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## Authors
Yu, Juehua
Liu, Shi-he
Sanchez, Robbi
et al.

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# Pancreatic cancer actionable genes and Precision Medicine and Personalized Surgery

**Juehua Yu**[a], **Shi-he Liu**[a], **Robbi Sanchez**[a], **John Nemunaitis**[b], **Enrique Rozengurt**[c], and **F. Charles Brunicardi**[a]

[a] Division of General Surgery, Department of Surgery, David Geffen School of Medicine at University of California Los Angeles, CA, USA

[b] Mary Crowley Cancer Research Center, Dallas, TX, USA

[c] Department of Medicine, David Geffen School of Medicine at University of California Los Angeles, CA, USA

## Abstract

Pancreatic ductal adenocarcinoma (PDAC) is a deadly cancer with an overall 5-year survival rate less than 5% due to the poor early diagnosis and lack of effective therapeutic options. The most effective therapy remains surgery, however post-operative survival could be enhanced with effective adjuvant therapy. The massive information gained from Omics techniques on PDAC at the beginning of the 21st century is a remarkable accomplishment. However, the information gained from the omics data, including next generation sequencing data, has yet to successfully affect care of patients suffering with PDAC. Therefore, we propose the development of an actionable genomic platform that matches a patient's PDAC clinically actionable genes with potential targeted adjuvant therapies. Using this platform, PDX1 has been identified PDX1 as a potential actionable gene for PDAC, therefore, RNAi therapy, gene therapy and small inhibitory drugs, all targeting PDX1, serve as potential targeted adjuvant therapies. Preclinical studies support the hypothesis that identification of PDAC actionable genes could permit translation of a patient's genomic information into precision targeted adjuvant therapy for PDAC.

## Introduction

Pancreatic ductal adenocarcinoma (PDAC) is an extremely aggressive and deadly cancer that ranks fourth among cancer-related deaths in the United States [1]. The overall 5-year survival rate of patients with PDAC is less than 5%. Only less than 20% of patients diagnosed with PDAC are eligible for potentially curative resection, however the 5-year survival for patients with resectable PDAC is only 25% [2-6]. Therefore, while the most effective therapy remains surgery, post-operative survival could be significantly enhanced with effective adjuvant therapy. It is believed that PDAC arises from changes in the DNA sequence of oncogenes and/or tumor suppressor genes in the genomes of a subset of adult pancreatic cells [2, 7-10]. The somatic oncogenic mutations accumulate and then disrupt normal functions of multiple central signaling pathways, including Ras, PI3K, Wnt, Notch, Hedgehog and others, which play multiple important roles in regulating cell growth, cell proliferation, cell apoptosis, cell survival, as well as cell migration and metastasis [11-15]. All of these genetic alterations can now be identified using the advanced techniques for genomics including next-generation

DNA/RNA sequencing and other proteomics tools, however none of them are actionable, ie., their identification does not affect choice, nor effectiveness, of care. To date, a list of gene mutations and PDAC biomarkers, including serologic patterns, aberrant overexpressed mRNAs, miRNAs and proteins, as well as epigenetic signatures including DNA methylation and histone modification profiles, have already been identified and associated with PDAC. In addition, a series of circulating tumor cell (CTC) and cell-free circulating tumor DNA (ctDNA) were discovered using state-of-the-art imaging techniques and high-throughput next-generation sequencing approaches using liquid biopsy from cancer patients [16-20]. These could be potentially used as future early diagnostic and therapeutic tools. However, the information gained from genomic sequencing data has yet to successfully affect care of patients suffering with PDAC. It remains undetermined how to translate genomic sequencing techniques and genomic information into targeted therapies and prophylactic surgery (like that of mastectomy for BRCA mutations or thyroidectomy for RET proto-oncogene mutations) for PDAC [21, 22]. Current adjuvant therapies for PDAC include Gemcitabine, Erlotinib, Capecitabine, FOLFORINOX (a combination of 5-fluorouracil, irinotecan, and oxaliplatin, plus the adjuvant folinic acid), and Gemcitabine + nab-Paclitaxel, which confer a survival advantage of only weeks to six months [23]. The hope the next generation sequencing would lead to more effective targeted adjuvant has not been realized and there remains an enormous gap between genomic data and their translation to clinical care for patients with this deadly malignancy. Thus, we propose the development of an actionable genomic platform in which identification of a patient's PDAC actionable genes can be matched to targeted therapies, and preclinical studies support the hypothesis of a precision medicine strategy for PDAC.

