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EPIGALLOCATECHIN GALLATE SENSITIZES PANCREATIC CANCER CELLS TO GEMCITABINE BY MODULATING MICRORNA EXPRESSION PROFILE

EPİGALLOKATEŞİN GALLAT, PANKREAS KANSER HÜCRELERİNİ GEMSİTABİN'E KARŞI MİKRORNA İFADE PROFİLİNİ DEĞİŞTİREREK HASSASLAŞTIRMAKTADIR

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Öz

Amaç

Pankreas kanser, gelişmiş ülkelerde kansere bağlı ölümlerin önde gelen nedenlerinden biri olup, 5 yıllık ortalama sağkalım oranının %5'ten az olduğu malign bir hastalıktır. Gemsitabin (GEM), FDA onaylı bir pirimidin antimetaboliti olup, pankreas kanser tedavisinde yaygın olarak kullanılmaktadır. Ancak, tüm bölünen hücreleri hedef alması sebebiyle, GEM tedavisi gören pankreas kanseri hastalarında ciddi yan etkiler sıklıkla gözlemlenmektedir. Sonuç olarak, meta-analizler, GEM'in diğer aktif bileşiklerle kombinasyonunun, pankreas kanser hastalarının 1 yıllık sağkalım oranını önemli ölçüde artırdığını göstermiştir. Epigallokateşin-3-gallat (EGCG), yeşil çayda (Camellia sinensis) bulunan aktif bir bileşik olup, pankreas kanserinde antikanser aktivitesi kanıtlanmıştır. Devamındaki çalışmalarda da EGCG'nin pankreas kanseri hücrelerinin GEM'e karşı hassasiyetini arttırdığı gösterilmiştir. Ancak, bugüne kadar yapılan çalışmalar arasında, EGCG ve GEM kombinasyonunun pankreatik kanser patolojisinde kritik bir epigenetik düzenleyici olan mikroRNA ifadesinin üzerine etkisi incelenmemiştir. Bu çalışmada, GEM ve EGCG kombinasyonunun PANC1 hücrelerindeki sitotoksik ve apoptotik etkilerinin belirlemesi ve kanser progresyonunda rol oynayan mikroRNA'ların ifade düzeyleri üzerindeki etkinliğinin incelenmesi amaçlanmıştır.

Gereç ve Yöntem

GEM ve EGCG'nin PANC1 hücrelerindeki sitotoksisitesi WST-1 testi kullanılarak değerlendirildi ve kombinasyon etkileri izobologram analizi kullanılarak analiz edildi. Apoptoz analizi, Annexin V yöntemi kullanılarak yapıldı. MikroRNA izolasyonu miRNeasy Kiti ile gerçekleştirildi ve miScript II Ters Transkriptaz Kiti kullanılarak cDNA sentezi yapıldı. Kanser hücresi proliferasyonu, apoptozis ve metastazda rol oynayan mikroRNA'ların ifadesindeki değişiklikler, gerçek zamanlı qRT-PCR analizi kullanılarak incelendi.

Bulgular

GEM'in 24, 48 ve 72 saatteki IC50 değerleri sırasıyla 72.85 µM, 26.55 µM ve 9.38 µM olarak belirlendi. EGCG'nin 24, 48 ve 72 saatteki IC50 değerleri sırasıyla 64.36 µM, 48.34 µM ve 19.73 µM olarak belirlendi. 24 ve 72 saatte GEM:EGCG oranı 2:3 olarak birleştirildiğinde sinerjistik bir etki gözlemlenirken, 48 saatte güçlü sinerjistik bir ilaç etkileşimi gözlendi. Sadece 26.55 µM konsantrasyonda GEM ile tedavi edilen grupta taze ortam verilen kontrol grubuna kıyasla

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apoptozda 4.2 kat artış meydana geldiği saptanırken, kombinasyon uygulaması (EGCG: 3.14 µM, GEM: 4.71 µM) apoptozda 12.04 kat artışa neden olmuştur. Kombinasyon tedavisi sonrası, tümör baskılayıcı mikroRNA'ların, miR-137 ve miR-130a-3p, ifadesi artarken, onkogenik mikroRNA'ların, miR-27a-3p, miR-425-5p, miR-183-5p, miR-187-3p, miR-21-5p, miR-324-5p ve miR-486-5p, ifadesi azaldı.

