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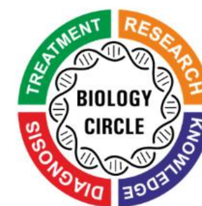

Research Article

In vitro evidence supports cell cycle arrest by alkyl-phospholipid erufosine in pancreatic cancer cell line

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Abstract

Background: Pancreatic cancer is an aggressive malignancy with limited therapeutic options and poor prognosis. Dysregulation of cell cycle control is a hallmark of tumor progression, making cell cycle–targeted therapies an attractive strategy. Erufosine, a synthetic alkyl phosphocholine, has shown promising antitumor activity in various cancer models by interfering with signaling pathways involved in cell survival and proliferation. However, its effects on cell cycle regulation in pancreatic cancer cells require investigation.

Methods: Human pancreatic cancer cells were treated with erufosine at defined concentrations for specified time interval. Cell cycle distribution was analyzed using propidium iodide-based staining of DNA followed by flow cytometry. The staining enabled quantification of DNA content to assess proportion of cells in different phases of the cell cycle.

Results: Erufosine treatment resulted in alteration of cell cycle progression in pancreatic cancer cells. Flow cytometric analysis revealed an accumulation of cells in a specific phase of the cell cycle (G2/M), indicating a halt in cell cycle progression compared to untreated controls. This arrest was accompanied by a corresponding reduction in the proportion of cells undergoing active proliferation.

Conclusion: These findings suggest that erufosine exerts its anti-tumor effects in pancreatic cancer, at least in part, by inducing cell cycle arrest. Erufosine may therefore represent a potential therapeutic agent targeting cell cycle dysregulation in pancreatic cancer.

Key Words: Pancreatic cancer, Anticancer drug, Cell cycle arrest, Erufosine

INTRODUCTION

Cell cycle is a tightly regulated series of events that control cell growth, DNA replication and division. Normal progression through the cycle depends on a complex interplay between regulatory proteins, checkpoints and signaling pathways that ensure genomic integrity and appropriate responses to environmental factors [1]. In cancer, this regulation is profoundly disrupted leading to uncontrolled proliferation, a hallmark of tumorigenesis [2]. Cell cycle progression is orchestrated by cyclin-dependent kinases (CDKs) and their regulatory partners, cyclins [3]. Cancer cells often bypass normal regulatory controls. One of the most common alterations includes overexpression of cyclins and CDKs, inactivation of tumor suppressors, cyclin-dependent kinase inhibitor dysfunction, checkpoint failure and genomic instability [4, 5].

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The combined effect of these alterations is uncontrolled cellular proliferation, genomic instability and tumor progression [6]. Cancer cells evade growth suppressors, reduce apoptotic responses, and accumulate mutations due to deficient checkpoints reinforcing malignant phenotypes [7]. This deregulation also interfaces with other cancer hallmarks, such as metabolic reprogramming and immune evasion, suggesting that cell cycle abnormalities have broad implications beyond simple proliferation [8].

Erufosine (ErPC3) is a synthetic alkyl phosphocholine and a 3rd analog of miltefosine that has attracted considerable interest as a membrane-targeting anticancer agent [9]. Unlike classical cytotoxic drugs that primarily damage DNA, erufosine integrates into cellular membranes and lipid rafts thereby modulating signal transduction pathways essential for tumor cell proliferation, survival, and cell cycle progression [10]. A number of *in vitro* studies have demonstrated that erufosine exerts broad spectrum of anticancer activity against solid and hematological malignancies, including breast, lung, colorectal, oral squamous cell

carcinoma and leukemia [11-14]. Its antineoplastic effects are characterized by dose- and time-dependent inhibition of cell proliferation, reduced clonogenic survival, and suppression of tumor growth in animal models, while exhibiting relatively low toxicity toward normal cells suggesting a favorable therapeutic index [15, 16]. Mechanistically, erufosine interferes with key oncogenic signaling cascades, most notably the PI3K/Akt/mTOR pathway, which plays a central role in regulating cell growth, metabolism, and survival [17, 18]. Inhibition of Akt phosphorylation and downstream mTOR signaling leads to decreased protein synthesis, impaired metabolic support for proliferation, sensitization of cancer cells to cell cycle arrest and programmed cell death [19]. In parallel, modulation of the Ras/Raf/MEK/ERK pathway and disruption of lipid raft–associated signaling further contribute to its antiproliferative effects [20].