## Potential Actionable Genes for PDAC

The definition of an "actionable gene" is quite variable and includes the use of biomarkers for imaging and early detection, surgery for prophylactic removal of tissues at risk for cancer, as well as those that guideg choice of targeted therapy [24, 25]. Dependent on the choice of actions taken, potential actionable genes for PDAC can be primarily categorized into 3 types: (1) oncogenes carrying gain-of-function mutations, (2) tumor suppressor genes carrying loss-of-function mutations, and (3) genes that are aberrantly overexpressed in PDAC compared to adjacent non-tumor pancreas tissue, including genes that are regulating global transcription network of pancreas development.

One of the most important type I genes for PDAC is KRAS. Oncogenic KRAS mutations (e.g. $KRAS^{G12D}$) occur in more than 90% of PDAC, and appear to be the signature event in PDAC, serving a critical role in tumor initiation [16]. Constitutive activated $KRAS^{G12D}$ protein promotes cell proliferation and cell survival in PDAC cells through the activation of the downstream MAPK and PI3K-mTOR signaling pathways, assigning KRAS gene as the driver oncogenic mutation for PDAC. While KRAS mutations are still largely considered undruggable for PDAC, RNA interference (RNAi) technologies, especially the small interfering RNA (siRNA) in combination with a nanoparticle delivery system, have proved feasibility in effectively silencing mutant KRAS expression *in vitro* and may potentially serve as an alternative choice of gene-targeted therapy for PDAC.

Type II genes are tumor suppressor genes, which have been associated with multiple key signaling pathways and related to cell proliferation and survival, including p53 pathway (TP53), cell cycle pathway (CDKN2A), TGFβ pathway (SMAD4, TGFBR1, TGFBR2) and DNA damage response pathway (ATM, BRCA2) [16, 17]. Several therapeutic platforms by restoration of tumor suppressive function of these type II genes have been tested at various preclinical and clinical stages, however none of these “actions” have yet to affect clinical practice for patients with PDAC.

Type III genes for PDAC are those genes aberrantly overexpressed in PDAC compared to adjacent non-tumor pancreatic tissues, including those that play an important role in regulating pancreas development. To date, a list of aberrant overexpressed genes have been identified to associate with PDAC states using microarray and next generation sequencing technologies, and validated by immunohistochemical staining on PDAC tumor specimens. Type III genes could be potentially used to design novel screening and imaging agents based on molecular contrast which would differentiate PDAC with adjacent non-tumor tissues, as well as putative gene targets for targeted therapies. In a series of preclinical studies, our team has demonstrated that PDX1 (Pancreatic and duodenal homeobox-1) represents a potential type III actionable gene of PDAC [26, 27].

## PDX1 as an actionable gene for PDAC

PDX1 plays a central role in regulating the pancreas global transcription network and is an essential transcription factor for pancreatic development, β-cell differentiation and the maintenance of mature β cell function [28]. PDX1 is well known to be a master regulator for expression of genes crucial for both exocrine and endocrine pancreatic development [29]. In adult pancreas, PDX1 regulates multiple islet-expressed genes such as insulin, islet amyloid polypeptide, somatostatin and glucokinase that maintain homeostasis of the endocrine and exocrine pancreas. NR5A2, which is regulated by PDX1, has been identified as a PDAC susceptibility gene from genome wide association studies [30]. Persistent expression of Pdx1 in acinar cells results in acinar-to-ductal cell metaplasia in mice [31, 32]. PDAC cells tend to form ductal structures *in vivo*, which support the concept that PDAC originates from ductal cells; however, the cells of origin of PDAC remain undetermined. Recently, a sub-population of Pdx1-expressing cells has been shown to be an origin of PDAC in mice. In contrast, mouse islet β cells, which also express Pdx1, are resistant to transformation with Kras activation, unless under contextual conditions of oxidative stress or inflammation [33, 34].

