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Abstract

Pancreatic cancer (PaCa) has an extremely poor prognosis. This malignancy rapidly acquires resistance to gemcitabine (GEM), a key chemotherapeutic agent; yet, the mechanisms underlying this resistance remain incompletely understood. We previously established GEM-resistant (GEM-R) PaCa cell lines and found that these cells exhibit constitutively elevated expression of matrix metalloproteinase-1 (MMP-1), which contributes to the invasion and metastasis of PaCa. Kaempferol, a natural flavonoid found in many plant species, has been reported to exert antitumor effects in several cancer types. In this study, we confirmed that non-cytotoxic concentrations of kaempferol significantly decrease MMP-1 protein expression in GEM-R PaCa and suppress their migration and invasion capacities. Western blot analysis demonstrated that MMP-1 protein expression was higher in GEM-R than GEM-sensitive (GEM-S) PaCa and was suppressed by kaempferol treatment. In Transwell migration/invasion and wound healing assays, GEM-R PaCa cell lines exhibited enhanced migration and invasion capacities compared with GEM-S cells, whereas kaempferol treatment suppressed these properties, similar to the effects observed by MMP-1 knockdown or treatment with the MMP inhibitor batimastat. Furthermore, kaempferol treatment reduced phosphorylated Akt expression and NF-κB p65 activity. These findings indicate that kaempferol suppresses the migration and invasion capacities of PaCa cells by downregulating MMP-1 via inhibition of Akt and NF-κB signaling pathways, and that kaempferol holds promise as a potential therapeutic agent for the treatment of GEM-R PaCa.

Keywords: pancreatic cancer; gemcitabine resistance; kaempferol; MMP-1; migrasion; invasion

1. Introduction

Pancreatic cancer (PaCa) remains one of the most lethal malignancies. In the United States, the median survival is approximately 4 months, and the 5-year survival rate is 13%. PaCa is currently the third leading cause of cancer-related death and is projected to become the second by 2030 [1]. In 2024, an estimated 66,400 new cases and 51,750 deaths were expected in the U.S. [2]. Due to its asymptomatic and aggressive nature, PaCa is often diagnosed at an advanced, incurable stage; thus, chemotherapy remains a cornerstone of treatment.

Gemcitabine (GEM) is a standard chemotherapeutic agent for PaCa; however, GEM resistance commonly develops early during therapy. Reported mechanisms of GEM resistance include reduced GEM uptake, increased detoxification, and elevated levels of endogenous substrates competing with GEM activation. Our previous work demonstrated that multidrug resistance-associated protein 1

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(MRP1), a transporter that effluxes anticancer drugs, is upregulated in GEM-resistant (GEM-R) PaCa cells [3]. Despite these findings, the mechanisms underlying GEM-R are still not fully elucidated, and new therapeutic strategies are urgently needed.

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases involved in extracellular matrix remodeling and are classified into collagenases, gelatinases, stromelysins, and matrilysins [4,5]. MMPs promote tumor invasion and metastasis by degrading extracellular matrix components and regulate the signaling pathways involved in inflammation, angiogenesis, and cellular proliferation [6]. MMP-1, the first identified MMP [7], plays a key role in collagen degradation and cancer cell invasion. Although MMPs have long been implicated in tumor progression, no effective MMP-targeted therapy has yet reached clinical application.

We previously developed GEM-R PaCa cell lines [3,8] and observed consistently elevated MMP-1 expression in these cells. Analysis of patient datasets using the Kaplan–Meier Plotter (Pan-cancer RNAseq, TCGA) further showed that high MMP-1 expression is associated with a poor prognosis in PaCa. These findings highlight MMP-1 as a potential therapeutic target in GEM-R PaCa.

Kaempferol, a naturally occurring flavonoid abundant in fruits, vegetables, and traditional medicinal plants, has attracted increasing attention due to its anti-inflammatory and anticancer properties. Its antitumor effects have been reported in various malignancies, including PaCa. In colorectal cancer, kaempferol has been shown to suppress MMP-1 expression [4]; however, whether kaempferol regulates MMP-1 in PaCa, particularly in GEM-R cells, remains unknown.

Therefore, this study aimed to investigate MMP-1 expression and the enhanced migration/invasion characteristics associated with GEM resistance, and to evaluate the inhibitory effects of kaempferol on MMP-1 and cell motility in GEM-R PaCa cell lines, comparing its effects with those of the MMP inhibitor batimastat and MMP-1 knockdown.

