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The Effects of Juglone on Cell Proliferation and Insulin-Like Growth Factor I Receptor/Phosphoinositide 3-Kinase/p85 (IGF-IR/PI3K/p85) Signaling Pathway in Pancreatic Cancer

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ABSTRACT

Objective: Pancreatic adenocarcinoma is a highly aggressive cancer with a poor prognosis, a high potential for invasion and metastasis, and resistance to therapy. We have previously demonstrated the cytotoxic, apoptotic, and antimetastatic effects of juglone. The insulin-like growth factor I receptor/phosphoinositide 3-kinase (IGF-IR/PI3K) signaling pathway plays an important role in tumor growth, metastasis, and therapeutic resistance in pancreatic cancer. In this study, we aimed to investigate the effect of juglone on cell proliferation in pancreatic cancer and to determine whether its potential effects occur via the IGF-IR/PI3K/p85 signaling pathway.

Materials and Methods: The PANC-1 pancreatic cancer cell line was cultured and treated with juglone at concentrations of 5, 10, 15, and 20 μM for 24 hours. The expressions levels of IGF-IR and proliferating cell nuclear antigen (PCNA), an indicator of cell proliferation, as well as p85/PIK3R1 gene transcription, a major downstream molecule of the IGF-IR/PI3K pathway, were evaluated by immunofluorescence analysis and quantitative polymerase chain reaction (qPCR), respectively.

Results: Juglone significantly downregulated IGF-IR and PCNA expressions, as well as p85/PIK3R1 transcription in PANC-1 pancreatic cancer cells. These findings suggest that juglone exhibits a potent antiproliferative effect and has the potential to suppress various processes involving the IGF-IR/PI3K/p85 signaling pathway, including tumor growth, metastasis, therapeutic resistance, stemness, and the epithelial-mesenchymal transition (EMT) phenotype of cancer cells.

Conclusion: Juglone exerts negative regulatory effects on pancreatic cancer, some of which are likely mediated through the IGF-IR/PI3K/p85 signaling pathway. Therefore, juglone may serve as a potential therapeutic option for the treatment and prevention of the progression of this devastating cancer.

Keywords: Insulin-like growth factor I receptor (IGF-IR), juglone, PANC-1, pancreatic cancer, phosphoinositide 3-kinase/p85 (PI3K/p85).

INTRODUCTION

Pancreatic adenocarcinoma is a highly aggressive cancer with a poor prognosis, a high potential for invasion and metastasis, and therapeutic resistance, making it a leading cause of cancer-related deaths worldwide. It is nearly impossible to cure due to its natural resistance to conventional chemotherapeutic drugs and insensitivity to radiotherapy.[1,2] For these reasons, new therapeutic strategies must be developed to combat pancreatic cancer. In recent years, the combination of anti-tumor agents derived from natural sources with conventional chemotherapeutics, targeting dysregulated signaling pathways involved in carcinogenesis and capable of suppressing the invasion and metastasis potential of cancer cells, has gained significant importance in cancer treatment. Juglone is a natural naphthoquinone found in trees of the *Juglandaceae* (Walnut) family. Numerous studies on various cancers have demonstrated the cytotoxic and apoptotic effects of juglone by blocking the cell cycle and/or inducing apoptosis via mitochondrial pathways, as well as its anti-metastatic properties.[3–8] However, there are limited studies on the role of juglone in pancreatic cancer and its potential underlying mechanisms. In our previous study, we reported for the first time that juglone exhibits a cytotoxic effect in pancreatic cancer and suppresses the invasion and metastasis potential of cancer cells by downregulating phosphatase and actin regulator 1 (Phactr-1), matrix metalloproteinase-2 (MMP-2), and MMP-9.[6] We also demonstrated that juglone induces reactive oxygen species-mediated (ROS-mediated) mitochondrial apoptosis,[7] inhibits the Wnt/β-catenin signaling pathway,[8] and suppresses the epithelial-mesenchymal transition (EMT) phenotype,[9] which play critical roles in tumor angiogenesis, invasion, and metastasis. However, further studies are needed to fully understand the molecular signals of juglone on the growth and invasion potential of pancreatic cancer cells.