Sonuç
EGCG, pankreatik kanseri GEM'e epigenetik mekanizmalar aracılığıyla hassaslaştırabilir ve yeni terapötik yaklaşımların ışığını göstermektedir.

Anahtar Kelimeler: Epigallokateşin gallat, Gemsitabin, mikroRNA, Pankreas kanseri

Abstract

Objective
Pancreatic cancer is a leading cause of cancer-related deaths in developed countries, with a 5-year average survival rate of less than 5%, making it a malignant disease. Gemcitabine (GEM), an FDA-approved pyrimidine antimetabolite, is widely used in pancreatic cancer treatment. However, due to its targeting of all dividing cells, severe side effects are frequently observed in patients undergoing GEM treatment for pancreatic cancer. Consequently, meta-analyses have shown that the combination of GEM with other active compounds significantly improves the 1-year survival rate of pancreatic cancer patients. Epigallocatechin-3-gallate (EGCG), an active compound found in green tea (Camellia sinensis), has proven anticancer activity in pancreatic cancer. Subsequent studies have demonstrated that EGCG enhances the sensitivity of pancreatic cancer cells to GEM. However, among the studies conducted to date, the impact of the combination of EGCG and GEM on the expression of critical microRNAs, which act as key epigenetic regulators in pancreatic cancer pathology, has not been investigated. This study aims to determine the cytotoxic and apoptotic effects of the combination of GEM and EGCG on PANC1 cells and to examine its effectiveness on the expression levels of microRNAs involved in cancer progression.

Material and Method
Cytotoxicity of GEM and EGCG in PANC1 cells was assessed using the WST-1 assay, and combination effects were analyzed using isobologram analysis. Apoptosis analysis was performed using the Annexin V method. miRNA isolation was conducted with the miRNeasy Kit, followed by cDNA synthesis using the miScript II Reverse Transcription Kit. Changes in the expression of miRNAs involved in cancer cell proliferation, apoptosis, and metastasis were examined using real-time qRT-PCR analysis.

Results
The IC50 values for GEM at 24, 48, and 72 hours were determined as 72.85 µM, 26.55 µM, and 9.38 µM, respectively. EGCG's IC50 values at 24, 48, and 72 hours were determined as 64.36 µM, 48.34 µM, and 19.73 µM, respectively. When combined at a 2:3 ratio (GEM: EGCG) at 24 and 72 hours, a synergistic effect was observed, while at 48 hours, a strong synergistic drug interaction was observed. At a concentration of only 26.55 µM, the group treated with GEM showed a 4.2-fold increase in apoptosis compared to the control group receiving fresh medium. In contrast, the combination treatment (EGCG: 4.71 µM, GEM: 3.14 µM) resulted in a remarkable 12.04-fold increase in apoptosis. After combination treatment, the expression of tumor suppressor miRNAs, miR-137, and miR-130a-3p, increased, while the expression of oncogenic miRNAs, including miR-27a-3p, miR-425-5p, miR-183-5p, miR-187-3p, miR-21-5p, miR-324-5p, and miR-486-5p, decreased.

Conclusion
EGCG can sensitize pancreatic cancer to GEM through epigenetic mechanisms, shedding light on novel therapeutic approaches.

Keywords: Epigallocatechin gallate, Gemcitabine, microRNA, Pancreatic cancer

Introduction

Pancreatic cancer, representing the 7th leading cause of cancer-related deaths according to 2020 GLOBOCAN data, remains a lethal malignancy with both its incidence and mortality rates consistently on the rise (1). Surgical resection can only be performed in approximately 15-20% of patients following initial diagnosis. Unfortunately, nearly 40% of patients are diagnosed at the metastatic stage, leaving unresectable tumors to be managed solely through chemotherapy and radiation therapy (2). Gemcitabine (GEM), the first drug FDA-approved for pancreatic cancer treatment, is considered a cornerstone in the management of pancreatic

ductal adenocarcinoma, especially in cases of local progression or metastatic tumors. However, its use as a single agent is associated with systemic toxicity, potential development of resistance, or recurrence. Consequently, researchers have directed their efforts towards evaluating different combination therapies, a trend supported by prospective clinical trials and meta-analyses that have shown enhanced clinical outcomes when gemcitabine is used in combination (3). Combinations of chemotherapeutic agents with distinct molecular targets have not only shown the potential to increase drug efficacy but also to reduce side effects that often result from high doses of single chemotherapeutics. This has led to increased interest in the combination of natural compounds with chemotherapeutics due to their potential to exhibit lower toxicity (4).