A prominent feature of erufosine mediated anticancer activity is the induction of cell cycle arrest most commonly at the G2/M phase, although G0/G1 arrest has also been reported in certain cellular contexts [21]. This arrest is associated with downregulation of key cyclin and cyclin-dependent kinases required for cell cycle progression. Concurrently, erufosine has been shown to upregulate CDK inhibitors, reinforcing checkpoint activation and preventing mitotic entry. The sustained cell cycle blockade imposed by erufosine often precedes the activation of apoptotic and autophagic pathways, marked by caspase activation, PARP cleavage, and modulation of Bcl-2 family proteins indicating that cell cycle arrest is functionally linked to cell death induction [22, 23]. Collectively, the available literature supports that erufosine exerts its anticancer effects through a multifaceted mechanism involving disruption of membrane-associated oncogenic signaling, induction of cell cycle arrest and subsequent activation of apoptosis and autophagy, positioning it as a promising candidate for further development as an anticancer agent.

To date, effect of erufosine on cell cycle machinery in pancreatic ductal adenocarcinoma has not been characterized at the molecular level. In this study, we investigated the impact of erufosine exposure on cell cycle machinery of pancreatic cancer. This is one of the most dangerous malignancies as advanced stages are imposing a major challenge to control the cancer associated deaths. Considering the known anticancer effects of erufosine and lethal nature of pancreatic cancer, the findings provided a platform to find the novel applications of erufosine as cytostatic agent against pancreatic cancer.

METHODS

Cell Culture of Cancer Cells

Human (BxPC-3) cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 Medium (Gibco, Thermo Fisher Scientific) supplemented with 1% penicillin (100U/mL), streptomycin (100µg/mL), 10% fetal bovine serum (FBS) and 2mM L-glutamine. Cells were maintained at 37°C in a humidified incubator containing 5% CO_2. Cells were sub-cultured at 60–80% confluence using 0.25% trypsin–EDTA after washing with sterile phosphate-buffered saline (PBS). The cells were centrifuged at 1500 rpm for 5 minutes, resuspended in fresh medium and seeded at appropriate densities depending on the experimental requirements.

Flowcytometry Analysis

The cells were cultured in a 25cm^2 flask (250,000 cells/5ml medium) and left to grow overnight. Next day, growing cultures were exposed with erufosine (5, 10 and 20µM) for a period of 48 h. After the time interval, cells were harvested at the indicated time point and washed twice with cold PBS and fixed by the addition of ice cold 70% ethanol. After vortexing gently, the cells were incubated at -20°C for 1 h. The fixed cells were centrifuged at 3000 rpm for 5 min and washed twice with PBS to remove residual ethanol. The obtained palettes were resuspended in staining solution (50µg/mL PI and 100µg/mL RNase A in PBS). The samples were incubated for 30 min at 37°C in the dark to allow RNA degradation and DNA staining. Prior to acquisition of data, cell suspensions were vortexed to remove aggregates and flow cytometric analysis was performed using flow cytometer (BD FACS Calibur) equipped with 488nm excitation laser. PI fluorescence signal was collected by using appropriate red fluorescence channel (FL2-H). A minimum of 10,000 single-cell events per sample were acquired.