The insulin-like growth factor (IGF) signaling pathway is complex and plays a crucial role in cancer development, prognosis, metastasis, and therapeutic resistance.[10] In particular, insulin-like growth factor I (IGF-I) and IGF-I receptor (IGF-IR) have been strongly associated with many types of cancer.[11] IGF-IR is overexpressed or activated in the majority of pancreatic cancers.[12] Furthermore, aberrant activation of IGF-IR in pancreatic cancer has been found to contribute to poor survival, high invasive and metastatic potential, the EMT phenotype, and therapeutic resistance, resulting in the extremely aggressive nature of this cancer.[13–15] Consequently, IGF-IR appears to be a potential prognostic and diagnostic factor for pancreatic cancer.[11]

IGF-IR is the primary receptor for IGF-I and IGF-II. IGF-IR is a tetramer composed of two identical α-subunits responsible for ligand binding and two identical β-subunits containing a transmembrane domain and an intracellular tyrosine kinase. The binding of IGF-IR to its ligands leads to autophosphorylation in the tyrosine kinase domain, resulting in the activation of the phosphatidylinositol-3 kinase/AKT (PI3K/AKT) and RAS/mitogen-activated protein kinase (RAS/MAPK) pathways.[10] Class IA PI3K consists of regulatory (p85) and catalytic (p110) subunits. The major regulatory subunit of PI3K, p85, is encoded by phosphoinositide-3-kinase regulatory subunit 1 (PIK3R1).[10] Activation of the IGF-IR/PI3K signaling pathway induces cell proliferation and growth while suppressing apoptosis, regulated by multiple mechanisms often involving cross-talk with other signaling pathways.[10,15,16] The clinical relevance of the PI3K signaling pathway is based on the fact that aberrant signaling within this pathway is involved in tumorigenesis.[16] Thus, IGF-IR and its downstream signaling pathways have become important targets in cancer treatment. There is substantial preclinical evidence that inhibition of the IGF-IR pathway prevents cancer growth, metastasis, and resistance to therapy.[17–19]

KEY MESSAGES

- Juglone exhibits a potent antiproliferative effect in pancreatic cancer by inhibiting PCNA expression.
- Juglone, through inhibition of the IGF-IR/PI3K/p85 signaling pathway in pancreatic cancer, has the potential to suppress processes mediated by this pathway, including cancer metastasis, therapeutic resistance, cancer stem cells, and EMT phenotype.
- Therefore, juglone has a negative regulatory effect on pancreatic cancer and may serve as a potential option for the treatment and prevention of the progression of this devastating cancer.

Based on the previously demonstrated strong anticancer activities of juglone and the critical role of the IGF-IR/PI3K signaling pathway in pancreatic cancer, the present study aimed to investigate the effect of juglone on cell proliferation and to determine whether its potential effects occur via the IGF-IR/PI3K/p85 signaling pathway.

MATERIALS AND METHODS

Cell Culture

The PANC-1 cell line was used as a human pancreatic adenocarcinoma cell line. The cell line was purchased from the American Type Culture Collection (ATCC; Manassass, VA, USA, Number CRL-1469, RRID: CVCL_0480). The PANC-1 cell line was derived from 56-year-old male patients with pancreatic adenocarcinoma; therefore, the current study includes only male samples. PANC-1 cells were cultured in

Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and containing 100 IU/mL penicillin and 100 µg/mL streptomycin. The culture was maintained in a humidified atmosphere at 37°C with 5% CO_2. Juglone was purchased from Sigma-Aldrich Chemical Company.

Approval for the study was obtained from Selçuk University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (Protocol number: 2016/131, Date: 27.04.2016).

Determination of Experimental Groups

In our previous study, in which we demonstrated the cytotoxic effect of juglone on pancreatic cancer (PANC-1 and BxPC3 cell lines) using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay,[6] we determined that the juglone concentration required to suppress cell vitality by 50% in PANC-1 cells was 21.25 µM. Based on this dose, we used juglone at concentrations of 5, 10, 15, and 20 µM in subsequent experiments.

In our current study, considering our previous findings, we designed the treatment groups as four groups, in which juglone was applied at concentrations of 5, 10, 15, and 20 µM for 24 hours, with a group without juglone serving as the control group.

Gene Expression Analysis

To determine the potential role of juglone on the p85 regulatory subunit of PI3K in pancreatic cancer, we analyzed the expression levels of PIK3R1, the gene encoding p85. To assess changes in PIK3R1 gene expression levels in PANC-1 cells (ATCC, CRL-1469, RRID: CVCL_0480), RNA isolation was first performed. Total RNA isolation was conducted using the classical protocol with TRIdity G reagent (Applichem, A4050). Subsequently, cDNA synthesis was performed from the total RNA obtained using the Vivantis 2-Step Reverse Transcription-Polymerase Chain Reaction (RT-PCR) Kit (Vivantis, RTPL2).

