Kaempferol-induced mitochondrial damage promotes NF-κB-NLRP3-caspase-1 signaling axis-mediated pyroptosis in gastric cancer cells

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Abstract

Purpose Gastric cancer GC is a gastrointestinal tumor with high morbidity and mortality. Owing to the high rate of postoperative recurrence associated with GC, the effectiveness of radiotherapy and chemotherapy may be compromised by the occurrence of severe undesirable side effects. In light of these circumstances, kaempferol (KP), a flavonoid abundantly present in diverse herbal and fruit sources, emerges as a promising therapeutic agent with inherent anti-tumor properties. This study endeavors to demonstrate the therapeutic potential of KP in the context of Gastric cancer while unraveling the intricate underlying mechanisms.

Methods We employed methods of bioinformatics, network pharmacology, and molecular docking, followed by multiple experimental validations.

Result Our investigations unveil that KP stimulation effectively promotes the activation of NLRP3 inflammatory vesicles within AGS cells by engaging the NF-κB signaling pathway. Consequently, the signal cascade triggers the cleavage of Caspase-1, culminating in the liberation of IL-18. Furthermore, we ascertain that KP facilitate AGS cell pyroptosis by inducing mitochondrial damage.

Conclusion Our findings showcase KP as a compelling candidate for the treatment of Gastric cancer-related diseases, heralding new possibilities for future therapeutic interventions.

1. Introduction

Gastric cancer (GC) is the third leading cause of cancer-related deaths worldwide. (Ajani, D'Amico et al. 2022) Most patients are diagnosed at advanced stages due to the subtle symptoms of earlier disease and the low rate of regular screening. (Guan, He et al. 2023) The coronavirus disease 2019 (COVID-19) pandemic caused delays in the diagnosis and treatment of cancer. (Siegel, Miller et al. 2023) Although the impact of the COVID-19 pandemic has gradually diminished, the resumption of healthcare services in its entirety remains incomplete, leaving many advanced-stage patients unable to endure the adverse effects associated with radiation and chemotherapy. Consequently, it is of paramount importance to explore novel approaches for clinical gastric cancer treatment through fundamental experiments, providing new insights and directions.

Cellular pyroptosis represents a form of programmed cell death characterized by cellular distension culminating in the rupture of the cell membrane, thereby leading to the release of cellular contents. This process elicits a potent activation of inflammatory and immune responses. (Wei, Xie et al. 2022) NLRP3 inflammasome, being the most prominent and extensively investigated inflammasome complex, exerts pivotal roles in this context. (Lv, Fan et al. 2021) Activation of NLRP3 triggers the secretion of caspase-1-dependent proinflammatory cytokines IL-1β and IL-18, instigating an inflammatory-related cell death process known as pyroptosis. (Chen, Lee et al. 2022)
Kaempferol (KP; 3,4',5,7-tetrahydroxyflavone), also known as indigo yellow, represents a nutrient abundantly present in numerous plants, fruits, and vegetables, such as tea, cabbage, broccoli, apples, and grapes. (Kubina, Krzykawski et al. 2023) Accumulating scholarly literature substantiates the favorable therapeutic effects of kaempferol on various malignant tumors, including gastric cancer (Ren, Lu et al. 2019), lung cancer (Jo, Park et al. 2015), liver cancer (Guo), breast cancer (Sindhu, Verma et al. 2021), and glioma (Chen, Zhao et al. 2023). Furthermore, multiple studies have elucidated the anti-cancer potential of kaempferol through various pathways, encompassing inhibition of angiogenesis and vascular endothelial growth factor (VEGF) expression, modulation of hypoxia-inducible factor-1α (HIF-1α)-mediated apoptosis induction, induction of G2/M cell cycle arrest, and caspase-3-dependent cellular apoptosis. Nonetheless, further refinement is warranted to unravel the precise mechanisms underlying the anti-gastric cancer effects of kaempferol. (Almatroudi, Allemailem et al. 2023) Nonetheless, further refinement is warranted to unravel the precise mechanisms underlying the anti-gastric cancer effects of kaempferol.