2. Materials and Methods

2.1. Reagents

Kaempferol was purchased from Santa Cruz Biotechnology (cat. no. sc-202679B; Dallas, TX, USA) and batimastat from Selleck Chemicals (cat. no. S7155; Houston, TX, USA). Both kaempferol and batimastat were dissolved in dimethyl sulfoxide (Sigma-Aldrich, St. Louis, MO, USA). Recombinant human interleukin-1β (IL-1β) was purchased from PeproTech Inc. (cat. no. 200-01B-100UG; Cranbury, NJ, USA) and dissolved in distilled water.

2.2. Cell Lines and Cell Culture

The human pancreatic duct epithelial (HPDE) cell line H6c7 (cat. no. ECA001-FP; Kerafast, Newark, CA, USA) was maintained in keratinocyte serum-free medium (Gibco/Thermo Fisher Scientific, Rockford, IL, USA). Human pancreatic ductal adenocarcinoma cell lines (AsPC-1, MIA PaCa-2, PANC-1, and SW1990) were obtained from ATCC (Manassas, VA). AsPC-1 cells were cultured in RPMI-1640 medium (Sigma Aldrich; Merck KGaA), while the other pancreatic ductal adenocarcinoma cell lines were maintained in Dulbecco's Modified Eagle Medium (Sigma Aldrich; Merck KGaA).

Unless otherwise specified, all media contained 10% fetal bovine serum (FBS; Gibco/Thermo Fisher Scientific), 100 U/mL penicillin, 100 μg/mL streptomycin, and 25 μg/mL amphotericin B. All cell lines were incubated at 37°C in a humidified atmosphere with 5% CO_2.

2.3. Establishment of GEM-R PaCa Cell Lines

GEM-R PaCa cell lines were established as described previously [3,9]. Briefly, parental PaCa cell lines (AsPC-1, MIA PaCa-2, PANC-1, and SW1990) were treated with increasing concentrations of GEM (Eli Lilly Japan K.K., Kanagawa, Japan) followed by repeated passaging.

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The half-maximal inhibitory concentration (IC_{50}) of GEM in each cell line was first determined using the WST-1 assay (cat. no. MK400; Takara Bio, Yamanashi, Japan). Cells were then exposed to GEM at their respective IC_{50} concentrations, and the IC_{50} was re-evaluated after each passage. Cell lines with a GEM IC_{50} value more than 50-fold higher than that of the parental cells were defined as GEM-R.

2.4. Cytotoxicity Assay

The cytotoxicity of kaempferol and batimastat was assessed using the WST-1 assay. GEM-sensitive (GEM-S) and GEM-R MIA PaCa-2 or SW1990 cells were seeded at 3×10^3/well in 96-well plates (100 μL/well). Cells were treated for 24 h with kaempferol (0–250 μM) or batimastat (0–4000 ng/mL). The absorbance was measured using the SpectraMax ABS microplate reader (Molecular Devices, San Jose, CA, USA) to determine cell viability.

2.5. siRNA Transfection

siRNA targeting MMP-1 (siMMP-1; cat. no. AM16708) and negative control siRNA (siNC; cat. no. 4390843) were obtained from Thermo Fisher Scientific. PaCa cells were seeded in 6-well plates and grown to 70–80% confluence without antibiotics. According to the manufacturer's protocol, 100 nM siRNA was transfected into cells using Lipofectamine RNAiMAX in Opti-MEM medium (Thermo Fisher Scientific) without antibiotics or FBS at 37°C for 24 h. After transfection, the cells were used for protein extraction, Transwell assays, and wound healing assays.

2.6. Western Blotting

Protein extraction was performed using RIPA Lysis and Extraction Buffer (cat. no. 89900; Thermo Fisher Scientific) supplemented with protease and phosphatase inhibitor cocktails. The protein concentration was determined using the Pierce BCA Protein Assay Kit. The protein samples (30 μg) were denatured at 90°C for 5 min, separated on 10% Mini-PROTEAN TGX precast gels (Bio-Rad Laboratories, Inc., Hayward, CA, USA), and then transferred to nitrocellulose membranes. Blocking and antibody incubation were performed using the iBind Flex Western System (Thermo Fisher Scientific).

