Response of Pancreatic Cancer Cell Lines to the Polyamine Analog, PG-11047

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ABSTRACT

Pancreatic ductal adenocarcinoma (PDA) is a deadly malignancy (1). While the etiology of this specific cancer type is not well known, it has been suspected that polyamine dysregulation may play a critical role in the proliferation of cancer cells (2). Polyamines are organic compounds that promote normal cell growth and survival, yet the dysfunction of their inherent regulatory controls has been identified as a recurrent component of several cancers (2). Here, we examine the effects of PG-11047, a drug targeted to interfere with polyamine regulation, on several PDA cell lines. Following exposure to PG-11047, 72-hour cancer cell growth inhibition was determined to produce a drug dose response curve. The PDA cell lines showed a variable range of response to PG-11047, with certain cell lines being sensitive to the drug and others being resistant. Genome-wide mRNA expression profiles of the cancer cell line were supervised with drug sensitivity data to discover molecular correlates of drug response. These variable responses indicate that certain cancer subtypes may proliferate due to polyamine dysregulation while the resistant cancer subtypes do not. These results have importance for the personalization of cancer therapy in PDA.

INTRODUCTION

Polyamines are amino acid-derived organic cations that are critical for eukaryotic cell growth (2, 3). Polyamines are synthesized in all tissues of the body, and they can also enter the cells via external food sources such as red meat and cheese (2). Some of the more specific roles that polyamines are involved with in relation to cell growth include interactions with nucleic acids, maintaining the proper structure of chromatin, regulating gene expression, controlling ion channel function, sustaining membrane stability, and scavenging free radicals (3). Polyamines have been implicated in various cancers, likely due to their essential role in eukaryotic cell growth and the resultant hyperproliferation that can occur when polyamine metabolism is dysregulated (4). Due to the important role polyamines can play in promoting cellular proliferation, several studies have focused on inhibiting polyamine biosynthesis as a method of cancer therapy and prevention (5).

The majority of polyamine-inhibiting studies have focused on the first enzyme of polyamine biosynthesis, ornithine decarboxylase, as a target for epithelial cancers of the skin, lung, prostate, and colon (2). Difluoromethylornithine has been tested in a variety of animal models and clinical trials as an inhibitor of ornithine decarboxylase and of polyamine metabolism (2). While difluoromethylornithine has shown some success in treating trypanosomiasis and brain tumors, other inhibitors of polyamine biosynthesis enzymes have been less effective in chemotherapeutic clinical trials (6). This study focuses on an alternative approach in which a polyamine analog is utilized to interfere with the over-activation of polyamine syn-

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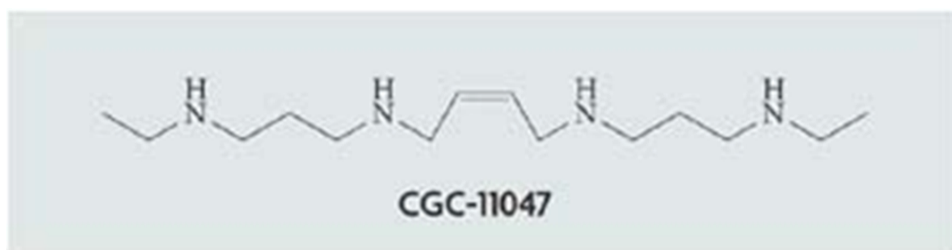

Figure 1. Chemical structure of the polyamine analog, PG-11047 (previously known as CGC-11047). This structure shows the cis double bond of the analog, which results in a conformational restriction that allows for the increased activity of the compound against cellular proliferation.
Reference: http://www.nature.com/nrd/journal/v6/n5/thumbs/nrd2243-f6.jpg

thesis that occurs in many cancer types. By acting as an analog, this less-widely explored approach takes advantage of the self-regulating activities of polyamines in order to interfere with their promotion of cell growth. Polyamine analogs have also prevented angiogenesis in mouse models, which further supports their use as an anti-proliferative cancer therapy (3).

The specific drug used in this study, PG-11047, is a polyamine analog of N1,N12-bisethylspermine (BESpm)(6). PG-11047 is similar to BESpm, but it has a cis double bond between the two central carbon atoms that introduces a conformational restriction (see Figure 1) (6). The double bond causes the polyamine analog to have increased activity against proliferation with only minimal levels of toxicity (3). PG-11047 has been in a phase I clinical trial as an independent compound as well as in phase Ib combination trials with bevacizumab, erlotinib, docetaxel, or gemcitabine. Additionally, PG-11047 has shown promising results in phase II trials for metastatic prostate cancer as well as in a phase I human clinical trial for lymphoma (3, 6). Based on the success of PG-11047 against the aforementioned cancer types and its ability to inhibit polyamine biosynthesis and cell proliferation, this drug could be a successful intervention against other types of cancer as well.

