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Research Article

Calcium channel blocker (Fendiline) induces cell cycle arrest in pancreatic cancer cells

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Abstract
Background: Pancreatic ductal adenocarcinoma is one of the most lethal malignancies worldwide, characterized by late diagnosis, aggressive progression, and resistance to conventional therapies. The urgent need for novel therapeutic strategies has prompted investigation into drug repurposing approach. Fendiline, a non-selective calcium channel blocker with reported anti-proliferative properties, has emerged as a potential candidate. This study aimed to evaluate its impact on cell cycle progression using flow cytometry.
Methods: Human pancreatic cancer cell line (BxPC-3) was treated with increasing concentrations of fendiline (5, 10, 15μM) for the defined time interval (48h). Cell cycle distribution was analyzed by propidium iodide staining followed by flow cytometric quantification of DNA content. Percentage of cells in different phases of the cell cycle were resented in comparison to untreated control.
Results: Compared with untreated control cells, fendiline treatment exhibited a marked increase in the G0/G1 population. At the same time, a concomitant reduction in the S phase fraction in response to exposure with drug was noticed. Quantitative analysis demonstrated a dose-dependent effect, with lower concentrations of fendiline producing a moderate increase in G0/G1 phase cells (49.2%), while higher concentrations resulted in pronounced G1 accumulation (73.2%) as compared to control cell fraction (43.2%).
Conclusion: These findings demonstrate that fendiline exerts anti-proliferative effects in pancreatic cancer cells by disrupting cell cycle progression. Study supports further investigation of fendiline as a repurposed therapeutic cytostatic agent for pancreatic cancer and provides mechanistic insight into its anti-tumor activity.
Key Words: Pancreatic cancer, Fendiline, Cell cycle arrest

INTRODUCTION

Pancreatic cancer is the most lethal malignancies worldwide and is characterized by aggressive tumor biology, late clinical presentation along with poor prognosis. Among its subtypes, pancreatic ductal adenocarcinoma constitutes with most cases and is responsible for the highest mortality rate relative to incidence of other major solid tumors [1]. Despite advances in oncology, the 5-year survival rate remains below 10%, reflecting the persistent challenges in early detection and effective therapeutic intervention [2]. Early-stage disease is typically asymptomatic; diagnosis frequently occurs at advanced stages when curative treatment is no longer feasible [3].

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Surgical resection remains the only potentially curative option for pancreatic cancer; however, fewer than 20% of patients present with resectable disease at diagnosis. For unresectable or metastatic disease, systemic therapies provide palliative benefit, but outcomes remain dismal. Significant challenges persist in pancreatic cancer management, including lack of early detection methods, intrinsic resistance to chemotherapy and immunosuppressive tumor microenvironment [4, 5]. Emerging avenues of research focus on molecular profiling for precision medicine, novel biomarkers for early diagnosis and novel therapeutic entities [6].
Fendiline is a non-selective calcium channel blocker originally developed as an antianginal agent [7]. Over time, it has gained attention in oncology domain because of its effects on membrane lipid organization and oncogenic signaling. Beyond modulating Ca^{2+} influx, fendiline alters plasma membrane phosphatidylserine which is required for proper localization and function of KRAS [8]. In KRAS-driven

cancers, including pancreatic ductal adenocarcinoma, fendiline has been shown to reduce cell proliferation and suppress tumor growth in xenograft models [9, 10]. Mechanistically, the effects are associated with decreased membrane microdomain stability and induction of apoptotic pathways, including caspase activation and mitochondrial dysfunction. Additionally, fendiline has been reported to induce cytostatic and cytotoxic effects in various cancer cell lines through disruption of calcium homeostasis and interference with survival signaling pathways [11, 12]. Although these findings highlight fendiline as promising repurposing candidate targeting membrane-dependent oncogenic signaling, its anticancer activity remains confined to preclinical studies, and no clinical trials have yet established its efficacy or safety in cancer patients [13].