The primary antibodies included anti-MMP-1 (1:500; cat. no. 54376; Cell Signaling Technology, Danvers, MA, USA), anti-GAPDH (1:2000; cat. no. 2118, Cell Signaling Technology), anti-Akt (1:1000; cat. no. 4691), and anti-phospho-Akt (1:2000; cat. no. 8200, Cell Signaling Technology). Horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibody (cat. no. P0448; DAKO/Agilent, Santa Clasa, CA, USA) was used at the appropriate dilution. The protein signals were visualized using SuperSignal West Pico PLUS or Pierce ECL substrates (Thermo Fisher Scientific) and detected using the Amersham Imager 600 (Cytiva, Uppsala, Sweden). Band intensities were quantified using ImageJ software (version 1.52v).

2.7. Transwell Migration and Invasion Assays

Transwell assays were performed using the Boyden chamber method. Falcon 8.0-μm pore inserts were used in the migration assays and Corning BioCoat Matrigel invasion chambers in the invasion assays. The upper chambers contained 500 μL serum-free medium and the lower chambers 750 μL medium supplemented with 10% FBS. PaCa cells (1×10^5) were seeded into the upper chamber and incubated at 37°C for 24 h. Cells that had migrated/invaded to the lower surface were stained with Diff-Quick, and the cells in nine random fields (×200) were imaged and counted.

2.8. Wound Healing Assay

GEM-S and GEM-R MIA PaCa-2 or SW1990 cells (1.0×10^5) were seeded into 6-well plates and grown to ≥ 90% confluence. A linear scratch was created across the cell layer using a sterile P10 pipette tip. Images were taken immediately (0 h) using the BZ-X710 microscope (Keyence, Osaka, Japan).

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After imaging, the cells were cultured in FBS-free medium containing kaempferol, batimastat, or IL-1β. The same fields were imaged at 24 h, and the wound area was quantified using ImageJ. The wound closure rate was calculated as follows:

$$\text{Closure rate (\%)} = (S_0h - S_{24}h) / S_0h \times 100$$

2.9. Nuclear Protein Extraction and NF-κB p65 Activity Assay

Nuclear protein extraction and NF-κB p65 activity analysis were performed as described previously. GEM-S and GEM-R MIA PaCa-2 and SW1990 cells were grown to 80–90% confluence, after which they were treated with kaempferol for 2 h and with IL-1β for 30 min. After treatment, nuclear proteins were extracted using the Nuclear Extraction Kit (Active Motif). Protein concentrations were determined using the BCA assay. NF-κB p65 activity was measured using the TransAM NF-κB p65/p50 Transcription Factor Assay Kit (cat. no. 40096; Active Motif).

2.10. Statistical Analysis

Statistical analyses were performed using EZR version 1.41 (Saitama Medical Center, Jichi Medical University, Japan). Data are presented as the mean ± standard deviation. Comparisons between two groups were performed using unpaired t-tests. For comparisons among three or more groups, one-way ANOVA followed by Bonferroni correction was used. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. IC_{50} Values of GEM in PaCa Cell Lines

WST-1 assays were performed to determine the IC_{50} values of GEM in the established GEM-R PaCa cell lines (AsPC-1, MIA PaCa-2, PANC-1, and SW1990). After treatment with various concentrations of GEM for 48 h, cell viability was assessed using the WST-1 assay. The IC_{50} values after 48 h of GEM treatment were as follows: 0.066 and 179 μM for GEM-S and GEM-R AsPC-1 cells; 0.64 and 61.6 μM for GEM-S and GEM-R MIA PaCa-2 cells; 0.047 and 67.6 μM for GEM-S and GEM-R PANC-1 cells; and 0.081 and 95.6 μM for GEM-S and GEM-R SW1990 cells, respectively (Figure 1a–e).

3.2. MMP-1 Protein Expression in the PaCa Cell Lines

The protein levels of MMP-1 in the HPDE cell line (H6c7) and PaCa cell lines (AsPC-1, MIA PaCa-2, PANC-1, and SW1990) were evaluated by western blotting. All GEM-S PaCa cell lines showed significantly higher MMP-1 expression compared with the H6c7 cell line (Figure 2a, b).

Furthermore, MMP-1 expression was markedly increased in all GEM-R PaCa cell lines compared with their corresponding GEM-S counterparts. Specifically, the MMP-1 level in GEM-R AsPC-1, GEM-R MIA PaCa-2, GEM-R PANC-1, and GEM-R SW1990 cells was elevated by 322%, 59%, 200%, and 179%, respectively, compared with the respective parental GEM-S cell lines (Figure 2c, d).