Pancreatic ductal adenocarcinoma (PDA) is a deadly cancer and is responsible for over 85% of pancreatic cancer cases (1). PDA is also the fourth leading cause of all deaths due to cancer in the United States, with a 5-year survival rate of less than 5% of those diagnosed (1). Several risk factors have been linked to PDA, including smoking, advanced age, diabetes, obesity, and chronic pancreatitis (7). Having a positive family history has been shown to be a predisposition in approximately 10% of patients with PDA (1). Due to the paucity of effective agents in PDA, this cancer type is a good candidate for treatment with a polyamine analog. In this paper, the effects of PG-11047 on the growth of pancreatic cancer cell lines will be examined. Sensitivity and resistance of several cell lines to this drug will be reported based on the dose required to inhibit 50% of cell growth, the GI_{50}.

MATERIALS AND METHODS

Twenty-two pancreatic cancer cell lines were investigated in this study. All cells were cultured in DMEM medium with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Each cell line was plated in a 96-well plate at a density of about 2500 cells/100 μL/well. Before treatment with a polyamine analog, the cells were given time to attach to the wells overnight (approximately 24 hours) while they were incubated at 37°C in 5% carbon dioxide. The drug compound, PG-11047, was provided by Progen Pharmceuticals (Redwood City, CA). PG-11047 was diluted with sterilized water to create a 100 mM stock solution. Then, the cells were treated with a set of nine different doses, each with a final concentration ranging from 3.3E-4 M to 8.5E-10 M. These doses were applied as a 1:5 serial dilution added in triplicate wells using BIO-TEK Precision Power, a robotic program of randomized drug application. The drug treatment program also included a random distribution of control wells (only DMEM was added) and blank wells (no media added). Following drug treatment, the cells were incubated at 37°C in 5% carbon dioxide for 72 hours. A Cell Titer-Glo (CTG) Luminescent Cell Viability Assay was used to quantify the number of cells present in each well at both day 0 (time of treatment) and day 3 (after 72 hours of drug exposure). The CTG reagent was diluted with PBS in a 1:1 dilution. After aspirating the media from the

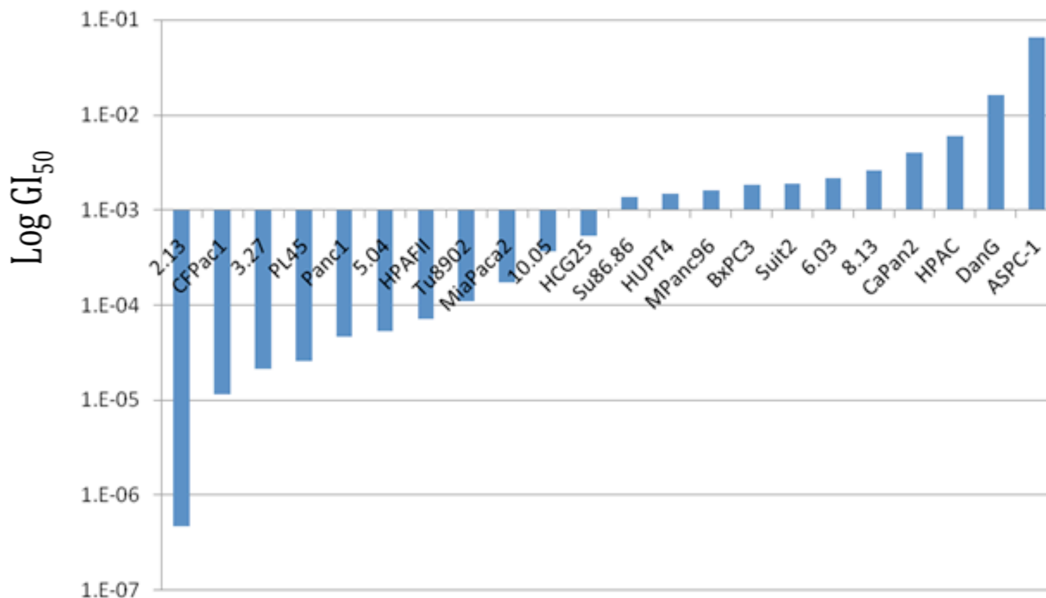

Figure 2. Chemosensitivity profile of PDA cell lines to PG-11047. The cell lines are arranged from sensitive (left) to resistant (right) based on their GI50 values (drug dose required for 50% inhibition of cell growth, compared to vehicle control.)