Quantitative real-time PCR (qPCR) was carried out using the Roche LightCycler96 device to determine the effects of juglone at different concentrations on PIK3R1 gene expression levels in the PANC-1 cell line.

SYBR Green qPCR Master Mix (Thermo Scientific, K0251) was used for qPCR analysis. The primers used for the PIK3R1 gene and the reference β-actin gene[20,21] are shown in Table 1.

The PIK3R1 gene expression level in PANC-1 cancer cells was interpreted based on cycle threshold (Ct) values. The β-actin gene was used for normalization, and the PIK3R1 expression level of the control group was determined using the $2^{-\Delta\Delta CT}$ method. The gene expression levels of the treatment groups were then compared. In the interpretation of qPCR results, a twofold or greater change was considered significant in accordance with the literature.

Table 1. Specific primer sequences used for the phosphoinositide-3-kinase regulatory subunit 1 (PIK3R1) and β-actin genes

Gene	Primer sequence	Reference
β-Actin	I: 5-ACTCTTCCAGCCTTCCTTC-3 G: 5-ATCTCCTTCTGCATCCTGTC-3	Hsu et al., 2005[20]
PIK3R1	I: 5- ACCACTACCGGAATGAATCTCT-3 G: 5- GGGATGTGCGGGTATATTCTTC-3	Wu et al., 2016[21]

Immunofluorescence Analysis

The protein expressions of IGF-IR and proliferating cell nuclear antigen (PCNA) in the control and treatment groups were analyzed using the indirect immunofluorescence method. Immunostaining was performed using primary antibodies for IGF-IR (mouse monoclonal, Santa Cruz Biotechnology, sc-271606, RRID: AB_10650003) and PCNA (mouse monoclonal, Santa Cruz Biotechnology, sc-56, RRID: AB_628110), along with fluorescein isothiocyanate (FITC)-labeled secondary antibodies (goat anti-mouse immunoglobulin G [IgG] H&L (Alexa Fluor® 488, Abcam, ab150113, RRID: AB_2576208) and 4',6-diamidino-2-phenylindole (DAPI) as a nuclear stain. Briefly, cultured cells were fixed in absolute methanol for 15 minutes and then treated with 0.2% Triton X-100 solution for 20 minutes to increase permeability. Next, cells were incubated in a serum block solution for 40 minutes to prevent nonspecific staining. Subsequently, cultured cells were incubated with primary antibodies and then with FITC-labeled secondary antibodies for 1 hour at room temperature. Finally, cells were coated with DAPI to label cell nuclei.

The stained cells were examined using fluorescence microscopy, and images were obtained. To detect statistically significant differences between the study groups, the one-way analysis of variance was performed at a 5% significance level, with 80% statistical power and a large effect size of 0.4. The required sample size was determined to be at least 16 per group. Thus, protein expression levels in each group were evaluated in at least 10 different randomly selected fields by an observer blinded to the experimental groups. Each experiment was repeated three times. IGF-IR and PCNA-positive stained cells were counted in each examined field, and IGF-IR and PCNA expression percentages were calculated by dividing the number of positive cells by the total cell count.

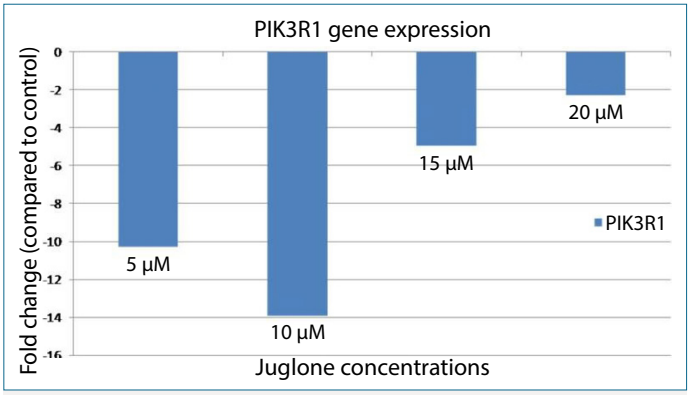

Figure 1. Effects of different concentrations of juglone treatments on phosphoinositide-3-kinase regulatory subunit 1 (PIK3R1) gene expression levels in PANC-1 cells. Data were analyzed using the $2^{-\Delta\Delta CT}$ method.