3.3. IC_{50} Values of Kaempferol and Batimastat in PaCa Cell Lines

Before performing subsequent experiments, we assessed the cytotoxicity of kaempferol and batimastat using the WST-1 assay. After 24 h of treatment, no significant cytotoxicity of kaempferol or batimastat was observed at concentrations up to 50 μM (Figure 3a–d) or 400 ng/mL, respectively (Figure 3e–h). Based on these findings, 50 μM kaempferol and 400 ng/mL batimastat were selected as the concentrations to use in subsequent experiments to avoid cytotoxic effects.

3.4. Effect of Kaempferol Treatment on MMP-1 Protein Expression in PaCa Cell Lines

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We evaluated the effects of MMP-1 knockdown, 400 ng/mL batimastat, and 50 μM kaempferol on MMP-1 protein expression using western blotting. In both GEM-S and GEM-R MIA PaCa-2 cells, MMP-1 expression was 31.1% and 11.0% lower in cells transfected with siMMP-1 than in those transfected with siNC, respectively. Similarly, in GEM-S and GEM-R SW1990 cells, MMP-1 expression was 70.3% and 46.7% lower in siMMP-1-transfected than siNC-transfected cells, respectively (Figure 4a, b).

Batimastat reduced MMP-1 protein levels in all PaCa cell lines. In GEM-S and GEM-R MIA PaCa-2 cells, MMP-1 expression was 19.4% and 64.5% lower compared with the control group, respectively. In GEM-S and GEM-R SW1990 cells, batimastat reduced MMP-1 expression by 64.7% and 83.9%, respectively (Figure 4c, d).

Kaempferol also reduced MMP-1 expression in all cell lines examined. Kaempferol decreased MMP-1 expression by 63.8% and 35.2% in GEM-S and GEM-R MIA PaCa-2 cells, respectively, and by 66.8% and 39.6% in GEM-S and GEM-R SW1990 cells, respectively (Figure 4e, f).

3.5. Effect of GEM-R and MMP-1 Knockdown on the Migration of PaCa Cells

To evaluate the effect of MMP-1 knockdown on cell migration, we performed Transwell migration and wound healing assays. In both MIA PaCa-2 and SW1990 cell lines, GEM-R cells exhibited increased migration ability compared with GEM-S cells, and MMP-1 knockdown significantly suppressed this enhancement.

In the Transwell migration assay, MMP-1 knockdown reduced the number of migrating cells by 27.9% and 46.3% among GEM-S and GEM-R MIA PaCa-2 cells, respectively, and by 77.9% and 72.1% among SW1990 GEM-S and GEM-R cells, respectively, compared with the siNC-transfected cells (Figure 5a–d).

Consistent results were observed in the wound healing assay. MMP-1 knockdown reduced relative wound closure in both cell lines, indicating suppressed migration. The wound closure rate decreased from 20.8% to 4.14% and from 34.0% to 10.4% in GEM-S and GEM-R MIA PaCa-2 cells, respectively, and from 54.6% to 33.1% and from 78.6% to 38.7% in GEM-S and GEM-R SW1990 cells, respectively, compared with siNC-transfected cells (Figure 6a–d).

3.6. Effect of Kaempferol and Batimastat on PaCa Cell Migration, Including IL-1β-Induced Migration

To examine the effects of kaempferol and batimastat on PaCa cell migration, Transwell migration and wound healing assays were conducted. Both agents suppressed the migration of PaCa cell lines, including that enhanced by IL-1β. In the Transwell migration assay, 50 μM kaempferol and 400 ng/mL batimastat significantly reduced the number of migrating cells among both GEM-S and GEM-R MIA PaCa-2 and SW1990 cells. IL-1β increased the number of migrating cells among all PaCa cell lines; however, this increase was inhibited by both kaempferol and batimastat. Kaempferol reduced the migrating cell numbers by 79.1% and 72.9% and batimastat by 81.3% and 82.0% among GEM-S and GEM-R MIA PaCa-2 cells, respectively. Among GEM-S and GEM-R SW1990 cells, kaempferol reduced migration by 70.1% and 66.0% and batimastat by 73.2% and 80.3%, respectively. Kaempferol and batimastat also suppressed IL-1β-induced migration. Kaempferol reduced the number of IL-1β-induced migrating cells by 70.1% and 65.9% among GEM-S and GEM-R MIA PaCa-2 cells and by 83.9% and 76.2% among GEM-S and GEM-R SW1990 cells, respectively, while batimastat reduced these numbers by 51.0% and 85.2% among GEM-S and GEM-R SW1990 cells, respectively (Figure 7a–d).