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96-well plate, 50 μL of the CTG mixture was added to each well. The plates sat on a shaker for 15 minutes to allow mixing of the cells with the CTG reagent. Viable cell counts were determined using a BIO-TEK FLx800 luminometer, and the data output was collected using a KC Junior computer program. The responsiveness of the cell lines to PG-11047 was established by calculating the GI_{50} from the following equation: $100 \times [(T-T_0) / (C-T_0)] = 50$, in which T is the number of cells after treatment (day 3), T_0 is the number of cells at day 0, and C is the number of untreated cells at day 3 (8). This equation gives the dose of PG-11047 that inhibits 50% of cell growth for a given cell line. By comparing all the cell lines, levels of sensitivity and resistance were analyzed according to these GI_{50} values. Genomic targets affected by the polyamine analog were determined using Ingenuity Pathway Analysis (IPA 5.0, www.ingenuity.com). This system of analysis is a database search that compares gene pathways that are known to be involved in certain cancers with target genes that are predicted to be affected based on shared mRNA expression profiles (9). Gene arrays were used to measure the expression of mRNA from exponentially growing PDA cell lines as described by Aguirre et al. (10). CEL files from the gene arrays were normalized using RMA. Normalized data was supervised with GI_{50} data using the adaptive spline technique (Das, D., unpublished methods). This technique resulted in predictors of sensitivity or resistance.

RESULTS

The twenty-two pancreatic cancer cell lines tested showed a variable range of response to PG-11047 after being treated with the polyamine analog. Each cell line was exposed to drug doses ranging from 0.85 nM to 0.33 mM for a period of 72 hours. These cell lines showed different levels of sensitivity and resistance to PG-11047 as reflected in Figure 2, spanning 4 logs of activity in which the most sensitive cell lines are at the left of the figure and the most resistant are toward the right end. The drug dose responding in 50% cell growth inhibition, the GI_{50} dose, ranged from 0.4 μM to 66 mM (Table 1). Ingenuity Pathway Analysis of mRNA targets in the pancreatic cancer lines identified eight genes as important predictors of response to PG-11047. Increased expression levels of the FLNB and IFNGR2 genes predict resistance to PG-11047, while the other six genes predict sensitivity to PG-11047 (Table 2). The biological functions related to this gene set include protein ubiquitination, endocytosis, interferon signaling, and antigen presentation pathways. Additionally, these genes showed similar responses to PG-11047 as a panel of breast cancer cell lines treated with PG-11047 (Kuo, *et al.*, in preparation).

DISCUSSION

Polyamines play a critical function in normal cell

Cell Line	GI_{50} (M)
2.13	4.71E-07
CFPac1	1.17E-05
3.27	2.12E-05
PL45	2.60E-05
Panc1	4.69E-05
5.04	5.35E-05
HPAFII	7.13E-05
Tu8902	1.09E-04
MiaPaca2	1.73E-04
10.05	3.65E-04
HCG25	5.37E-04
Su86.86	1.38E-03
HUPT4	1.49E-03
MPanc96	1.59E-03
BxPC3	1.83E-03
Suit2	1.91E-03
6.03	2.17E-03
8.13	2.63E-03
CaPan2	4.03E-03
HPAC	5.99E-03
DanG	1.63E-02
ASPC-1	6.60E-02

Table 1. 50% Growth Inhibition Values for PDA Cell Lines.

Ingenuity Canonical Pathways	p-value	mRNA predictors in the pathway
Protein Ubiquitination Pathway	0.0013	MED20, TAP1, PSMD3, HLA-B, USP9X
Caveolar-mediated Endocytosis	0.0052	**FLNB**, COPB1, HLA-B
Interferon Signaling	0.0076	TAP1, **IFNGR2**
Antigen Presentation Pathway	0.0095	TAP1, HLA-B

Table 2. Pathway Enrichment for CGC-11047 Predictors in PDA Cell Lines. Note: Genes in normal font and bold font are predictors of sensitivity and resistance, respectively

growth and maintenance, but are frequently found to be overactive in many cancer types (2). An inappropriate increase in polyamine activity with resultant hyperproliferation of cells leads to cancer, such as in pancreatic ductal adenocarcinoma (PDA). Polyamine analogs that prevent such uncontrolled growth have been synthesized as a method of cancer therapy (4). PG-11047 (previously named SL-11047 and CGC-11047) is a second generation polyamine analog of N1,N12-bisethylspermine that acts to inhibit cell proliferation (3,6). In prior studies, this compound was reported to be effective against breast and lung cancer cell lines (11, 12). However, much less research has been done to examine the effects of PG-11047 on pancreatic cancer cells. The purpose of this study was to examine the potential benefits of this drug as a therapeutic agent for PDA.