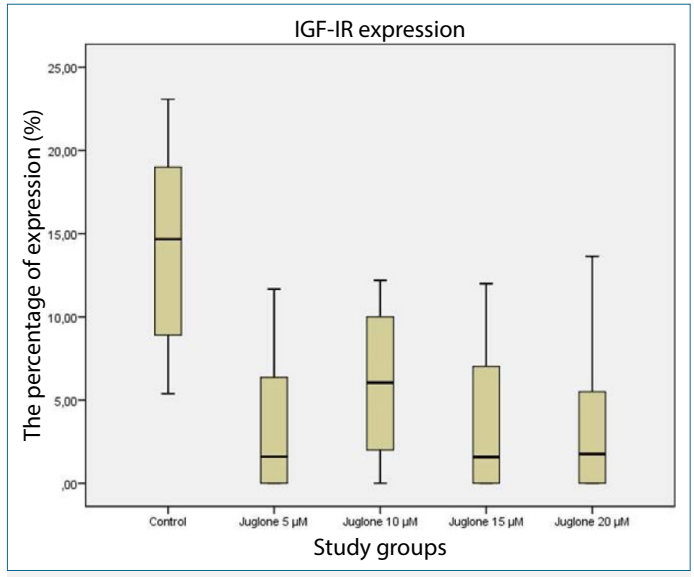

Figure 2. Effect of juglone treatment at different concentrations on insulin-like growth factor I receptor (IGF-IR) protein expression levels in PANC-1 cells. IGF-IR expression levels were significantly decreased in the juglone treatment groups at 5, 15, and 20 µM concentrations compared to the control group (p=0.006, p=0.009, p=0.006, respectively).

Statistical Analysis

Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS) version 22.0 (IBM Corp. released 2013, IBM SPSS Statistics for Windows, version 22.0; IBM Corp., Armonk, NY, USA). The sample size of the study was calculated using R version 3.6.0 (The R Foundation for Statistical Computing, RRID: SCR_001905, Vienna, Austria; https://www.r-project.org/) to detect statistically significant differences between the study groups at a 5% significance level, 80% statistical power, and a large effect size of 0.4. The Kolmogorov-Smirnov test was applied to assess the distribution of variables. Differences in IGF-IR and PCNA expression levels between study groups were evaluated using the Kruskal-Wallis test with multiple comparisons. Values are presented as mean±standard deviation, median, and 25th and 75th percentiles. A p value of less than 0.05 was considered statistically significant.

RESULTS

Effect of Juglone on PIK3R1 Gene Expression Level in the PANC-1 Cells

Juglone treatment in PANC-1 cells resulted in a significant reduction in PIK3R1 gene expression at all treated doses. Compared with the control group, treatment with 5, 10, 15, and 20 µM juglone led to a reduction of 10.3-, 13.9-, 4.9-, and 2.3-fold, respectively (Fig. 1).

Effect of Juglone on IGF-IR Protein Expression Level in PANC-1 Cells

Immunofluorescence staining results demonstrated that juglone significantly downregulated IGF-IR protein expression in PANC-1 pancreatic cancer cells (p=0.002). Table 2 presents the mean, median, and 25th and 75th percentile values of IGF-IR protein expression across study groups. While IGF-IR protein was expressed in 13.9% of cancer cells (median: 14.7%) in the control group, it was expressed in 3.2% (median: 1.6%), 5.7% (median: 6%), 3.6% (median: 1.6%), and 3.3% (median: 1.7%) of cancer cells in the juglone treatment groups at concentrations of 5, 10, 15, and 20 µM, respectively. The decrease in IGF-IR expression was statistically significant in the juglone treatment groups at 5, 15, and 20 µM concentrations compared to the control group, but not at 10 µM concentration (Table 2, Fig. 2, 3).

Effect of Juglone on PCNA Protein Expression Level in PANC-1 Cells

Juglone significantly reduced the number of PCNA-positive cells, a direct marker of cell proliferation in PANC-1 cells (p=0.001). PCNA protein was expressed in 21.1% (median: 21.4%) of cancer cells in the control group, compared to 7.1% (median: 4.3%), 4.9% (median: 5.1%), and 1.3% (median: 0%) of cancer cells in the 5, 10, and 15 µM juglone treatment groups, respectively. No PCNA expression was observed in the 20 µM juglone treatment group, indicating the ability of juglone to completely inhibit cell proliferation (Table 2, Fig. 4, 5). The decrease in PCNA expression across all juglone treatment groups compared to the control group was statistically significant. These findings highlight the strong anti-proliferative effect of juglone on pancreatic cancer cells.