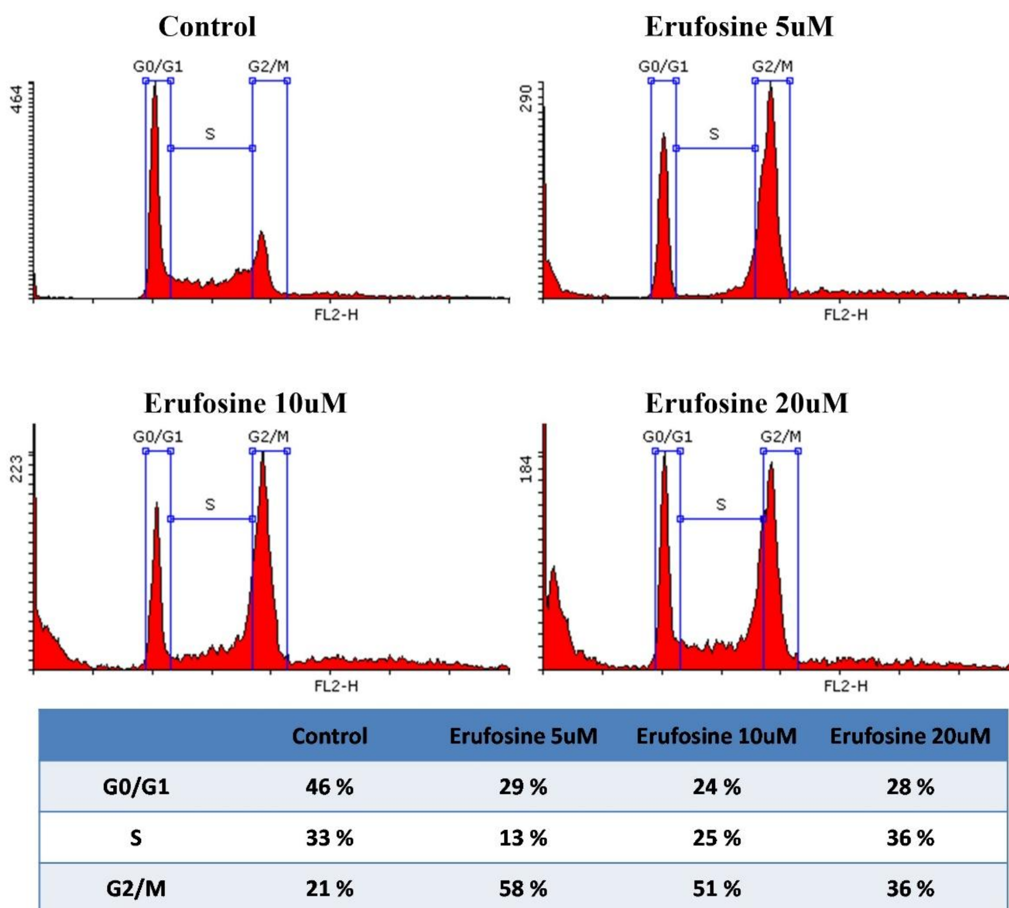
Data Analysis

Cell cycle distribution (G0/G1, S, and G2/M phases) was analyzed using cell cycle modeling software. For this purpose, data sets were uploaded to Flowing Software 2.5.1 and the percentages of cells in the three stages of cell cycle were identified. The values were compared with the data obtained from control untreated cells processed in parallel.

RESULTS

Erufosine Induced Cell Cycle Arrest

PI staining followed by flow cytometric analysis was performed to determine the effect of erufosine on cell cycle progression in pancreatic cells. DNA content analysis revealed a marked alteration in cell cycle distribution upon erufosine treatment compared with untreated controls. The data obtained and analyzed showed the potential of erufosine to induce cell cycle arrest in the pancreatic cells as shown in Figure 1. Erufosine exposure resulted in accumulation of cells in the G2/M phase, accompanied by a concomitant reduction in G0/G1 population, indicating induction of G2/M cell cycle arrest. In addition, a moderate decrease in the S-phase fraction was also observed, suggesting impaired DNA synthesis and progression through cell cycle. The effect was not concentration-dependent, as with lower doses exposure producing a more pronounced shift in cell cycle distribution. Furthermore, an increase in the sub-G1 population was detected in treated samples, consistent with the induction of apoptotic cell death induced by erufosine. This increase in sub-G1 population was concentration dependent which showed marked ability of erufosine to initiate the apoptotic machinery in the pancreatic cancer cells as well. Collectively, these findings demonstrate that erufosine effectively disrupts cell cycle progression primarily by inducing G2/M phase arrest and promoting apoptotic cell death.



	Control	Erufosine 5uM	Erufosine 10uM	Erufosine 20uM
G0/G1	46 %	29 %	24 %	28 %
S	33 %	13 %	25 %	36 %
G2/M	21 %	58 %	51 %	36 %

Figure 1: Erufosine induces G2/M cell cycle arrest and apoptosis in pancreatic cells. Cell cycle distribution was analyzed by propidium iodide (PI) staining followed by flow cytometry after treatment with erufosine for indicated concentrations. Representative DNA content histograms are shown and summarize the distribution of cells in G0/G1, S, G2/M, and sub-G1 phases. Erufosine treatment resulted in a significant accumulation of cells in the G2/M phase, accompanied by a reduction in the G0/G1 and S-phase populations, indicating induction of G2/M cell cycle arrest. Additionally, an increase in the sub-G1 fraction was observed, consistent with apoptotic cell death. The data was also shown as percentages while comparing the values with untreated control cells.