In this study, we have demonstrated the robust ability of KP to induce pyroptosis in gastric cancer cells. The mechanism underlying this phenomenon involves KP-mediated induction of mitochondrial damage, which subsequently activates the NF-κB signaling pathway, ultimately leading to the activation of the NLPR3-caspase-1 axis and promoting pyroptosis in AGS cells. In light of these findings, it can be concluded that KP, widely present in various fruits and vegetables, exhibits a high level of safety, minimal side effects, superior bioavailability, and potent anti-tumor properties (Lomphithak, Jaikla et al. 2023). Consequently, KP holds significant potential as a therapeutic and preventive agent for neoplastic diseases.

2. Materials and Methods

2.1 Reagents and Materials


2.2 Cell and Cultures

AGS (Human gastric adenocarcinoma cells, National Collection of Authenticated Cell Cultures) were incubated with 1640 medium (KeyGEN BioTECH) containing 10% fetal bovine serum (FBS, abw) with 1% streptomycin-penicillin in an incubator at 37 degrees Celsius, 5% CO2 level.

2.3 Cell Viability Assay
Cell viability was assessed to evaluate the responsiveness of cells to kaempferol stimulation, utilizing the CCK8 kit. The cells were cultured in 96-well plates at a seeding density of 5 * 10^4 cells per well. Upon reaching 80–85% confluency, the culture medium was replaced, and varying concentrations of kaempferol (10 µM/ml, 20 µM/ml, 40 µM/ml, 70 µM/ml, 140 µM/ml) were introduced. Following a 24-hour incubation period, the medium was aspirated, and each well was treated with 10 µl of the CCK8 working solution, subsequently incubated at 37°C under light-obstructed conditions for a duration of 2 hours. Absorbance measurements (OD) were obtained at a wavelength of 490 nm using a Microplate Reader. The presented data are representative of a minimum of three independent experimental replicates.

### 2.4 Molecular Docking

High-resolution crystal structures of Caspase-1 (ID: 6MFQ, 7AEH), NLRP3 (ID: 5YQO), GSDMD (ID: 6BZ9), ASC (ID: 3VLN), and NF-κB (ID: 6P0Z) were selected from the PDB database, ensuring their structural integrity. Prior to docking simulations, the crystals underwent pretreatment using AutoDock Tools software, which included dehydration and hydrogenation processes. The Autogrid software was employed for the docking simulations, generating binding energies. Visualization and analysis of the obtained results were conducted using pymol software.

### 2.5 Network Pharmacology Analysis

The identification of therapeutic targets for kaempferol was accomplished by leveraging the Traditional Chinese Medicine Database and Analysis Platform (TCMSP) (Ru, Li et al. 2014). Subsequently, the active targets associated with gastric cancer were curated from the DrugBank database (Wishart, Feunang et al. 2018) and the DisGeNET v6.0 database (Piñero, Ramírez-Anguita et al. 2020). The VENNY 2.1.0 Online Interactive Software (Oliveros (2007–2015) ) facilitated the determination of the intersection between the datasets pertaining to kaempferol and disease-related targets. To gain further insights into the functional implications of these identified targets, comprehensive analyses encompassing KEGG, GO-CC, GO-BP, as well as GO-MF were conducted employing the Maspetace database (Zhou, Zhou et al. 2019). By integrating the comprehensive dataset, a holistic "kaempferol - therapeutic target - pathway of action" diagram was successfully generated, depicting the interplay between kaempferol, its specific targets, and the intricately orchestrated signaling cascades modulated by this bioactive compound.

### 2.6 Bioinformatics Analysis

The retrieval of GSE54129 and GSE62254 datasets from the Gene Expression Omnibus (GEO) database was conducted using the GEOquery package (version 2.54.1) in the R programming environment (version 3.6.3). To ensure data integrity, probes corresponding to multiple molecules were eliminated, with preference given to the probe exhibiting the highest signal value for a particular molecule. Subsequently, the filtered dataset underwent batch-to-batch variation removal utilizing the ComBat function from the sva package (version 3.34.0), considering the distinct datasets as sources of such variation. To gain insights into the sample grouping patterns, principal component analysis (PCA) plots and UMAP plots were generated, enabling the visualization of clustering trends among the different sample groups.
Furthermore, leveraging the limma package, differential analyses were performed to identify significant distinctions between the two aforementioned groups.