Table 2. Percentages of insulin-like growth factor I receptor (IGF-IR) and proliferating cell nuclear antigen (PCNA) expression in study groups in PANC-1 cells

Study groups	IGF-IR eexpression, %				PCNA expression, %			
	N	Mean±SD	Median (25th; 75th per.)	p*	N	Mean±SD	Median (25th; 75th per.)	p*
Control	10	13.9±6.1	14.7 (8.9; 19.0)		10	21.1±9.9	21.4 (12.5; 26.3)	
Juglone 5 µM	10	3.2±4.0	1.6 (0.0; 6.4)	0.006	10	7.1±6.5	4.3 (2.8; 12.5)	0.01
Juglone 10 µM	10	5.7±4.3	6.0 (2.0; 10.0)	>0.05	10	4.9±3.2	5.1 (4.4; 6.3)	0.01
Juglone 15 µM	10	3.6±4.5	1.6 (0.0; 7.0)	0.009	10	1.3±1.9	0.0 (0.0; 2.1)	<0.001
Juglone 20 µM	10	3.3±4.4	1.7 (0.0; 5.5)	0.006	10	0.0±0.0	0.0 (0.0; 0.0)	<0.001

IGF-IR expression levels were significantly different between the study groups (p=0.002). PCNA expression levels were significantly different between the study groups (p<0.001). *: P values are for the juglone treatment groups compared to the control group; SD: Standard deviation; Per: Percentiles.

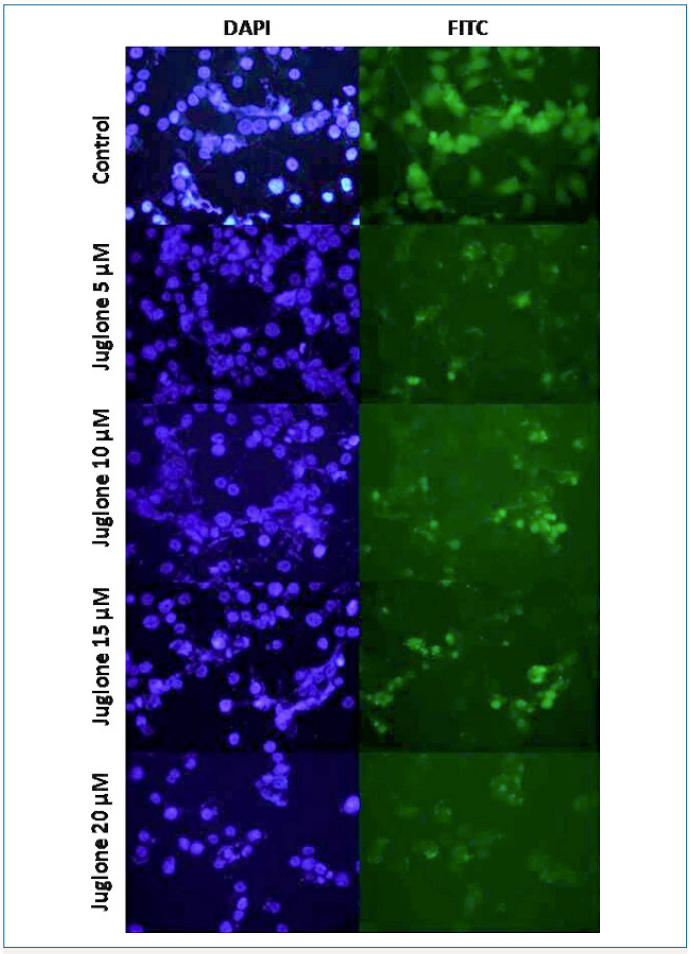

Figure 3. Immunofluorescence images of insulin-like growth factor I receptor (IGF-IR) protein expression in the control and juglone treatment groups in PANC-1 cells. The 4′,6-diamidino-2-phenylindole (DAPI) images are in the left panel, and the fluorescein isothiocyanate (FITC) images are in the right panel. Scale bar: 25 µm.