DISCUSSION

Erufosine demonstrated significant antiproliferative and cell cycle arrest effects across various cancer cell types. Multiple studies report that erufosine exposure leads to accumulation of cancer cells in the G2/M phase, reflecting disruption of cell cycle progression and activation of checkpoint controls that hinder mitotic entry, thereby suppressing tumor cell proliferation. In oral squamous carcinoma cells, for example, erufosine significantly increased the proportion of cells in G2/M while reducing cyclin D1 expression, indicating interruption of cell cycle regulatory machinery alongside induction of apoptosis and autophagy through modulation of the Akt-mTOR signaling pathway [13].

Similar cytostatic effects have been observed in other cancer models, where erufosine not only inhibited proliferation but also downregulated key cyclins and cyclin-dependent kinases, reinforcing its role as a cell cycle inhibitor. Additionally, transcriptomic and gene expression analyses show that erufosine alters the expression of multiple cell cycle-related genes, further supporting its capacity to arrest cancer cells in specific phases of the cycle and impair progression [24, 25].

The present study demonstrates that erufosine markedly disrupts cell cycle progression in pancreatic cells, primarily by inducing G2/M phase arrest and promoting apoptotic cell death. Flow cytometric analysis following PI staining revealed a significant accumulation of cells in the G2/M phase, accompanied by a concomitant reduction in the G0/G1 population and a moderate decline in S-phase cells. These findings indicate that erufosine interferes with normal cell cycle progression, preventing cells from successfully completing mitosis and thereby suppressing cellular proliferation in tested pancreatic cancer cells.

The observed G2/M arrest suggests that erufosine may activate cell cycle checkpoint mechanisms that monitor DNA integrity and mitotic spindle formation. The G2/M transition is tightly regulated by cyclin-dependent kinases, particularly the cyclin B1–CDK1 complex and is highly sensitive to cellular stress and DNA damage signals [26]. Arrest at this checkpoint often reflects activation of regulatory pathways that halt cell cycle progression to allow DNA repair or alternatively, to direct cells toward apoptosis if the damage is irreparable. The concomitant reduction in S-phase cells further supports the notion that erufosine impairs DNA synthesis and progression through replication, thereby limiting proliferative capacity.

Interestingly, the induction of G2/M arrest was not concentration-dependent, as lower doses of erufosine produced a more pronounced shift in cell cycle distribution. This finding suggests that erufosine may exert maximal checkpoint activation at submaximal concentrations, potentially through early signaling events that saturate at relatively low doses. Alternatively, higher concentrations may accelerate apoptotic progression, thereby reducing the detectable fraction of cells accumulated at G2/M. This interpretation is supported by the concentration-dependent increase observed in the sub-G1 population, which is indicative of DNA fragmentation and apoptotic cell death.

The marked elevation in the sub-G1 fraction confirms the pro-apoptotic activity of erufosine in pancreatic cancer cells. The concentration-dependent nature of this increase highlight ability of erufosine to activate apoptotic machinery in a dose-responsive manner. Together, the dual effects of G2/M arrest and apoptosis suggest that erufosine not only halts cell proliferation but also actively eliminates malignant cells. This is therapeutically advantageous as cell cycle arrest alone may be reversible, whereas apoptosis ensures irreversible loss of tumor cells.

Collectively, these findings support the potential of erufosine as an effective anti-proliferative agent in pancreatic cancer. By targeting critical regulatory checkpoints and inducing apoptosis, erufosine disrupts the survival and expansion of pancreatic tumor cells. Further mechanistic studies investigating the involvement of checkpoint regulators, cyclins, CDKs, and apoptotic signaling pathways will be essential to fully elucidate the molecular basis of its anticancer activity. Additionally, expanded *in vitro* and *in vivo* validation and combination studies with standard chemotherapeutic agents may help determine its translational potential in pancreatic cancer therapy.

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Competing Interests: There are no competing interests among authors.

Data Availability Statement: The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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