2.7 Transmission Electron Microscope (TEM)

The AGS cells were prepared for analysis following a conventional pre-embedding procedure involving the utilization of agar. Subsequently, the samples were fixed for a duration of 2 hours at room temperature, ensuring proper fixation while shielded from light. Following fixation, dehydration and osmotic embedding procedures were employed to prepare the samples for further analysis. To facilitate polymerization, the embedded plates were subjected to an incubation period within an oven set to a controlled temperature of 60°C, spanning a duration of 48 hours. Once the polymerization process was complete, sections were carefully excised from the embedded samples, taking meticulous care to ensure their integrity. Utilizing transmission electron microscopy (TEM) techniques, performed using an HT7800 instrument manufactured by Hitachi in Japan, each stained section underwent observation and documentation through photographic imaging.

2.8 Western blot

The cells were subjected to a 24-hour stimulation with kaempferol, after which they were harvested for protein extraction, enabling the determination of total protein concentration. The extracted proteins underwent separation via electrophoresis using SDS-PAGE gels with varying concentrations of 10%, 12%, and 15%. Following electrophoretic separation, the proteins were transferred onto PVDF membranes. To prevent non-specific binding, the PVDF membrane was blocked and subsequently incubated overnight at 4°C with specific antibodies, including anti-GAPDH, anti-NLRP3, anti-Caspase-1, anti-GSDMD, anti-IL-18, anti-NF-κB, and anti-ASC. After thorough washing, the membrane was further incubated with a secondary antibody for a duration of 2 hours at room temperature, facilitating the detection of primary antibody-bound protein bands. Visualization of the protein bands was achieved utilizing a luminescence imaging system, enabling the capture of accurate and reproducible data.

2.9 Quantitative real-time PCR (qRT-PCR) analysis

The isolation of total RNA was performed utilizing TRNzol regent (TIANGEN, China), a proven reagent renowned for its efficacy in extracting high-quality RNA samples. To facilitate cDNA synthesis, the Reverse Transcription Kit (Vazyme, R323-01), a well-established and reliable kit, was employed. In order to ascertain the expression levels of specific target genes via quantitative polymerase chain reaction (Q-PCR) analysis within this study, The primer sequences employed in this investigation are presented as follows:
Table 1
The primer sequences

<table>
<thead>
<tr>
<th>GENE</th>
<th>primer sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAPDH</td>
<td>Forward 5'-AGAACATCATCCCTGCCTCTACTGG-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse 5'-CGCCTGCTTCACCACCTTCTTG-3'</td>
</tr>
<tr>
<td>Caspase-1</td>
<td>Forward 5'-TCCTCAGGCTCAGAAGGAATGTC-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse 5'-GTGCAGGCTTGACTTGTCATTATTG-3'</td>
</tr>
<tr>
<td>NLRP3</td>
<td>Forward 5'-GCCAGGAAGACACGCTATTGAAGAGG-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse 5'-AGTCGTGTGCTAGGGTTGAGG-3'</td>
</tr>
<tr>
<td>GSDMD</td>
<td>Forward 5'-ACAGCTCCAGCCTCAATGAATG-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse 5'-GCACCTCAGTCCACCACGTACAC-3'</td>
</tr>
<tr>
<td>NF-κB</td>
<td>Forward 5'-TCTCTCGCTGCCTCCACAAG-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse 5'-GATGTCTCCAGGCCGCTGTC-3'</td>
</tr>
<tr>
<td>ASC</td>
<td>Forward 5'-GCCCACCACCCAAGCAAGATG-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse 5'-CTCCGCTCCAGGTCTCCAC-3'</td>
</tr>
<tr>
<td>IL-18</td>
<td>Forward 5'-ATGGCTGCTGAACCAGTAAGAGC-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse 5'-AGAGGCCGATTTCCTTGGTCATAATG-3'</td>
</tr>
</tbody>
</table>

The relative expression levels of the target genes were analyzed using the 2-ΔΔCt method. To facilitate this analysis, PCR primers were synthesized by Shanghai Sangon Biotech (Shanghai, China), a reputable provider of molecular biology services.