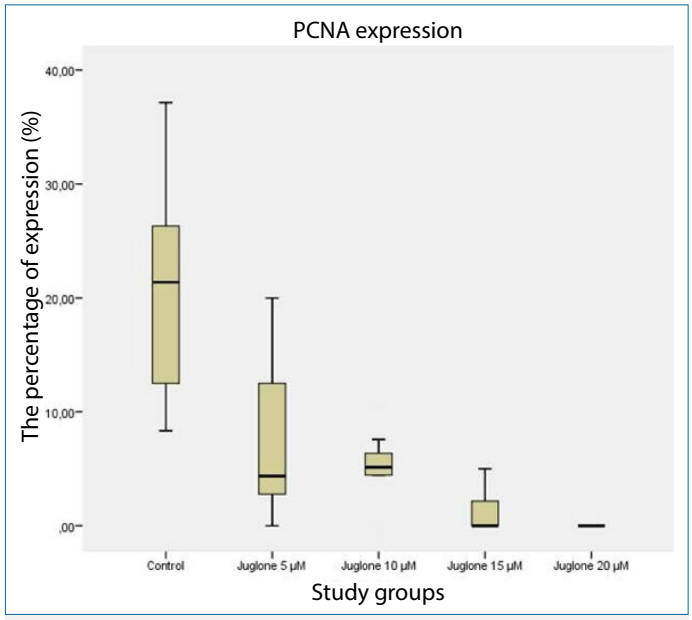

Figure 4. Effect of juglone treatment at different concentrations on proliferating cell nuclear antigen (PCNA) protein expression levels in PANC-1 cells. PCNA expression levels were significantly decreased in the juglone treatment groups at 5, 10, 15, and 20 µM concentrations compared to the control group (p=0.01, p=0.01, p<0.001, p<0.001, respectively).

DISCUSSION

The treatment of pancreatic cancer, which responds poorly to conventional therapies, remains a significant challenge. The development of resistance to cancer therapy is a complex process involving multiple signaling pathways, and the underlying downstream mechanisms are not yet fully understood. Several pathways, including Kirsten rat sarcoma viral oncogene homolog (KRAS) mutations,[22] PI3K/AKT[23]

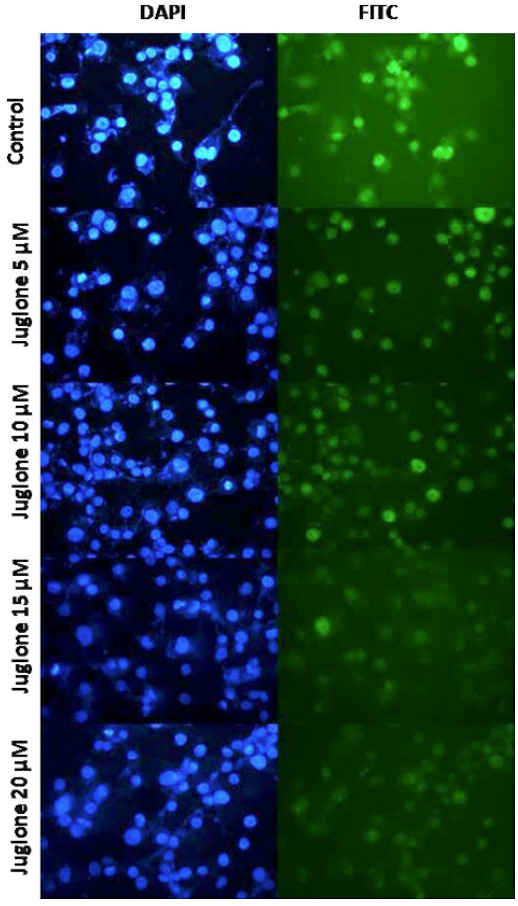

Figure 5. Immunofluorescence images of proliferating cell nuclear antigen (PCNA) protein expression in the control and juglone treatment groups in PANC-1 cells. The 4′,6-diamidino-2-phenylindole (DAPI) images are in the left panel, and the fluorescein isothiocyanate (FITC) images are in the right panel. Scale bar: 25 µm.