In the wound healing assay, suppression of migration by kaempferol and batimastat was also observed. Kaempferol reduced the wound closure rate from 61.8% to 13.7% and 45.1% and from 79.5% to 42.7% and 71.6% in GEM-S and GEM-R MIA PaCa-2 cells, respectively, and from 43.6% to 25.9% and 29.6% and from 56.5% to 22.8% and 31.3% in GEM-S and GEM-R SW1990 cells, respectively. Kaempferol and batimastat also suppressed IL-1β-induced wound closure from 69.1% to 18.4% and 51.9% and from 86.7% to 47.4% and 73.6% in GEM-S and GEM-R MIA PaCa-2 cells,

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respectively, and from 64.7% to 22.9% and 27.7% and from 80.6% to 31.2% and 33.7% in GEM-S and GEM-R SW1990 cells, respectively (Figure 8a–d).

These findings suggest that kaempferol effectively suppresses both the baseline and IL-1β-enhanced migration of PaCa cells, comparable with the effect of the MMP inhibitor batimastat.

3.7. Effect of GEM Resistance and MMP-1 Knockdown on the Invasion of PaCa Cells

To evaluate the effect of MMP-1 knockdown on the invasion capacity of PaCa cell lines, we performed a Matrigel invasion assay, similar to the Transwell migration assay. In both MIA PaCa-2 and SW1990 cell lines, GEM-R cells exhibited enhanced invasion compared with their respective GEM-S cells.

Compared with the siNC-transfected cells, knockdown of MMP-1 significantly reduced the number of invading cells in both cell lines. In MIA PaCa-2 cells, the number of invading MMP-1-knockdown cells was decreased by 40.1% in GEM-S cells and by 54.0% in GEM-R cells (Figure 9a, b). In SW1990 cells, MMP-1 knockdown reduced the number of invading cells by 31.3% in GEM-S cells and by 25.4% in GEM-R cells (Figure 9c, d).

These findings indicate that MMP-1 contributes to the enhanced invasion observed in GEM-R PaCa cells.

3.8. Effect of Kaempferol on the IL-1β-Induced Invasion of PaCa Cells

Following the invasion assay under MMP-1 knockdown conditions, we next examined changes in the invasion ability of PaCa cells after treatment with kaempferol or the MMP inhibitor batimastat. Consistent with the Transwell migration assay, treatment with 50 μM kaempferol or 400 ng/mL batimastat reduced the number of invading cells. IL-1β increased the invasion ability of PaCa cells, and both kaempferol and batimastat effectively inhibited this increase. In MIA PaCa-2 cells, kaempferol and batimastat reduced the number of invading GEM-S cells by 73.6% and 74.5% and the number of GEM-R cells by 68.9% and 82.0%, respectively. In SW1990 cells, kaempferol and batimastat decreased invasion by 70.5% and 57.6% in GEM-S cells and by 74.2% and 68.0% in GEM-R cells, respectively.

Kaempferol and batimastat also suppressed the IL-1β-induced increase in invasion. In MIA PaCa-2 cells, kaempferol reduced IL-1β-enhanced invasion by 85.5% in GEM-S cells and 76.2% in GEM-R cells, while batimastat reduced invasion by 76.8% in GEM-S and 85.2% in GEM-R cells. In SW1990 cells, kaempferol reduced IL-1β-stimulated invasion by 60.3% in GEM-S cells and 53.4% in GEM-R cells, whereas batimastat reduced invasion by 64.2% in GEM-S cells and 72.6% in GEM-R cells, respectively (Figure 10a–d).

These findings suggest that kaempferol effectively suppresses both the baseline and IL-1β-enhanced invasion ability of PaCa cells, comparable with the effect of the MMP inhibitor batimastat.