### 2.10 Lactate dehydrogenase (LDH) release assay

The release of lactate dehydrogenase (LDH) serves as a reliable indicator of cellular membrane integrity and potential rupture. To assess the extent of LDH release, an LDH assay kit (Nanjing Jiancheng Bioengineering Institute, China) was employed. AGS cells were subjected to stimulation with varying concentrations (50 µM, 70 µM, 90 µM) of kaempferol for a duration of 24 hours. In order to evaluate the impact of inhibition, a separate group was pretreated with 20 µM MS for 2 hours, followed by subsequent stimulation with different concentrations of kaempferol for 24 hours. For each experimental group, cell supernatants were collected and processed according to the instructions provided with the LDH assay kit. Subsequently, the absorbance (OD) of the samples was measured at a wavelength of 450 nm using a Microplate Reader.

### 2.11 ELISA analysis
The assessment of cellular release of inflammatory factors was performed using ELISA kits (Jianglai Bio, China). AGS cells were seeded at a density of $4 \times 10^5$ cells per well in 96-well plates, followed by stimulation with various concentrations of kaempferol (KP) for a duration of 24 hours. Subsequently, the cell supernatant was collected and subjected to experimental procedures following the instructions provided by the ELISA kit with absorbance measured at a wavelength of 450 nm.

### 2.11 Statistical analysis

The acquired data were obtained from three independent experiments, ensuring reliable and robust statistical analysis. GraphPad Prism 8 software was employed for the statistical analyses conducted in this study. To compare two groups, the independent samples t-test was utilized, with a significance level set at $P < 0.05$ to determine statistical significance. For comparisons involving multiple groups, Tukey's test based on one-way analysis of variance (ANOVA) was employed.

### 3. Results

#### 3.1 KP elicits mitochondrial damage, thereby facilitating pyroptosis in gastric cancer cells.

The chemical structure of kaempferol (KP) is illustrated in Fig. 2a. The cell viability assay demonstrated (Fig. 2b-2d) that gastric cancer cells exhibited an IC50 value of 112 µM after 24 hours of KP stimulation, leading us to select a working concentration of 70 µM for this study. Microscopic examination (Fig. 2e) revealed distinct observations following 24 hours of KP stimulation in AGS cells compared to the control group. Noticeably, the administered group displayed a significant decrease in cell numbers, along with signs of cellular inflammation and swelling. Furthermore, the cells exhibited numerous vacuoles, ruptured cell membranes, and dispersed cellular contents within the culture medium. Transmission electron microscopy (TEM) analysis further corroborated these findings, illustrating ruptured cell membranes and altered cellular morphology, as indicated by the release of cellular contents (highlighted by red arrows). Notably, mitochondrial alterations were also observed, characterized by structural changes, loss of body cristae, internal swelling, and complete loss of structural features in certain mitochondria (indicated by yellow arrows) (Fig. 2f). Collectively, our results elucidate the effective promotion of gastric cancer cell pyroptosis by KP, potentially mediated through the induction of mitochondrial damage.

#### 3.2 KP induces the activation of NLRP3 inflammatory vesicles, leading to the induction of pyroptosis.

Pyroptosis, an inflammatory form of necrosis, is characterized by the activation of the NLRP3 inflammasome, followed by downstream caspase-1 activation, ultimately resulting in the release of the inflammatory factor IL-18 (Al Mamun, Suchi et al. 2022). The transition of NLRP3 from an inactive homo-oligomeric multimer to an active multimeric inflammasome triggers the helical oligomeric assembly of the adaptor molecule ASC, which serves as a platform for caspase-1 activation (Akbal, Demst et al.)
2022). Notably, several studies have demonstrated the regulatory role of KP in NLRP3 activation in various diseases, highlighting its potential for preventive and therapeutic interventions (Lim, Min et al. 2018, Han, Sun et al. 2019, Ling, Wu et al. 2019, Liu, Yao et al. 2021). To investigate the impact of KP on NLRP3 inflammasome activation, we initially conducted molecular docking simulations to predict the binding sites between KP and key molecules such as NLRP3, GSDMD, Caspase-1, ASC, and NF-κB (Fig. 3a-e). The results revealed that KP exhibited the strongest binding energy with NLRP3 (-8.7 kJ), followed by ASC (-7.4 kJ) and Caspase-1 (-8.1 kJ). Lower docking energy indicates a more stable binding conformation between the protein ligand and the drug molecule. Based on these findings, we postulated that KP-induced NLRP3 activation led to Caspase-1 cleavage, thereby promoting pyroptosis in gastric cancer cells.