Upon entering the cell, GEM is metabolized by nucleoside kinases into dFdCDP and dFdCTP. This metabolic process inhibits the activity of ribonucleotide reductase, responsible for DNTP synthesis, thereby preventing cells from transitioning through the G1/S phase of the cell cycle and inhibiting DNA synthesis. As a result, Gemcitabine induces cell death (5).

The main catechin in green tea, Epigallocatechin-3-gallate (EGCG), has been identified as a polyphenolic compound with anti-cancer activity due to its anti-inflammatory, pro-oxidant, cell cycle modulation, and cell death-inducing properties (6). In the case of pancreatic cancer, it has been revealed that EGCG reduces cell proliferation, invasion, metastasis, and migration, leading to cell cycle arrest and stimulation of apoptosis (7). When investigating the combined effects of GEM and EGCG on pancreatic cancer in both in vivo and in vitro research, it has been observed that the combination suppresses Akt and EMT, thereby reducing migration and invasion (8). Additionally, the combination of GEM with EGCG suppresses glycolysis, leading to decreased cell proliferation (9). Furthermore, the combination has been found to inhibit the ERK pathway, which is responsible for drug resistance (10). The impact of EGCG and GEM in combination on miRNA expression, which contributes to malignant processes, remains an area of ongoing research and is lacking in the literature.

This study aims to assess the cytotoxic and apoptotic effects resulting from the combination of Gemcitabine and EGCG on PANC1 cells. Subsequently, the effectiveness of this combination in influencing miRNA expression levels associated with cancer progression will be investigated.

Material and Method

Cell Culture and Chemicals

The PANC1 cell line (Cat. No: CRL-1469), which will be used as the pancreatic ductal adenocarcinoma, was purchased from ATCC. To support the proliferation of PANC1 cells, Dulbecco's Eagle's Minimum Essential Medium growth medium was supplemented with 1% 2 mM L-glutamine, 1% penicillin/streptomycin, 100 µL of plasmocin, and 10% Fetal Bovine Serum. The cells were cultured under conditions with 95% humidity, 37°C, and 5% CO2. EGCG (Cat. No: E4143, Sigma-Aldrich) and Gemcitabine (Cat. No: 95058-81-4, Selleckchem), obtained in powdered form, were dissolved in sterile water to prepare a 5 mM stock solution, which was then stored at -80°C. Each experiment was conducted with cells corresponding to the fourth passage, involving the thawing of cells preserved at -80°C as the third passage.

WST-1 Test

The WST-1 test was conducted to examine the impact of each drug individually and in combination on cell viability. PANC1 cells were seeded in a 96-well plate with a density of 1×10^3 cells/well. Initially, a cytotoxicity test was established to determine the individual IC50 values of GEM and EGCG, revealing the cytotoxic effects of both agents separately on PANC1 cells. In the subsequent step, considering the previously determined IC50 values, experiments were set up for both the GEM treatment group and the EGCG treatment group individually on the same plate. Additionally, a combination group (IC50(GEM):IC50(EGCG)) was established on the same plate based on the predetermined ratios. After 24 hours (h) of seeding, the cells were treated with EGCG in a concentration range of 9.37 µM to 300 µM for 24, 48, and 72 h. Similarly, GEM was applied at concentrations ranging from 6.25 µM to 200 µM. Following the incubation period, WST solution was added to each well with 10 µL, and the plate was incubated for 0.5 to 4 h. During this incubation, microplate readings were taken every 30 minutes at a reference wavelength range of 450 nm to 620 nm using a microplate reader (Multiskan FC, Thermo). The experiments were independently conducted in triplicate, and the results were presented on dose-response graphs.

Isobologram Analysis

Cells were seeded into 96-well plates at a density of 1×10^3 cells/well. Based on the IC50 values, GEM and EGCG were combined in a 2:3 ratio for PANC1 cells. Following the combination treatment, absorbance values obtained at 24, 48, and 72 h were analyzed

using CalcuSyn 2.0 software (Biosoft) utilizing the Chou-Talalay theorem. Dose-response curves were plotted, and parameters including the fraction affected by the applied doses (Fa), the dose at which 50% effect is achieved (ED50), combination indices (CI), dose reduction indices (DRI), and the slope of the dose-response curve (m) were determined. The experiments were conducted in three separate replicates. The experiment results have been presented through Dose-Effect, Median-Effect, and Fraction (Fa)-Combination index (CI) graphs. The synergistic effect of the combination is illustrated on the Fraction -Combination index graph.

