The Mechanism by Which *Pereskia aculeata* Mill. Regulates Ferroptosis and Interferes with Colon Cancer Based on Network Pharmacology

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Abstract [**Objectives**] The paper was to investigate the mechanism by which *Pereskia aculeata* Mill. regulates ferroptosis and interferes with colon cancer using network pharmacology. [**Methods**] The effect of *P. aculeata* on HCT116 cells was observed by examining cell morphology. The intersection of *P. aculeata*, colon cancer, and ferroptosis targets was determined using the Venny 2.1.0 online platform. DAVID database was used to perform GO and KEGG enrichment analyses. Cytoscape_v3.10.0 was used for network mapping and PPI analysis. [**Results**] The observation of cell morphology indicated that *P. aculeata* suppressed the growth of HCT116 cells. GO and KEGG analysis identified 67 genes involved in potential pathways, including the MAPK signaling pathway and Apoptosis, 18 of which were located on the MAPK pathway. After conducting PPI visualization analysis and ranking based on Degree values, we identified *TP53*, *TNF*, *EGFR*, *MAPK14*, and *AKT1* as the top five targets with the highest Degree values. [**Conclusions**] *P. aculeata* may activate the *p53* gene through the MAPK signaling pathway, inducing ferroptosis and ultimately resulting in tumor cell death.

Key words *Pereskia aculeata* Mill., Ferroptosis, MAPK signal pathway, Colon cancer

1 Introduction

Ferroptosis is a widespread form of cell death that is iron-dependent and non-apoptotic[1]. It is a byproduct of cellular metabolism, and the production of reactive oxygen species (ROS) is inevitable due to the importance of oxygen and iron in metabolism. If phospholipid hydroperoxides (PLOOH) are not effectively neutralized and accumulated, they can disrupt plasma membrane integrity, leading to ferroptosis[2-3]. In addition, various factors can induce ferroptosis, including the inhibition of GPX4[4]. Ferroptosis has been implicated in numerous diseases, such as tumor suppression, neurodegeneration, ischemic organ damage, cardiac injury, and organ transplantation, by triggering its occurrence[5].

Colon cancer is a prevalent gastrointestinal malignancy and ranks third in terms of frequency of occurrence[6]. It is also the second leading cause of death in the United States, following lung cancer. Its incidence rate is only surpassed by breast, lung, and prostate cancers[7]. Although there have been significant advances in the treatment of colon cancer, its incidence continues to increase[8]. Currently, only chemotherapeutic agents such as oxaliplatin and fluoropyrimidines have been proven effective[9], and drug development is imminent. Regulating SLC7A11 can induce ferroptosis in colorectal cancer stem cells[10]. Triggering ferroptosis can inhibit the growth of colon cancer cells.

Pereskia aculeata Mill. is a plant native to tropical America and is cultivated in Guangxi, Yunnan, Guangdong, Fujian, and other parts of China. Studies have shown that *P. aculeata* has low acute toxicity in rats[11]. *P. aculeata* is a medicinal plant with analgesic potential[12]. Additionally, research has found that *P. aculeata* significantly reduces ADCY1 expression in SH-SY5Y cells[13]. Its powder has been shown to improve intestinal health[14]. However, it is currently unknown whether *P. aculeata* can have an anti-colon cancer effect through ferroptosis and the MAPK pathway. Therefore, we investigated this using network pharmacology.

2 Materials and methods

2.1 Materials

2.1.1 Reagents. Ethanolic extract of *P. aculeata* (self-extracted); DMEM (GIBCO Inc.); fetal bovine serum (BI); trypsin digestion solution (Solarbio Technology Ltd.); double antibody (GIBCO Inc.).

2.1.2 Laboratory instruments. Inverted electron microscope (Olympus); electrothermal constant temperature water bath (Tianjin Taiste Instrument Co., Ltd.); carbon dioxide incubator (PHC Co., Ltd.); ultra-clean bench (Sujeong Group); low-speed centrifuge (Shanghai Anting Scientific Instrument Co., Ltd.).

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2.2 Methods

2.2.1 Effect of ethanol extract of *P. aculeata* on HCT116 cells observed by an inverted microscope. The ethanol extract of *P. aculeata* was administered at concentrations of 250, 500, and 1 000 μg/mL, representing low, medium, and high concentrations, respectively. A total of 1.5×10^6 cells were incubated in 6-well plates at 5% CO_2 and 37 ℃ for 24 h. After 24 h, the drug was administered, and morphological changes were observed after 48 h.