Subsequently, we assessed the release of lactate dehydrogenase (LDH), a major product released during pyroptosis, in AGS cells stimulated with kaempferol. Remarkably, LDH release significantly increased upon kaempferol stimulation. Furthermore, the inhibitory effect of MS on LDH release was counteracted by KP (Fig. 3f). We then evaluated the activation levels of NLRP3 and Caspase-1 in the administered group and MS group cells using immunoblotting and PCR techniques. The results demonstrated that KP promoted the activation of NLRP3 in AGS cells (Fig. 3g), subsequently activating Caspase-1 (Fig. 3h) and releasing the active N-terminus of GSDMD (Fig. 3i). Additionally, KP upregulated the level of ASC (Fig. 3j) and facilitated the secretion of IL-18 (Fig. 3k). Collectively, these findings highlight the ability of KP to induce NLRP3 inflammasome activation, resulting in caspase-1 cleavage and subsequent pyroptosis in gastric cancer cells.

Furthermore, KP exhibited a mitigating effect on the inhibitory actions of MS regarding the expression levels of Caspase-1 (Fig. 4a, 4e), ASC (Fig. 4a, 4d), and IL-18 (Fig. 4a, 4b). The NLRP3 inflammatory vesicle (Fig. 4a, 4f) is responsible for processing pro-precursor IL-18 into its biologically active form, enabling extracellular secretion through plasma membrane pores (Wang, Yang et al., 2021). To ensure experimental precision, we quantified the release of IL-18 from AGS cells using an Elisa kit, and the results aligned consistently with the findings obtained through Western blotting and quantitative polymerase chain reaction (qPCR) analyses (Fig. 4g).

This information highlights that KP not only counteracted the inhibitory effects of MS on the expression of key pyroptosis-related molecules, such as Caspase-1, ASC, and IL-18, but also underscores the role of the NLRP3 inflammatory vesicle in the conversion of pro-precursor IL-18 to its active form, subsequently facilitating its extracellular release via plasma membrane pores. Additionally, the agreement between ELISA, Western blotting, and qPCR results reinforces the reliability and consistency of our experimental observations.

3.3 KP facilitates pyroptosis via modulation of the NF-κB-NLRP3-caspase-1 signaling axis.
The activation of the NF-κB signaling pathway plays a critical role in initiating inflammasome assembly and subsequently triggering pyroptosis (Tan, Sun et al. 2021). Notably, this classical inflammatory pathway is closely linked to the activation of the NLRP3 inflammasome (Li, Gu et al. 2023). In order to investigate the impact of kaempferol (KP) on NF-κB activation, we employed bioinformatics analyses (Fig. 5a-c), network pharmacology approaches (Fig. 5d-f, Fig. 6), as well as molecular docking predictions to guide our subsequent experimental investigations. The results obtained from these preliminary analyses have revealed that NF-κB serves as a pivotal pathway connecting gastric cancer and pyroptosis. Furthermore, through interaction analysis with KP's target molecules, we identified that KP specifically acts on the pyroptosis-related targets within gastric cancer cells. Notably, our findings indicate a strong docking affinity between KP and the RELA protein, which is a classical component of the NF-κB pathway. Our initial investigations suggest that kaempferol may exert its effects on cellular pyroptosis in gastric cancer cells through the modulation of the NF-κB-NLRP3-caspase-1 signaling axis.

Furthermore, through transmission electron microscopy (TEM) analysis (Fig. 7), we observed that kaempferol (KP) induced notable cellular abnormalities, including cell membrane rupture and mitochondrial alterations. Intriguingly, these morphological changes were substantially attenuated when co-treated with BAY, a specific inhibitor of NF-κB signaling. Conversely, AGS cells stimulated solely with BAY exhibited no discernible morphological alterations. These findings provide compelling evidence supporting the significance of NF-κB as a crucial target in mediating KP-induced promotion of pyroptosis.

