisms in the treatment of pancreatic cancer were investigated based on network pharmacology. The exploration of underlying mechanism of Xiaoji recipe may provide evidence for the TCM therapy in pancreatic cancer.

Chinese herbal medicine (CHM) is indicated as a promising treatment option for multiple malignant diseases with unique clinical effects [40, 41]. As reported previously, Xiaoji recipe has been to inhibit cell migration and growth of lung adenocarcinoma and gastric cancer [42, 43]. Consistently, our study found that Xiaoji recipe repressed cell proliferation and migration while promoted cell apoptosis in pancreatic cancer. More importantly, the main active components of Xiaoji recipe were explored in our study. Bisdemethoxy-curcumin, luteolin, quercetin, flavone, and beta-ecdysone
were the top five active ingredients ranked by degree according to the component-target-disease network, which were potentially the main active ingredients in the treatment of pancreatic cancer. Bisdemethoxycurcumin is reported with anti-fibrosis, anti-apoptosis, antioxidant, and anti-inflammatory characteristic in various diseases [44–46]. Bisdemethoxycurcumin is also reported with antitumor effects in hepatocellular carcinoma, breast cancer, and non-small-cell lung cancer [47–49]. Luteolin is a flavonoid with anti-inflammation, antiallergy, and anticancer properties and suppresses cell transformation, metastasis, invasion, and angiogenesis in carcinogenesis [50–52]. It has also been reported to inhibit the invasion and epithelial-mesenchymal transition of pancreatic cancer cells by inactivating the STAT3 pathway [53]. Quercetin is reported with cytotoxic and antitumor effects and suppresses pancreatic cancer progression by inhibiting the STAT3 pathway activation [54]. The active ingredients possess different degrees of therapeutic effects on pancreatic cancer and are involved in various signaling pathways.

The component-disease network showed the target genes regulated by these active components in pancreatic cancer. The genes include EGFR, CDK1, MMP7, BCL2, and PARP1. Moreover, the PPI networks revealed that TP53, HRAS, VEGFA, AKT1, STAT3, EGFR, and SRC were the core target genes in the PPI network. The target genes were not mutually independent but were interacted with each other. The Xiaoji recipe may inhibit the progression of pancreatic cancer by regulating the multiple proteins. In line with our finding, all mentioned core target genes have been reported as major driver genes for pancreatic cancer [55–60].

GO and KEGG enrichment analysis revealed the biological process, molecular functions, cellular component as well as the related signaling pathways in the occurrence and progression of pancreatic cancer. There were 2144 BP, 50 CC expression process, and 132 MF-related process of these 116 component-disease targets. We also found that the enrichment of the 116 component-disease targets in the PI3K/AKT and MAPK pathways according to the KEGG analysis. PI3K/AKT pathway is critically involved in the regulation of malignant behaviors in pancreatic cancer, indicating that PI3K/AKT is a valuable target for pancreatic cancer therapy [30, 61]. The MAPK pathways are reported to regulate the tumor cell proliferation, differentiation, apoptosis, and resistance to drug therapy, and PI3K/AKT and Ras/MEK/MAPK pathways were the two main signaling in the downstream of EGFR [62]. In our study, we found that EGF is one of the component-disease targets, and Xiaoji recipe may inhibit the activation of MAPK signaling by targeting EGF in pancreatic cancer. In addition, we performed in vitro assays to demonstrate that Xiaoji recipe exerted inhibitory effects on the activation of PI3K/AKT, MAPK,
and STAT3 signaling pathways. For all we know, these three signaling pathways are widely reported in the involvement of pancreatic cancer. Moreover, many literatures have supported that TCMs inhibit tumor progression via these signaling pathways [63–65].

In this study, the active ingredients and related targets of Xiaoji recipe in pancreatic cancer were explored, and the biological process, molecular function, cellular process, and related signaling pathways were under investigation based on the network pharmacology approach. Moreover, we also verified the effect of Xiaoji recipe on pancreatic cancer cell proliferation, migration, apoptosis, and relevant signaling pathways using in vitro assays. Xiaoji recipe significantly inhibited the proliferation, migration, AKT, MAP, and
STAT3 signaling pathways and induced the apoptosis of pancreatic cancer cells, which indicated its potential in clinical therapy of pancreatic cancer patients.

However, our study also exists some limitations. First, the in vivo experiments of Xiaoji recipe on tumor growth were lack. Second, the specific mechanism of Xiaoji recipe on regulating the PI3K/AKT, MAPK, and STAT3 signaling pathways were not explored. More, the potential mechanism of Xiaoji recipe on regulating the core target genes of TP53, HRAS, VEGFA, AKT1, STAT3, EGFR, and SRC in pancreatic cancer were not shown. All these limitations will be perfected in the near future.

Data Availability

Data generated in this study are available from the corresponding author under reasonable requests.

Conflicts of Interest

There is no any conflict of interest.

Authors’ Contributions

Cunbing Xia and Dexuan Chen contributed equally to this work.

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