2.2.2 Target acquisition. Colon Cancer: Searching for the keyword "Colon Cancer" yielded 22 356 results. Ferroptosis: Searching for the keyword "Ferroptosis" in GeneCards resulted in 1 354 genes. The Ferrdb database (http://www.zhounan.org/ferrdb/current/) provided a list of 483 genes that promoted, prevented, or indicated the occurrence of ferroptosis. Combining these results with another database and removing duplicates yielded a total of 1 572 genes.

2.2.3 Intersection of *P. aculeate* and colon cancer targets. A total of 67 genes were identified through the Venny 2.1.0 online platform at https://bioinfogp.cnb.csic.es/tools/venny/, which were found to be intersecting.

2.2.4 GO and KEGG enrichment. The DAVID database (https://david.ncifcrf.gov/) was used to perform KEGG analysis on 67 intersecting genes, and the bioinformatics online visualization cloud platform (https://www.bioinformatics.com.cn/) was used for mapping. The analysis revealed that 18 genes were in the MAPK pathway. Additionally, GO analysis was conducted, including analysis of cellular component (CC), molecular function (MF), and biological process (BP).

2.2.5 PPI network construction. PPI analysis was conducted on the 18 genes in the MAPK pathway using the STRING database (https://cn.string-db.org/). Network mapping was performed using Cytoscape_v3.10.0, and the network analyzer plugin was used to calculate the Degree value. The Degree value is indicated by the darkness of the color and the size of the area in the graph.

3 Results and analysis

3.1 Ethanol extract of *P. aculeate* inhibits the growth of HCT116 cells Following the administration of the ethanol extract of *P. aculeate*, inverted microscopy revealed a decrease in the number of HCT116 cells (Fig. 1). This suggests that *P. aculeate* may inhibit the growth of HCT116 cells.

3.2 Common targets of *P. aculeate*, colon cancer and ferroptosis A total of 22 356 targets related to colon cancer were identified using the Genecard online platform. To narrow down the results, only the top quartile with the highest relevance, totaling 5 590 genes, was selected. The genes were intersected with 1 572 ferroptosis genes and 1 500 targets predicted by 15 compounds of *P. aculeate*. This resulted in 67 common targets (Fig. 2). The evidence indicates that ferroptosis may play a role in the treatment of colon cancer by *P. aculeate*.

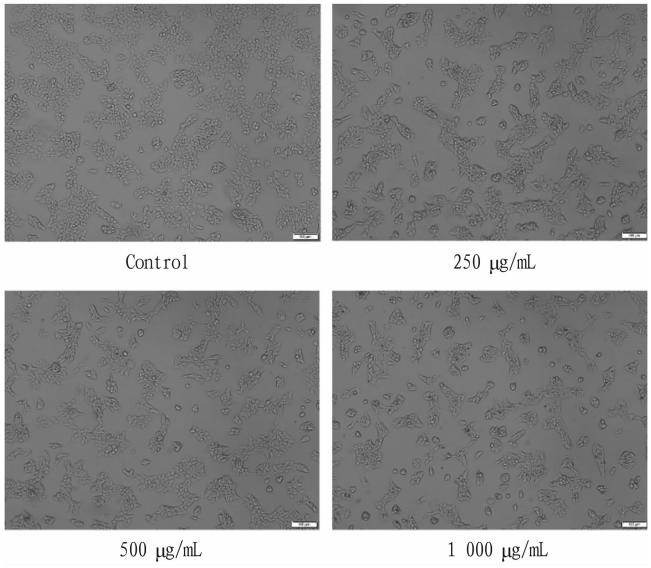


Fig. 1 Effect of *Pereskia aculeata* ethanol extract on the morphology of HCT116 cells (100 ×)