To further corroborate the impact of kaempferol (KP) on the NF-κB signaling pathway, we conducted Western blotting (WB) and quantitative polymerase chain reaction (qPCR) analyses to assess the expression levels of key molecules involved. Specifically, we examined the protein and mRNA levels of NF-κB P65, NLRP3, Caspase-1, ASC, GSDMD, and IL-18 in each experimental group (Fig. 8a-g). The results demonstrated a significant upregulation of NF-κB, NLRP3, Caspase-1, ASC, GSDMD, and IL-18 at the protein and mRNA levels upon KP treatment (Fig. 8h-m). Moreover, when AGS cells were pretreated with BAY, an inhibitor of NF-κB signaling, KP effectively counteracted the inhibitory effects on NLRP3, Caspase-1, ASC, GSDMD, and IL-18 expression, displaying an observable trend of upregulation. These findings provide strong evidence for the involvement of KP in modulating the NF-κB signaling pathway. The upregulation of NF-κB, NLRP3, Caspase-1, ASC, GSDMD, and IL-18 at both the protein and mRNA levels further supports the role of KP in promoting pyroptosis-related processes. Additionally, the antagonistic effect of KP against the inhibitory action of BAY highlights its ability to override the suppressive effects on the expression of critical pyroptotic molecules.

4. Discussions

Gastric cancer (GC) is a prevalent gastrointestinal tumor associated with high morbidity and mortality rates (Wang, Liu et al. 2022). Conventional treatment strategies for GC encompass surgical interventions, radiotherapy, and chemotherapy. However, the efficacy of these approaches may be compromised due to postoperative recurrence and severe adverse effects associated with radiotherapy and chemotherapy.
(Fan, Zhang et al. 2022). Consequently, there is growing interest in exploring alternative therapies with reduced side effects, such as traditional herbal medicines and their constituent compounds.

Kaempferol, a natural flavonoid commonly found in traditional herbal medicine, has attracted attention for its anticancer properties (Kim, Lee et al. 2018). Given its therapeutic potential, our research team aims to investigate the underlying anti-tumor mechanisms of kaempferol. Among various factors influencing gastric cancer progression, cell death holds significant importance (Wang, Liu et al., 2022). Pyroptosis, a form of programmed cell death, plays a crucial role in tumorigenesis, disease progression, and metastasis (Zaffaroni and Beretta 2023).

In our study, we sought to explore the relationship between kaempferol (KP), gastric cancer, and pyroptosis. By conducting transmission electron microscopy (TEM) observations, we confirmed that KP-induced cell death in gastric cancer cells exhibited characteristic features of pyroptosis. Additionally, morphological alterations in mitochondria suggested that KP may promote pyroptosis by inducing mitochondrial damage.

In our study, we sought to explore the relationship between KP, gastric cancer, and pyroptosis. By conducting transmission electron microscopy (TEM) observations, we confirmed that KP-induced cell death in gastric cancer cells exhibited characteristic features of pyroptosis. Additionally, morphological alterations in mitochondria suggested that KP may promote pyroptosis by inducing mitochondrial damage.

The NLRP3 inflammasome comprises three key components: the sensor NLRP3, adaptor ASC, and effector caspase-1 (Wen, Xuan et al. 2023). To investigate the impact of KP on NLRP3 inflammasome activation, we assessed intracellular levels of NLRP3, caspase-1, ASC, and IL-18 using Western blotting (WB) and quantitative polymerase chain reaction (Q-PCR). Our findings demonstrated that KP indeed facilitates pyroptosis in AGS cells through the NLRP3-Caspase-1 axis.

Based on our experimental results, we employed bioinformatics, network pharmacology, and molecular docking analyses to predict that KP activates the NLRP3 inflammasome via the NF-κB signaling pathway. This activation leads to the cleavage of downstream effector caspase-1 and subsequent release of the proinflammatory cytokine IL-18.

In summary, our experimental data strongly support the notion that KP induces cellular pyroptosis in GC cells through the NF-κB-mediated NLRP3-Caspase-1 axis. Moreover, our microscopic observations of cellular morphology suggest that KP promote pyroptosis in gastric cancer cells by inducing mitochondrial damage. A deeper understanding of the molecular mechanisms underlying the actions of KP could potentially pave the way for novel and effective therapeutic strategies for cancer treatment.

Declarations

Statements & Declarations
Funding

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

The authors declare that there are no conflict of interests. We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service, or company that could be construed as influencing the position presented in the review of the manuscript entitled.