3.9. The role of Phosphorylated Akt and p65 Activity in the Altered MMP-1 Protein Expression in PaCa

To evaluate the signaling pathways involved in the inhibition of MMP-1 by kaempferol, we first analyzed MMP-1 and phosphorylated Akt (p-Akt) levels in PaCa cell lines by western blotting. Treatment with 50 μM kaempferol suppressed the levels of both MMP-1 and p-Akt in GEM-S and GEM-R MIA PaCa-2 cells and SW1990 cells. IL-1β treatment increased MMP-1 and p-Akt levels in MIA PaCa-2 cells, whereas no significant change was observed in SW1990 cells compared with the control. Kaempferol attenuated the IL-1β-induced increases in both MMP-1 and p-Akt levels.

Next, we tested the effects of kaempferol on MMP-1 and p-Akt levels in the PaCa cells. In MIA PaCa-2 cells, kaempferol reduced MMP-1 and p-Akt levels by 52.0% and 32.4% in GEM-S cells and by 50.5% and 20.0% in GEM-R cells, respectively. Kaempferol also suppressed the IL-1β-enhanced expression of MMP-1 and p-Akt by 50.7% and 29.9% in GEM-S cells and by 21.1% and 19.1% in GEM-R cells, respectively (Figure 11a, b). In SW1990 cells, kaempferol reduced MMP-1 and p-Akt levels by 30.6% and 15.8% in GEM-S cells and 33.8% and 32.0% in GEM-R cells, respectively (Figure 11c, d).

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Next, nuclear extracts were prepared following the kaempferol and IL-1β treatments, and NF-κB p65 activity was evaluated using the TransAM NF-κB p65 assay. In both cell lines, p65 activity was higher in GEM-R than GEM-S cells. Kaempferol significantly decreased p65 activity. In MIA PaCa-2 cells, kaempferol reduced p65 activity by 37.1% in GEM-S cells and 32.5% in GEM-R cells compared with the control. Kaempferol also suppressed IL-1β-induced p65 activation by 16.5% in GEM-S cells and 34.9% in GEM-R cells. In SW1990 cells, p65 activity was reduced by 24.1% in GEM-S cells and 33.4% in GEM-R cells following kaempferol treatment, and the IL-1β-enhanced p65 activity was decreased by 27.1% in GEM-S cells and 10.7% in GEM-R cells (Figure 11e).

These results indicate that kaempferol suppresses MMP-1 expression in PaCa partly via inhibition of Akt phosphorylation and NF-κB p65 activation.

4. Discussion

Among the MMP family members, MMP-2 and MMP-9 have traditionally received the most attention; however, MMP-1 is also an important member with significant clinical relevance. Elevated MMP-1 expression is associated with a poor prognosis in PaCa patients [10–12]. Zhou et al. reported that the MMP1-1607 (1G>2G) polymorphism is associated with increased cancer risk [13], and Kaplan–Meier analyses indicated significantly reduced overall survival and recurrence-free survival in PaCa patients with high MMP-1 expression. The serum MMP-1 level has been correlated with disease stage and lymph node metastasis in PaCa [14]. Furthermore, MMP-1 has been implicated in perineural invasion [15], and Chen et al. demonstrated that suppressing MMP-1 inhibits PaCa cell migration, invasion, and metastasis both in vitro and in vivo [16].

Several broad-spectrum MMP inhibitors, including batimastat [17] and marimastat [18], have been developed and evaluated in clinical trials; however, none of these inhibitors have demonstrated satisfactory clinical efficacy. The major reasons for these failures include insufficient inhibitor specificity and inadequate understanding of the complex tumor biology underlying MMP-related pathways [19].

Kaempferol is a dietary flavonoid abundant in various fruits and vegetables. In addition to its anti-inflammatory [8], antibacterial [20], antioxidant [21], osteogenic [22], and neuroprotective properties [23], its anticancer activity has attracted considerable attention. Epidemiological studies suggest that a diet rich in flavonoids, including kaempferol, may reduce the risk of PaCa, particularly among smokers [24,25]. Other than PaCa, kaempferol demonstrates anticancer effects in gastric [26,27], colorectal [3,28], bladder [29,30], prostate [30], ovarian [31], and breast [32] cancers. In colorectal cancer, kaempferol suppresses migration and invasion by inhibiting MMPs [3]. In PaCa, kaempferol has been shown to induce apoptosis [33–35] and inhibit proliferation and migration [36]; however, its effects on migration and invasion as mediated by MMP-1 suppression, especially under GEM-R conditions, have not been investigated. Additionally, although several reports highlight the involvement of MMP-1 in PaCa progression, altered MMP-1 expression after GEM resistance development and the consequences of MMP-1 suppression have not been fully elucidated.