Apoptosis Analysis

The evaluation of the apoptotic effects of GEM, EGCG, and their combinations in pancreatic cancer cells involved treating cells with the designated doses. After treatment, the cells were subjected to the Annexin V method following the kit protocol. Subsequently, the cells were evaluated using BD ACCURI C6 flow cytometry (BD Biosciences Pharmingen). This analysis aimed to assess the population of cells undergoing apoptosis based on Annexin V staining.

miRNA Microarray Analysis

Following 48 h of treatment with EGCG, GEM, and their combination, total RNA isolation was carried out from both the dose and control groups using the miRNeasy Kit (Cat. No: 217084, Qiagen). The concentration and purity of the obtained RNA samples were assessed by measuring absorbance at wavelengths of 260/280 nm and 230/260 nm using a Nanodrop device (Thermo Scientific). From the isolated high-quality small RNAs, cDNA synthesis was performed using the miScript II Reverse Transcription Kit (Cat. No: 218193, Qiagen). Changes in miRNA expression in pancreatic cancer were analyzed using a miRNA PCR Array (Qiagen) containing 84 miRNAs implicated in cancer pathogenesis. The Roche Light Cycler 480 platform was used for this analysis. Internal controls, including SNORD61, SNORD72, SNORD96A, and RNU6-6P, were used. The analyses were conducted using the 2-ΔΔCt method. This method enables relative quantification of miRNA expression levels, normalized to internal controls, and presented relative to control groups.

Statistical Analysis

The relative expression of miRNAs was calculated using the 2−ΔΔCt method. In this calculation, all dose-treated groups were compared to the untreated control group. The fold changes and significances of miRNA expressions were calculated using the student t-test. MiRNA expressions that changed ± two-fold or more compared to the untreated control group, with p-values < 0.05, were considered significant. The IC50 values (half-maximal inhibitory concentration) and ED50 (median effective dose) values of the active substance on the cells were calculated using CalcuSyn v.2 software.

Results

Evaluation of the Effects of Gemcitabine, EGCG, and Combinations on Cell Viability

In PANC1 cells, the IC50 value of Gemcitabine was determined to be 72.85 µM (r: 0.94; m: 0.49437 +/- 0.083729) at 24 h, 26.55 µM (r: 0.94; m: 0.77940 +/- 0.066475) at 48 h, and 9.38 µM (r: 0.97; m: 0.49791 +/- 0.058230) at 72 h (Fig. 1A, Fig. 1B). For the EGCG compound, the IC50 values in PANC1 cells were 64.36 µM (r: 0.94; m: 0.49159 +/- 0.086826) at 24 h, 48.34 µM (r: 0.98; m: 0.77940 +/- 0.066475) at 48 h, and 19.73 µM (r: 0.99; m: 0.95816 +/- 0.061960) at 72 h.

Isobologram analysis was conducted for GEM and EGCG combinations at 24, 48, and 72 h. According to this analysis, the CI value of the Fa50 for the combination was 0.326 (synergistic) at 24 h, 0.216 (strongly synergistic) at 48 h, and the combination index for the Fa50 at 72 h was found to be 0.311 (synergistic). Further experiments were carried out based on the strong synergism data observed at the 48th h. When looking at the dose reduction index (DRI) at the 48th h, EGCG exhibited a DRI of 10.26-fold (from 48.34 µM to 4.71 µM), while GEM showed a DRI of 8.85-fold (from 26.55 µM to 3.14 µM) (Fig. 2A-C; Table 1).