Author Contributions

Xiafei Qi Performed the experiments and drafted the manuscript; Jiatong Liu co-authored the experiments; Liu-Xiang Wang Completed animal experiments and data collection; Pei-Xing Gu performed the data analysis; Si-Yuan Song revised the manuscript; Peng Shu Conceived the experiments and designed the experiments.

Data Availability

Datasets of gastric cancer targets generated during this study are available in DrugBank database and the DisGeNET database repository, [DrugBank database : https://go.drugbank.com/]. [DisGeNET database : https://www.disgenet.org ].

The dataset of baicalein targets generated during this study is available in the TCMSP database, [https://tcmsp-e.com/].

The dataset of gastric cancer samples analyzed in this study is available in the GEO database [https://www.ncbi.nlm.nih.gov/geo/]

The dataset of focal death gene samples analyzed in this study is available in the Genecard database [https://www.genecards.org/]

Ethics approval
The experimental subjects of this study consisted of purchased cell lines, and no human data or animal experiments were involved.

**Consent to participate**

Not applicable

No human data or animal experiments were involved.

**Consent to publish**

Not applicable

No human data or animal experiments were involved.

**References**


9. Guo, J. Inhibiting migration of liver cancer cells useful for treating liver cancer, comprises adding kaempferol-3-O-glucose rhamnoside and/or methyl ferulic acid to suspension of liver cancer cells to culture liver cancer cells, GUO J (GUOJ-Individual).


Figure 1

Technology roadmap for this study
Figure 2

Promotion of AGS cell pyroptosis by Kaempferol. a. Chemical structure of Kaempferol (KP). b-d. IC50 values of AGS cells under various drug stimuli, including KP, MS, and BAY. e. Microscopic examination depicting the morphological alterations of AGS cells at different magnifications (10X, 20X, 40X). f. Transmission electron microscopy (TEM) images illustrating the changes in AGS cell morphology. Red arrows indicate AGS cell membrane rupture, while yellow arrows highlight AGS cell mitochondrial changes.
the pivotal role played by KP in activating NLRP3 inflammatory vesicles, thereby inducing pyroptosis in gastric cancer cells. a-e. Molecular docking simulations depicting the interaction between Kaempferol (KP) and key molecules involved in pyroptosis, including NLRP3, GSDMMD, Caspase-1, ASC, and NF-κB. f. Quantification of lactate dehydrogenase (LDH) release from AGS cells following stimulation with
kaempferol. g-k. Analysis of mRNA expression levels for NLRP3, Caspase-1, GSDMD, ASC, and IL-18 in AGS cells treated with pharmacological agents.

Figure 4

The impact of kaempferol on the expression levels of key pyroptosis-related pathway proteins in AGS cells. a. Protein banding diagrams showing the expression levels of IL-18, GSDMD-N, ASC, Caspase-1, and NLRP3. b-f. Results of banding analysis representing the expression levels of IL-18, GSDMD-N, ASC, Caspase-1, and NLRP3. g. Quantification of the total amount of IL-18 released from AGS cells.
Figure 5

Comprehensive bioinformatics analyses exploring the intricate relationships between KP, gastric cancer, and pyroptosis. a. Identification of interacting genes linking gastric cancer and pyroptosis. b. GO pathway and KEGG pathway analysis depicting the functional and regulatory pathways associated with the interacting genes involved in gastric cancer and pyroptosis. c. Network diagram illustrating the interconnections among the GO pathways and KEGG pathways identified in gastric cancer and

Figure 6

Gene GO analysis of KP action in gastric cancer
Figure 7

Morphological modifications occurring in AGS cells upon stimulation with kaempferol (KP) a. Visualization of morphological changes in AGS cells using an inverted fluorescence microscope. b. Morphological alterations in AGS cells observed through transmission electron microscopy (TEM).
Figure 8

Expression Levels of NF-κB P65, NLRP3, GSDMD, Caspase-1, and IL-18 in AGS Cells

a. Western blot (WB) bands depicting the expression of NF-κB P65, NLRP3, GSDMD, Caspase-1, and IL-18 in AGS cells. b-g. Protein expression results showcasing the levels of NF-κB P65, NLRP3, GSDMD, Caspase-1, and IL-18 in AGS cells. h-m. Results of mRNA expression analysis indicating the levels of NF-κB P65, NLRP3, GSDMD, Caspase-1, and IL-18 in AGS cells.