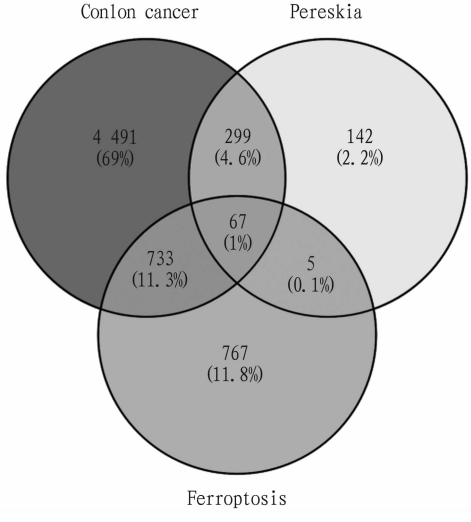
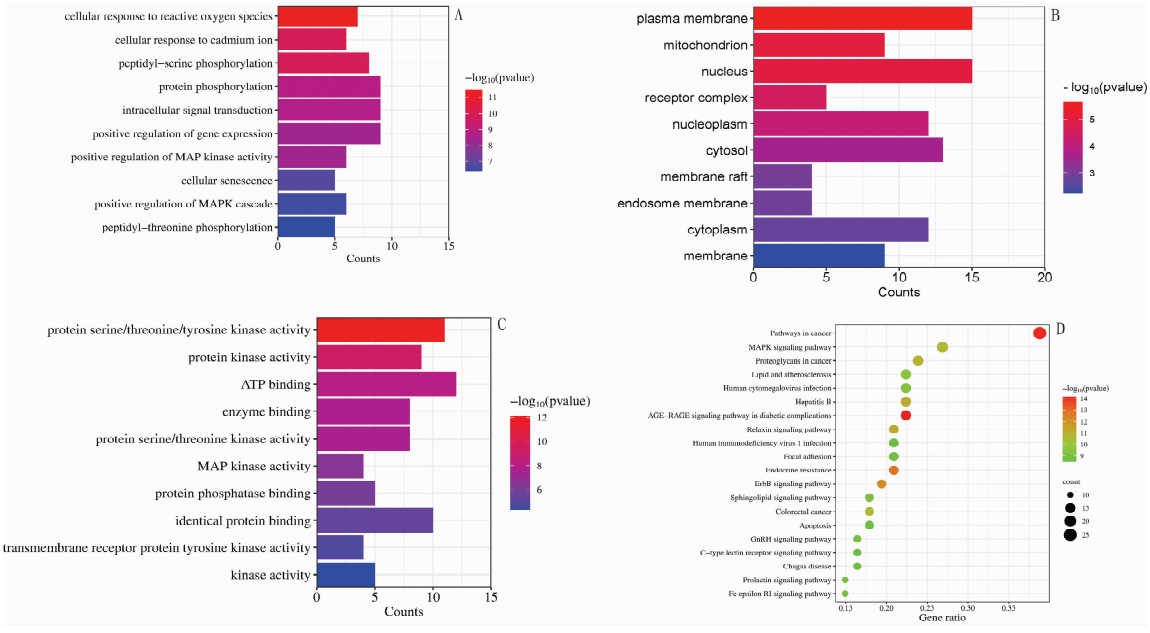


Fig. 2 Intersection of target genes related to colon cancer and ferroptosis in the action of *Pereskia aculeata*

3.3 Mechanism analysis of potential targets of *P. aculeate*, colon cancer and ferroptosis A bioinformatics online visualization cloud platform was used to map the potential targets. The results of the GO enrichment analysis of 67 potential targets showed that BP was primarily involved in the cellular response to reactive oxygen species, cellular response to cadmium ion, peptidyl-serine phosphorylation, protein phosphorylation, intracellular signal transduction, positive regulation of gene expression, positive regulation of MAP kinase activity, cellular senescence, positive regulation of MAPK cascade, and peptidyl-threonine phosphorylation (Fig. 3A); CC was primarily involved in plasma membrane, mitochondrion, nucleus, receptor complex, nucleoplasm, cytosol, membrane raft, endosome membrane, cytoplasm, and membrane (Fig. 3B); MF was primarily involved in protein serine/threonine/tyrosine kinase activity, protein kinase activity ATP binding, enzyme binding, protein serine/threonine kinase activity, MAP

kinase activity, protein phosphatase binding, identical protein binding, transmembrane receptor protein tyrosine kinase activity, and kinase activity (Fig. 3C). The KEGG enrichment analysis revealed significant associations between the targets and pathways in cancer, MAPK signaling pathway, Colorectal cancer, and Apoptosis (Fig. 3D).



NOTE A. GO enrichment analysis of identified targets from BP; B. GO enrichment analysis on identified targets from CC; C. GO enrichment analysis on identified targets based on MF, with the importance order sorted by $-\log_{10}(p\ \text{value})$ on the bar chart; D. Ranking the relationship between KEGG pathway enrichment results and potential targets in order of importance by the count value on the bar chart.

Fig. 3 GO and KEGG enrichment analysis

3.4 Visualization analysis of common targets of *P. aculeate*, colon cancer, ferroptosis and MAPK pathway The DAVID database was used to perform KEGG analysis on 67 intersecting genes, revealing 18 targets on the MAPK pathway (Table 1). The PPI analysis ranked 18 targets based on their Degree value. The tumor protein *p53* gene (*TP53*) had the highest Degree value, followed by the family member of human epidermal growth factor receptor (*EGFR*), tumor necrosis factor (*TNF*-α), serine/threonine protein kinase (*AKT1*), and mitogen-activated protein kinase 14 (*MAPK14*) (Fig. 4).