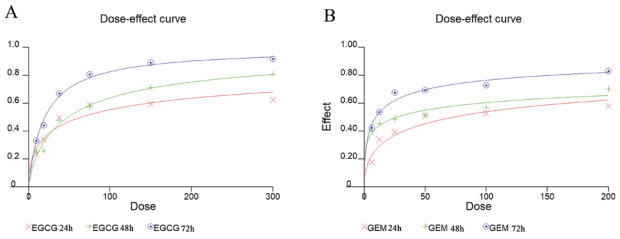

Figure 1
Dose-Effect Curve of GEM (Gemcitabine) and EGCG (Epigallocatechin-3-gallate) at 24, 48, and 72 hours

Evaluation of Apoptotic Effects of Single and Combination Agent Administration on PANC1 Cells

When evaluating the apoptotic effects of GEM and EGCG's IC50 doses and the combination's ED50 value on PANC1 cells at 48 h, compared to the

Table 1 Effect of EGCG, GEM and their combinations in PANC1 cells. DRI: Dose reduction index, CI: Combination index, RoM: Regression value of single drug administration, RoC: Regression value of combination

Time (hour)	24 h		48 h		72 h	
Drug	**GEM**	**EGCG**	**GEM**	**EGCG**	**GEM**	**EGCG**
IC_{50}	72.85 µM	64.36 µM	26.55 µM	48.34 µM	9.38 µM	19.73 µM
ED_{50}	8.8 µM	13.21 µM	3.14 µM	4.71 µM	1.70 µM	2.55 µM
RoM	0.94	0.94	0.94	0.98	0.97	0.99
DRI	8.27	4.87	8.85	10.26	5.51	7.73
CI	0.33 “synergistic”		0.22 “strongly synergistic”		0.31“synergistic”	
RoC	0.97		0.98		0.98	

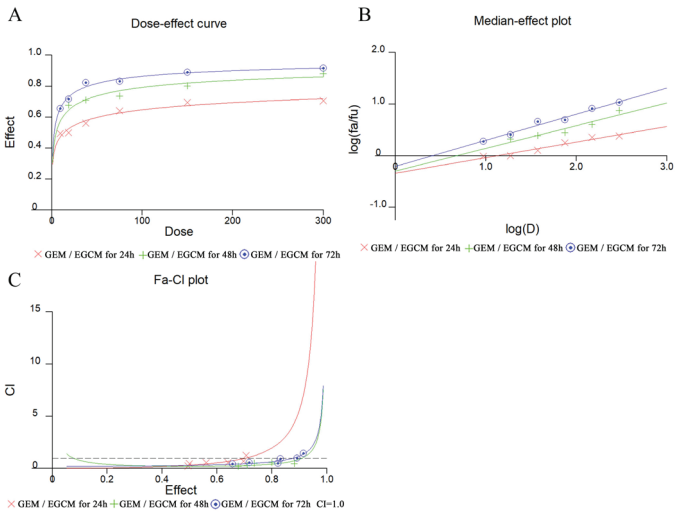

Figure 2
Dose-Effect, Median-Effect, Fraction (Fa)-Combination index (CI) plot of GEM (Gemcitabine) and EGCG (Epigallocatechin-3-gallate) combination at 24, 48, and 72 hours

Table 2 Demonstration of the effect of GEM, EGCG and their combination on miRNA expression in PANC1 cells

miRNA	GEM		EGCG		GEM/EGCG	
	Fold change	**p value**	**Fold change**	**p value**	**Fold change**	**p value**
miR137	9.8	0.000026	12.84	0.000024	19.54	0.000033
miR130a-3p	3.3	0	3.4	0	4.4	0
miR27a-3p	-3.5	0.0000001	-3.9	0.0000001	-5.2	0.0000004
miR425-5p	-2.9	0.0000005	-2.9	0.0000002	-3.9	0.000002
miR183-5p	-3.7	0.000002	-4.06	0.000002	-5.63	0.000002
miR187-3p	-3.61	0	-4.5	0	-5.64	0
miR21-5p	-1.09	0.000001	-3.1	0.0000001	-3.7	0.0000001
miR324-5p	-2.53	0.000001	-	-	-3.4	0.000001
miR486-5p	-2.5	0.000001	-	-	-4.5	0.000001
miR30b-5p	2.07	0	-	-	-	-

untreated group, it was found that the apoptotic rate was 4.2% in cells treated with EGCG, 10.6% in cells treated with GEM, and 30.1% in the combination group (Fig. 3A-D). EGCG showed a 1.68-fold increase in apoptosis, GEM exhibited a 4.24-fold increase, and the combination demonstrated a remarkable 12.04-fold increase in apoptosis, highlighting the enhanced apoptotic effects of the combination treatment.