In this study, we demonstrated that MMP-1 expression is increased in PaCa cells and further elevated in GEM-R PaCa cells. Kaempferol reduced MMP-1 expression and suppressed migration and invasion even in GEM-R PaCa cells, accompanied by inhibition of Akt phosphorylation and NF-κB p65 activity. Supporting our findings, previous reports have shown that kaempferol modulates key oncogenic pathways. Lee et al. reported that kaempferol induces apoptosis by downregulating the Src/Akt/ERK pathway via EGFR inhibition [36]. Wang et al. showed that kaempferol induces apoptosis by inhibiting the Akt/mTOR pathway via TGM-2, while other studies reported that kaempferol inhibits the STAT3 pathway by increasing reactive oxygen species production and decreasing SHP-1 expression [37]. Interestingly, kaempferol exhibited antioxidant properties in a neuroprotection study [21], yet induced oxidative stress to exert anticancer effects in PaCa [34,37]. NF-κB, which interacts with the Akt/mTOR pathway, regulates MMP expression [38]. We previously reported that NF-κB contributed to GEM resistance in PaCa [8,39]. The present study supports this

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relationship by showing increased MMP-1 expression via Akt and NF-κB signaling in GEM-R PaCa, an effect that was effectively suppressed by kaempferol.

In colorectal cancer, kaempferol reduced Akt/mTOR phosphorylation and suppressed MMP-1, MMP-2, and MMP-9 expression, thereby inhibiting migration and invasion [3]. However, that study used kaempferol at its IC_{50} concentration, and the cytotoxicity potentially induced by this concentration may confound motility assessments. In contrast, Kai et al. reported that MMP-1 knockdown suppressed PI3K/Akt/c-Myc pathway activation in colorectal cancer [28]. Similarly, in breast cancer, increased MMP-1 expression activated NF-κB signaling, promoting epithelial–mesenchymal transition [27]. Those findings suggest that similar mechanisms likely occur in PaCa, in which kaempferol may suppress MMP-1 expression via pathways involving reactive oxygen species generation, PI3K/Akt/mTOR signaling, NF-κB signaling, and STAT3 inhibition. In addition, reduced MMP-1 expression may further downregulate Akt/mTOR signaling, potentially creating a positive regulatory loop.

Several limitations must be acknowledged. First, the signaling pathways by which kaempferol suppresses MMP-1 remain incompletely understood. If, as reported in colorectal cancer [28], MMP-1 inhibition is found to attenuate Akt/mTOR phosphorylation in PaCa, the therapeutic potential of kaempferol would be even greater due to its secondary effects. Second, kaempferol has poor bioavailability, reportedly below 2% [40], which is a major challenge for clinical application. Strategies such as nanoparticle formulation [41], complex formation with hydroxypropyl-β-cyclodextrin, and liposomal encapsulation [42] may improve absorption. Future studies are needed to verify whether the MMP-1 inhibitory effect of kaempferol is achievable in vivo and to explore methods to enhance its bioavailability.

In conclusion, this study is the first to report altered MMP-1 expression in GEM-R PaCa and the therapeutic potential of kaempferol for GEM-R disease. GEM-R PaCa exhibited increased MMP-1 expression, contributing to enhanced migration and invasion. Kaempferol suppressed MMP-1 expression via inhibition of the Akt and NF-κB pathways, thereby reducing the migration and invasion capacities of GEM-R PaCa cells, similar to the established effects of the MMP inhibitor batimastat. These findings suggest MMP-1 to be a promising therapeutic target in GEM-R PaCa, and kaempferol may serve as a novel therapeutic agent for GEM-R PaCa by targeting MMP-1.

5. Conclusions

MMP-1 expression is elevated in PaCa cells compared with normal pancreatic ductal epithelial cells and increases further upon the acquisition of GEM resistance, contributing to enhanced migration and invasion capacities. Kaempferol suppresses this process at non-cytotoxic concentrations, exerting clear antitumor effects through MMP-1 inhibition. These findings suggest that kaempferol may serve as a promising MMP-1–targeted therapeutic agent for PaCa, potentially offering fewer adverse effects than current chemotherapeutic options.

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