mutations, cancer cell plasticity, cancer stem cells (CSC),[24] and the EMT,[25] contribute to the development of treatment resistance. Therefore, it is crucial to develop therapeutic strategies that target multiple molecular pathways or exhibit multiple mechanisms of action to overcome therapeutic resistance. EMT and CSCs are two interrelated programs that significantly contribute to the enhanced migration and invasion abilities of tumor cells, ultimately leading to tumor invasion, metastasis, and therapeutic resistance.[24,25] EMT can also promote the stemness of cancer cells. The IGF-IR signaling pathway is involved in numerous oncogenic events, including tumor proliferation, angiogenesis, metastasis, and therapeutic resistance. Moreover, increasing evidence suggests that IGF-IR signaling plays a crucial role in the induction of EMT and CSC programs.[17,26] Studies in various human cancer cell lines and xenograft models have demonstrated that IGF-IR inhibition prevents cancer growth, metastasis, and resistance to therapy.[16–19] Silencing IGF-IR has been shown to inhibit cell proliferation, growth, and metastasis in pancreatic cancer by blocking PI3K/AKT and EMT signaling pathways.[17] However, although some clinical trials of IGF-IR inhibitors have shown promising results,[27] most clinical trials have failed to improve patient outcomes.[28,29] The disappointing results of clinical trials targeting IGF-IR may be attributed to the high complexity of the IGF pathway, which is interconnected with other growth signaling pathways. Due to crosstalk between IGF-IR and particularly insulin receptor (IR), epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF), human epidermal growth factor receptor 2 (HER2), and common downstream pathways such as phosphoinositide-3 kinase/protein kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR) and rat sarcoma virus/rapidly accelerated fibrosarcoma/mitogen-activated protein kinase kinase/extracellular signal-regulated kinase (RAS/RAF/MEK/ERK), inhibition of IGF-IR alone is likely insufficient to arrest tumor growth and progression. To prevent potential crosstalk, the combined inhibition of IGF-IR along with other growth signaling pathways and/or common downstream pathways has been explored, yielding successful outcomes.[19] Furthermore, evidence that resistance to IGF-IR inhibition occurs via activation of PI3K/AKT/mTOR signaling suggests that additional targeting of this pathway is required to overcome resistance to IGF-IR inhibition.[23]

The PI3K/AKT/mTOR signaling pathway is a major downstream mediator of the fundamental oncogenic effects of IGF-IR signaling and contributes to EMT- and CSC-driven therapeutic resistance, particularly in pancreatic cancer.[15,30] Knockdown of IGF-IR and inhibition of the downstream PI3K/AKT/mTOR pathway have been shown to reduce the CSC population and suppress the EMT phenotype in breast cancer.[31] In pancreatic cancer, which frequently harbors KRAS mutations, KRAS mutation may also lead to activation of the PI3K/AKT/mTOR signaling pathway. PI3K has been identified as a key effector of oncogenic KRAS signaling, particularly in pancreatic cancer, and has been suggested as a potential biomarker for identifying patients suitable for targeted treatment.[32] The potential role of the p85/PIK3R1 subunit of PI3K in cancer is less well understood. Mutant or upregulated p85 has been associated with poor survival and resistance to therapy in several cancers and has been suggested as a prognostic biomarker.[33–35] Direct inhibition of p85/PIK3R1 transcription has been shown to limit PI3K/AKT signaling in hepatocellular carcinoma, suppressing cancer cell survival and motility.[34] Furthermore, PI3K/p85 has demonstrated predictive value in resistance to chemo-radiation therapy, including IGF-IR inhibitors.[35] In light of these findings, particularly in KRAS-mutated pancreatic cancer, the downstream PI3K/p85 molecule of the IGF-IR pathway

may serve as a crucial target to prevent EMT, CSC, and tumor metastasis, as well as to overcome drug resistance, including resistance to IGF-IR inhibitors.

In the present study, we demonstrated that juglone significantly downregulated the expressions of IGF-IR protein and p85/PIK3R1 gene, a major downstream molecule of the IGF-IR/PI3K/AKT pathway, in PANC-1 pancreatic cancer cells. The simultaneous downregulation of IGF-IR and PI3K/p85 by juglone suggests that juglone may eliminate potential crosstalks in the IGF-IR/PI3K pathway and mediate multiple signaling mechanisms in pancreatic cancer cells. The dual effect of juglone on the IGF-IR/PI3K pathway may represent an important mechanism for overcoming potential resistance that could arise from IGF-IR inhibition alone. Juglone may inhibit the activation of the IGF-IR/PI3K/AKT signaling pathway and suppresses multiple processes mediated by this pathway, including CSCs, EMT, metastasis, and therapeutic resistance. Furthermore, it is significant that juglone exerted these inhibitory effects in KRAS-mutated and gemcitabine-resistant PANC-1 pancreatic cancer cells.