The twenty-two PDA cell lines tested showed a variable range of response to PG-11047, with the GI_{50} values measured to be between 0.4 μM and 66 mM. Correlating the GI_{50} doses with mRNA expression profiles produced a set of eight genes that predicted either sensitivity or resistance to PG-11047. The cell lines that were sensitive to PG-11047 treatment showed a generally high level of expression of MED20, TAP1, PSMD3, HLA-B, USP9X, and COP-B1 genes and a low level of expression of FLNB and IFNGR2. Studies of FLNB and IFNGR2 have shown that both of these genes function in apoptotic signaling pathways (13, 14), which explains why a low level of expression of these genes would allow for resistance to PG-11047. With apoptotic genes expressed at low levels, continued proliferation of pancreatic cancer cells reflects a resistance to PG-11047. An Ingenuity Pathway Analysis (IPA) of the eight predictor genes was utilized to find molecular markers associated with drug response. This pathway search indicated links between MED20, TAP1, PSMD3, HLA-B, and USP9X for protein ubiquitination. The majority of polyamine biosynthetic enzymes are degraded through an ubiquitination pathway (15). PG-11047 may therefore function by enhancing polyamine enzyme ubiquitination followed by degradation, which would inhibit cell proliferation. FLNB, COPB1, and HLA-B exhibited an association through caveolar-mediated endocytosis. This type of endocytosis has been reported to mediate the uptake of polyamines in colon cancer cells (16). Based on this information, cell lines that were sensitive to the polyamine analog, PG-11047, are likely to have responded to the drug based on their higher levels of expression of genes associated with caveolar-mediated endocytosis. Finally, TAP1 was additionally linked to IFNGR2 through interferon signaling and to HLA-B through a pathway of antigen presentation. The loss of expression of both HLA (human leukocyte antigen B) and TAP (the transporter for antigen presentation) has been implicated in pancreatic cancer (17). Furthermore, dysregulation of TAP has also been reported to result in evasion of immune system controls leading to tumor progression in cervical, colorectal, non-small cell lung, breast, and renal cancers (17). Recent research by Kuo, W.L., et al., discovered TAP1 as an mRNA predictor of response of breast cancer cell lines to treatment by PG-11047 (unpublished results). This similarity of action

of PG-11047 against both PDA and breast cancer cell lines suggests that this polyamine analog may be effective against several cancer types depending on their expression of TAP1 and other related targets. Due to the variation in the expression profiles of the PDA cell lines and their wide range of sensitivity to PG-11047, it seems that the treatment of PDA by this polyamine analog would be most effective if personalized according to gene expression profiling. Hence, PDA cell lines that express sensitive mRNA predictors would be most suited for treatment with PG-11047 as a cancer therapy. This finding is significant in that it suggests the need for a more personalized form of cancer therapy that requires gene expression profiling prior to treatment.

CONCLUSION

The treatment of twenty-two PDA cell lines with a polyamine analog, PG-11047, resulted in a variable range of response. Chemosensitivity profiling based on GI_{50} doses revealed that some cell lines were very sensitive to the drug compound while others were resistant. Ingenuity Pathway Analysis produced a set of eight genes as mRNA predictors of sensitivity and resistance to PG-11047. Comparison of this study to another study of breast cancer cell line treatment with PG-11047 revealed a common gene, TAP1, which was targeted by the drug in both cancer types. The identical probe being identified amongst over 20,000 probe sets on the Hu133A array strongly suggests that this gene is central in the response or resistance to polyamine analogs. This protein is a member of the MDR/TAP subfamily (18). Members of the MDR/TAP subfamily are involved in multidrug resistance, yet interestingly this gene confers sensitivity to PG-11047. The protein encoded by this gene is involved in the pumping of degraded cytosolic peptides across the endoplasmic reticulum into the membrane-bound compartment where MHC class I molecules assemble (19). Mutations in this gene may be associated with ankylosing spondylitis, insulin-dependent diabetes mellitus, and celiac disease (20). These studies suggest that the link between TAP1 and polyamine metabolism warrants further investigation. Finally, it has been demonstrated that a preclinical system using PDA cell lines offers a mechanism to predict sensitivity or resistance to a given compound. These findings have direct relevance to clinical trial design in PDA.

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