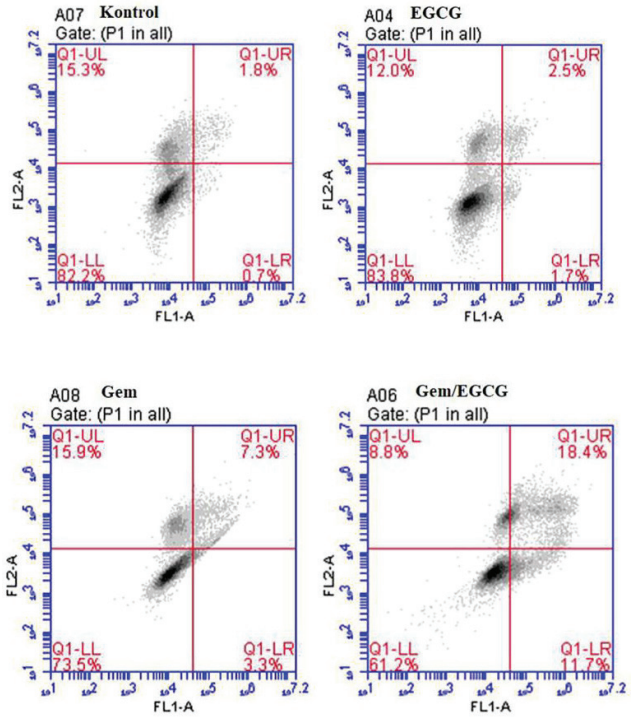

Figure 3
Visualization of the apoptotic effect of Untreated Control, EGCG, GEM, and combination in PANC1 cells. Bottom left: live cells, bottom right: early apoptotic, top right: late apoptotic, top left: necrotic cell percentages are shown.

miRNA Expression Changes Caused by Active Compounds in Cells

In PANC1 cells treated with IC50 value of GEM (26.55 μM) for 48 h, it was observed that the expression of miR137, miR130a-3p, and miR30b-5p increased, while the expression of miR27a-3p, miR183-5p, miR425-5p, miR187-3p, miR324-5p, miR486-5p, and miR21-5p decreased. After treatment with EGCG at its IC50 dose (48.34 μM) for 48 h, PANC1 cells exhibited increased expression of miR137 and miR130a-3p, while the expression of miR425-5p, miR183-5p, miR187-3p and miR21-5p decreased. Furthermore, the combination treatment (GEM: 3.14 μM and EGCG: 4.71 μM) for 48 h led to increased expression of miR137 and miR130a-3p, while the expression of miR27a-3p, miR425-5p, miR183-5p, miR187-3p, miR21-5p, miR324-5p and miR486-5p decreased (Table 2).

Discussion

MicroRNAs (miRNAs) binding to the 3'-UTR end of target mRNA play a pivotal role in governing biological processes by either inducing mRNA degradation or suppressing translation. Due to their ability to regulate the expression of oncogenes and tumor suppressor mRNAs, miRNAs significantly impact cellular functions. Notably, in pancreatic cancer, their expressions and oscillations display significant variations when compared to healthy pancreatic tissue (11).

In this study, the cytotoxic and apoptotic effects of the combination of GEM and EGCG were investigated in the pancreatic cancer model, PANC1 cells. The aim was to uncover the alterations in miRNA expression that might contribute to the efficacy of this combination in controlling cancer progression. Following the determination of the IC50 values of the active compounds, EGCG demonstrated a remarkable 3.14-fold reduction in GEM's ED50 value, indicating a strong synergistic effect. This combination exhibited robust synergistic activity in PANC1 cells, ranging from Fa10 to Fa85. These findings underscore the broad therapeutic potential of GEM and EGCG in the context of the pancreatic cancer model. Furthermore, it suggests that dynamic miRNA regulation may play a crucial role in EGCG's therapeutic efficacy, both individually and in combination with GEM.

miR137, a tumor suppressor, effectively inhibits cancer cell proliferation and invasion by targeting multiple oncogenes. Previous studies have demonstrated that miR137 directly targets Akt, a key player in conferring GEM resistance in pancreatic cancer (12). In this study, it was observed that miR137 expression significantly increased in the combination treatment compared to individual applications. Furthermore, miR27a-3p expression, upregulated in pancreatic cancer tissues and cell lines, is associated with lymph node and peritoneal metastasis. It has been shown that GATA6 mRNA, an inhibitor of the epithelial-mesenchymal transition (EMT) process, is negatively correlated with miR27a-3 in pancreatic cancer patients' samples (13). Consistent with these studies, our results suggested that treatment of PANC1 cells with both GEM and EGCG, either individually or in combination, leads to decreased miR27a-3p expression. This highlights miR27a-3p as a potential oncogenic target that can be modulated effectively by the combination of GEM and EGCG in pancreatic cancer.