Fendiline has demonstrated promising preclinical anticancer activity against pancreatic ductal adenocarcinoma. The effects largely occur through disruption of oncogenic KRAS signaling [14]. Because more than 90% of pancreatic ductal adenocarcinoma tumors harbor activating KRAS mutations, fendiline is a considerable candidate for targeting the tumor cells. At the same time, membrane localization of KRAS is essential for maintaining downstream signaling pathways (MAPK/ERK and PI3K/AKT) that drive tumor growth and survival [15, 16]. Fendiline has been shown to interfere with the localization of KRAS from the inner leaflet of the plasma membrane and attenuating the signaling capacity. In pancreatic cancer cell lines, this disruption results in decreased proliferation and impaired colony formation. Additionally, fendiline alters sphingolipid metabolism and membrane microdomain organization, further compromising oncogenic signal transduction [17]. In xenograft mouse models of KRAS-mutant pancreatic cancer, fendiline treatment suppressed tumor growth without systemic toxicity, supporting its potential as a repurposed therapeutic agent [18]. Mechanistically, fendiline has also been associated with induction of apoptosis, mitochondrial dysfunction, and perturbation of calcium homeostasis [19]. All these factors contribute to reduced tumor cell viability. Although these findings position fendiline as a promising membrane-targeting strategy for KRAS-driven pancreatic cancer, its effects on functional properties of pancreatic cancer cells including cell cycle need to be explored.

METHODS

Pancreatic Cancer Cell Line Cultures

The human pancreatic adenocarcinoma cell line BxPC-3 (ATCC® CRL-1687™) was obtained from the American Type Culture Collection (ATCC, USA) and cultured according to the supplier's recommendations. RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100µg/mL streptomycin, 100 U/mL penicillin along with 2mM L glutamine were used for this purpose. Cells were grown in humidified incubator at 37°C supported with 5% CO_2 and were routinely monitored for confluency via microscopic observations. The medium was replaced every 2–3 days, and cells were passaged at approximately 60–80% confluence using 0.25% trypsin–EDTA solution. During subculturing, the cells were washed with sterile phosphate-buffered saline (PBS), detached from the culture flasks with trypsin–EDTA at 37°C, neutralized with complete medium, and centrifuged at 1,500rpm for 5min. The resulting cell pellet was resuspended in the fresh complete medium and seeded at an appropriate density as per experimental requirements. For all experiments, cells were used between passages 3 and 20 to ensure phenotypic consistency.

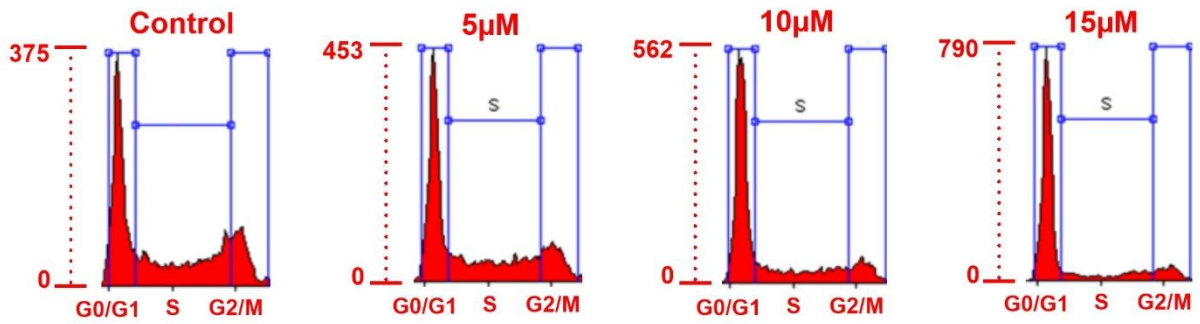
Flow Cytometry Analysis for Cell Cycle

Cell cycle distribution was analyzed by flow cytometry using propidium iodide (PI) staining of the DNA content. Briefly, the cells were seeded in appropriate culture plates (6 well) and treated with fendiline according to the experimental design (5, 10, 15µM, 48h). Following the treatment interval, both adherent and floating cells were collected to ensure accurate representation of all cell populations. Cells were washed with PBS and fixed in 70% ice-cold ethanol added dropwise while vortexing to prevent cell clumping. Fixation was carried out at 4°C for 2h. After fixation, the cells were centrifuged at 1,500rpm for 5min, washed twice with PBS to remove residual ethanol, and resuspended in staining solution containing 50µg/mL propidium iodide along with 100µg/mL RNase A, and 0.1% Triton X-100 in PBS. Samples were incubated at 37°C for 30min in the dark to allow for complete RNA degradation and DNA staining. Flow cytometric analysis was performed using a flow cytometer (BD FACS Caliber) equipped with a 488nm laser, and at least 10,000 events per sample were acquired. Cell cycle distribution in G0/G1, S, and G2/M phases was determined based on the DNA content histograms using cell cycle analysis software (FlowJo). The percentage of cells in each phase was calculated and compared with the untreated controls.