Previous studies have shown that juglone inhibits cell growth and induces apoptosis via mitochondrial pathways in pancreatic cancer.[5–8] However, apart from evidence demonstrating the inhibitory effect of juglone on the proliferation marker Ki67 by Shah et al.,[36] there is no data on the molecular mechanisms involved in juglone-mediated blockade of cell proliferation in pancreatic cancer. The present study demonstrated that juglone significantly suppressed the expression of PCNA, a direct marker of cell proliferation. Recent studies indicate that pancreatic cancer cells express high levels of PCNA and that its overexpression is associated with poor prognosis, highlighting the importance of targeting PCNA to prevent tumor growth.[37] Additionally, it has been established that PCNA mediates IGF-IR/PI3K-induced cell proliferation in liver oval cells, and that juglone treatment effectively blocks this proliferative response.[38] Our findings suggest that PCNA likely mediates cell proliferation stimulated by IGF-IR/PI3K signaling and that juglone can significantly abrogate IGF-IR/PI3K-mediated proliferative responses in pancreatic cancer by inhibiting PCNA expression.

Our previous studies, along with several others, have demonstrated the antimetastatic, antiangiogenic, and EMT-inhibitory effects of juglone in pancreatic cancer.[5–9,36] These effects were, in part, achieved by inhibiting the Wnt/β-catenin[8] and oncogenic MYC signaling pathways,[36] both of which promote EMT and CSC progression. Furthermore, the combination of juglone with selenium exhibited a synergistic effect and effectively suppressed the EMT phenotype.[9] Despite these findings, the molecular mechanisms mediating the effects of juglone on EMT, CSC, and tumor metastasis are not yet fully understood. Juglone has been shown to inhibit CSC and EMT in cancer cells by suppressing peptidyl-prolyl cis-trans isomerase NIMA-interacting 1 (Pin1).[39] Additionally, the inhibition of PI3K/AKT phosphorylation by juglone has been found to increase glycogen synthase kinase 3β (GSK 3β) activity and suppress EMT in prostate cancer.[4] Moreover, juglone has been reported to suppress PI3K activity via direct binding to PI3K, thereby inhibiting tumorigenesis.[40] Our results demonstrated that juglone directly downregulates p85 transcription in pancreatic cancer, suggesting that it may prevent the activation of PI3K signaling. Given that the IGF-IR/PI3K signaling pathway triggers EMT/CSC phenotype, our study suggests that juglone may suppress EMT and CSC characteristics by inhibiting the IGF-IR/PI3K/p85 pathway and potentially reversing cancer resistance mediated by these pathways.

CONCLUSION

The effects of juglone, which has been proven to be effective in many types of cancer, on pancreatic cancer and the molecular mechanisms underlying its activity are not yet fully elucidated. Our study demonstrated that juglone has a strong antiproliferative effect in pancreatic cancer. Additionally, juglone may disrupt the IGF-IR/PI3K signaling pathway by simultaneously downregulating IGF-IR and PI3K/p85 expression. The simultaneous downregulation of IGF-IR and PI3K/p85 by juglone is particularly significant, as it suggests that juglone may eliminate potential crosstalk within the IGF-IR/PI3K pathway and prevent possible resistance mechanisms. Moreover, juglone may suppress tumor growth, EMT, CSC phenotype, invasion, metastasis, and therapeutic resistance by targeting multiple cancer-driving pathways mediated by the IGF-IR/PI3K signaling pathway. Therefore, juglone exerts a negative regulatory effect on pancreatic cancer and may potentiate the effect of conventional chemotherapeutics when used in combination.

Ethics Committee Approval: The Selçuk University Non-Interventional Clinical Research Ethics Committee granted approval for this study (date: 27.04.2016, number: 2016/131).

Author Contributions: Concept – DD, HA, DEK; Design – DD, HA, DEK; Supervision – DD, HA, DEK; Resource – DD; Materials – DD, HA, DEK, FG; Data Collection and/or Processing – DD, HA, FG; Analysis and/or Interpretation – DD, HA, DEK; Literature Search – DD, HA, DEK; Writing – DD; Critical Reviews – DD, HA.

Conflict of Interest: The authors have no conflict of interest to declare.

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