miR183-5p, by controlling SOCS-6 expression, has been shown to enhance cell proliferation, migration, and invasion in pancreatic cancer models. The downregulation of miR183-5p has been associated with reduced oncogenic effects (14). This study demonstrated a more significant reduction in miR183-5p expression in PANC1 cells when the combination treatment was applied, compared to single-agent applications. Additionally, miR187, a positive regulator of the EMT process, exhibited decreased expression in response to the combination of GEM and EGCG.

The overexpression of miR30c-5p and miR30c-2-3p has been found to reduce cell proliferation, migration, and invasion in PANC1 cells upon transfection (15). Otherwise, miR425-5p's expression in pancreatic cancer tumor tissues has been correlated with lymph node metastasis, clinical stage, and differentiation degree (16). In the current study, the combination treatment of PANC1 cells with GEM and EGCG led to a decrease in miR425-5p expression, while miR30 expression increased. Taken together, the data from this study suggest that the combination of GEM and EGCG may reverse the invasive nature of pancreatic cancer through the regulation of miRNA expression levels.

miR130a, known for its role in chemoresistance development, exhibits reduced expression in high-risk premalignant pancreatic lesions (17). Moreover, miR130a-3p has tumor-suppressive activity and is known to target EPS8, ZWINT, SMC4, LDHA, GJB2, ZCCHC24, TOP2A, ANLN, and ADCY3 genes, which are associated with poor prognosis in pancreatic ductal adenocarcinoma (18). In the present study, the expression of miR130a-3p increased in the pancreatic cancer model treated with GEM and EGCG. Giovannetti et al. have revealed that miR21 is upregulated in GEM-resistant pancreatic cancer cells and that silencing miR21 sensitizes pancreatic cancer cells to GEM (19). In this study, both EGCG alone and in combination with GEM decreased the expression of miR130a-3p and miR21. Consequently, EGCG may enhance sensitivity to GEM in pancreatic cancer.

Wang et al. observed that the downregulation of miR486-5p coincided with increased apoptosis when they treated PANC1 cells with 5-Fluorouracil in vitro. This suggests that the downregulation of miR486-5p could enhance the anti-tumor effect of GEM (20). In our study, while GEM alone led to the downregulation of miR486-5p, the combination of GEM and EGCG further reduced miR486-5p expression compared to single-agent treatment. miR324-5p acts as a tumor suppressor by targeting different genes. It has been shown to induce apoptosis by targeting FGFR3 in colon cancer and inhibiting cancer cell proliferation by targeting FOS in cervical cancer (21, 22).

In the current study, EGCG, either alone or in combination with GEM, reduced the expression of miR324-5p. When investigating the correlation between the expression levels of apoptosis-associated miRNAs and cell death, it was observed that untreated control cells had significantly lower apoptosis rates compared to cells treated with GEM, EGCG, or their combination. The combination of GEM and EGCG displayed a synergistic effect, increasing apoptosis by 12.04-fold.

Conclusions

The effectiveness of the EGCG and GEM combination in pancreatic cancer has been established in previous studies, yet the miRNA profile supporting its anticancer activities has not been elucidated. In this study, regulators contributing to the upregulation of tumor-suppressor miRNAs and the downregulation of oncogenic miRNAs in the cytotoxic and apoptotic activities of the combination have been revealed. Determining the correlation between the altered expression of miRNAs and the targets of these miRNAs following the administration of GEM and EGCG in pancreatic cancer is an explicit goal for future studies.

Conflict of Interest Statement
The authors have no conflicts of interest to declare.

Ethical Approval
This article does not contain any studies with human or animal subjects.

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Availability of Data and Materials
The data and materials used in this study are available.

Authors Contributions
Ç. B.A: funding acquisition, resources, supervision, and review & editing draft.

A.H.K: formal analysis, investigation, and writing-original draft.

F.S: formal analysis, investigation, and writing-original draft.

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