RESULTS

Fendiline Induced Cell Cycle Arrest in G1 Phase of Cell Cycle

Flow cytometric analysis of DNA content revealed that fendiline treatment induced a significant accumulation of pancreatic cancer cells in the G0/G1 phase of the cell cycle, indicating G1 phase arrest. Compared with untreated control cells, which displayed a typical distribution across G0/G1 (43.2%), S (37.2%), and G2/M (19.6%) phases, fendiline-treated cells exhibited a marked increase in the G0/G1 population accompanied by a concomitant reduction in the S phase fraction in response to exposure with drug. Quantitative analysis demonstrated a dose-dependent effect, with lower concentrations of fendiline producing a moderate increase in G0/G1 phase cells (49.2%), while higher concentrations resulted in pronounced G1 accumulation (73.2%). The percentage of cells in G2/M phase was either modestly reduced or unchanged, suggesting that fendiline primarily interferes with the G1 to S phase transition rather than mitotic progression. These findings indicate that fendiline suppresses pancreatic cancer cell proliferation by blocking cell cycle progression at the G1 checkpoint, thereby limiting entry into S phase and subsequent DNA replication. The observed G1 arrest supports the proposed antiproliferative mechanism of fendiline and suggests inhibitory effects on oncogenic signaling pathways that regulate cyclin-dependent kinase activity and G1/S transition.



	Control	5μM	10μM	15μM
G0/G1	43.2	49.2	62.1	73.2
S	37.2	34.4	25.3	16.4
G2/M	19.6	16.4	12.6	10.4

Figure 1. Pancreatic cancer cells were exposed to three different concentrations of fendiline and resulting effects on cell cycle were identified by DNA staining and flow cytometry. The cells in different phases of the cell cycle are shown as histograms and numerically as percentages.

DISCUSSION

The present findings demonstrate that fendiline markedly suppresses pancreatic cancer cell proliferation by inducing G0/G1 cell cycle arrest. Flow cytometric analysis revealed a significant accumulation of cells in the G1 phase with a reduction in the S phase population, indicating impaired G1-to-S phase transition. These results suggest that fendiline interferes with regulatory checkpoints governing DNA synthesis and cell cycle progression. The observed decrease in S phase fraction reflects reduced replicative capacity, consistent with the antiproliferative effects reported for membrane-targeting agents in KRAS-driven malignancies.

Mechanistically, G1 arrest is commonly associated with modulation of cyclin-dependent kinase (CDK) activity, particularly CDK4/6–cyclin D and CDK2–cyclin E complexes, which are essential for retinoblastoma (Rb) phosphorylation and progression into S phase [20]. Although specific cell cycle regulators were not directly examined in this study, the marked G1 accumulation suggests that fendiline may downregulate cyclin D1 and/or cyclin E expression, inhibit CDK activation, or enhance CDK inhibitor proteins such as p21 and p27. Given that pancreatic ductal adenocarcinoma frequently exhibits dysregulated KRAS signaling and that KRAS activity

promotes cyclin D1 expression through MAPK/ERK pathways, the G1 arrest observed here may result from fendiline-mediated disruption of oncogenic signaling cascades.

As we know, fendiline is known to alter plasma membrane phosphatidylserine distribution, thereby mislocalizing KRAS and attenuating downstream signaling. Since sustained ERK and PI3K/AKT pathway activation drives cell cycle progression and suppresses checkpoint control, inhibition of these pathways provides a plausible explanation for the reduced S phase entry observed in treated cells. Furthermore, calcium signaling plays a regulatory role in cell cycle progression; thus, fendiline's modulation of intracellular calcium levels may additionally contribute to checkpoint activation and proliferative inhibition.

Collectively, these findings indicate that fendiline exerts significant antiproliferative effects in pancreatic cancer cells by enforcing G1 phase arrest, thereby limiting DNA replication and tumor cell expansion. Further investigation into molecular regulators of the G1/S checkpoint, including cyclins, CDKs, CDK inhibitors, and Rb phosphorylation status, will be essential to delineate the precise mechanisms underlying fendiline-mediated cell cycle control and to evaluate its translational potential as a repurposed therapeutic strategy for pancreatic cancer.

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