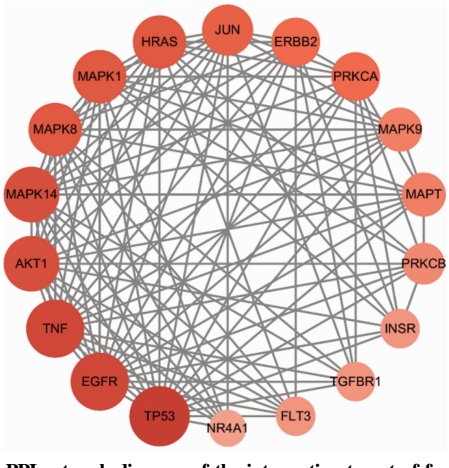


Fig. 4 PPI network diagram of the intersection target of ferroptosis and MAPK pathway in colon cancer treated with *Pereskia aculeata*

Table 1 Degree values of the common targets of ferroptosis and MAPK pathway in colon cancer treated with *Pereskia aculeata*

Gene	Degree	Gene	Degree
TP53	17	*PRKCA*	12
TNF	16	*ERBB2*	12
EGFR	16	*MAPT*	10
MAPK14	15	*MAPK9*	10
AKT1	15	*PRKCB*	9
MAPK8	14	*TGFBR1*	8
MAPK1	14	*INSR*	8
HRAS	14	*FLT3*	8
JUN	13	*NR4A1*	7

4 Discussion

After comparing the morphology of NCT116 cells before and after administering *P. aculeate*, it was discovered that *P. aculeate* had a stronger inhibitory effect on HCT116 cells. The cells were inhibited to varying degrees after administration of *P. aculeate* ethanol extract at different concentrations, with the highest inhibitory effect observed in the high-dose group. Pre-laboratory studies have shown that *P. aculeate* may be effective in treating arthritis by regulating the MAPK pathway[15]. Additionally, MAPK can activate ferroptosis through multiple pathways[16]. Ferroptosis is a common form of cell death. There is a significant amount of literature suggesting that ferroptosis may play a role in suppressing colon cancer[17-18]. Therefore, it is hypothesized that *P. aculeate* may activate ferroptosis through the MAPK pathway, thus inhibiting the

growth of colon cancer. To clarify the antitumor mechanism of action of *P. aculeate*, we used a network pharmacology approach to predict and analyze potential targets and pathways of *P. aculeate*'s anti-colon cancer effects[19].

A total of 67 common targets were obtained by intersecting predicted targets of *P. aculeate*, therapeutic targets of colon cancer and targets of ferroptosis through online platforms, suggesting that *P. aculeate* is able to inhibit the growth of colon cancer through ferroptosis. Subsequent GO and KEGG enrichment analysis revealed that two related signaling pathways, pathways in cancer, and MAPK signaling pathway, had high count values. The mechanism by which *P. aculeate* intervenes with colon cancer through ferroptosis may be related to MAPK signaling pathway and Apoptosis.

Analysis of the DAVID database revealed 18 common targets of the MAPK pathway and ferroptosis. *TP53*, *EGFR* and *TNF* were the three targets with the highest Degree values. *TP53* gene, also known as *p53*, is a tumor suppressor gene. Previous literatures suggest that inhibiting the MAPK signaling pathway and activating the LKB1-AMPK signaling pathway can activate the *p53* gene, thus inducing ferroptosis. SLC7A11 is a target of the *p53* gene and a crucial factor in the occurrence of ferroptosis. The *p53* gene inhibits System Xc- by suppressing the expression of the key protein SLC7A11, which leads to the impairment of cystine and affects the biosynthesis of GSH, inhibiting GPX4 and ultimately leading to cellular ferroptosis[16]. *EGFR* is extensively distributed in cell membranes of human tissues and is an epidermal growth factor receptor. Activation of *EGFR* enhances the expression of SLC7A11 in glioma cells, which in turn enhances the transport activity of System Xc- and inhibits ferroptosis[20]. The activity of the MAPK pathway is also suppressed while inhibiting *EGFR*[17]. *TNF* was the first cytokine causing hemorrhagic necrosis in tumor tissue cells, and it can activate the NF-κB and c-Jun pathways and the MAPK pathway. *TNF* can protect fibroblasts from oxidative stress and excess iron by enhancing cystine uptake and consequently cellular GSH production through activation of the NF-κB pathway[21].

5 Conclusions

In summary, the mechanism by which *P. aculeate* inhibits colon cancer cell growth may be that it activates the *p53* gene through the MAPK signaling pathway, inducing ferroptosis and ultimately resulting in tumor cell death. The information provides ideas and directions for further in-depth study of the mechanism of action of *P. aculeate* against colon cancer.

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