Our team has presented evidence supporting the hypothesis that PDX1 is an oncogenic transcription factor regulating PDAC and a potential type III actionable gene for PDAC. PDX1 was shown to be over-expressed in all human PDAC specimens studied to date, and was shown by others to be associated with advanced clinical pathological stages and poor prognosis. Furthermore, PDX1 was shown to be aberrantly overexpressed in other solid tumors including gastric, colon, breast, prostate, ovary, colorectal and kidney by tissue microarray assay [35]. We demonstrated that PDX1 regulates cell proliferation and invasion both *in vitro* and *in vivo* and that over-expression of PDX1 significantly increased cell proliferation and invasion in human PDAC cells, as well as in benign human pancreatic ductal epithelial cells and HEK293 cells. Furthermore, the application of PDX1-RNAi to

suppress PDX1 expression reversed these effects. Moreover, over-expression of PDX1 in human PDAC, as well as benign HEK293 cells, resulted in a significant increase in area of formed colonies and in volume of human PDAC tumors grown in mice. PDX1 overexpression resulted in significantly enhanced RAS and PI3K signaling by up-regulating expression of genes KRAS, EGFR, INS, etc, and by down-regulating expression of RAS-signaling negative regulators RASA1 and axon guidance genes SLIT2, ROBO2, etc, as well as decreasing p53 levels. Notably, PDX1 degradation is suppressed by PI3K/AKT phosphorylation of GSK3β and further stabilized by the RAS downstream kinase, MAPK p38. We have proposed a PDX1 amplification loop model (Figure 1) in which PDX1 enhances the RAS and PI3K pathways, which, in turn, inhibit PDX1 degradation resulting in PDX1 overexpression [26-28, 36]. In a series of preclinical studies, we have demonstrated that therapeutic actions targeting PDX1 could be achieved using RNAi, gene therapy and small molecules, such as metformin (Figure 1). These PDX1 targeted preclinical therapies will be reviewed.

## RNAi therapeutic platform targeting PDX1 in PDAC

RNA interference (RNAi) is a naturally occurring intracellular pathway by which small RNA molecules bind to mRNA and trigger its degradation. In last decade, RNAi has been used to demonstrate the role of a number of genes in carcinogenesis, cancer cell survival, proliferation, invasion, metastasis and resistance to chemotherapy. Having demonstrated PDX1 is a potential actionable gene for PDAC, we developed in collaboration with StrikeBio, Inc. PDX1 bi-functional shRNA therapy [37-39]. Bi-functional shRNA consists of two stem-loop shRNA (short-hairpin RNA) structures: one cleavage-dependent unit with a perfectly matched passenger-strand and guide-strand, and one cleavage-independent unit composed of a mismatched double strand. Bi-functional shRNA is able to induce both RNase-H like cleavage and non-cleavage mediated degradation of the target mRNA and inhibit translation concurrently, leading to more rapid onset of target gene silencing, higher efficacy and greater durability [40-43]. We designed and tested a series of bi-shRNA$^{PDX1}$ vectors and demonstrated *in vitro* that bi-shRNA$^{PDX1}$ was more effective than conventional shRNA$^{PDX1}$ in silencing PDX1 gene expression in PDAC cells. Pre-clinical *in vivo* therapeutic effects of bi-shRNA$^{PDX1}$ were demonstrated in mice using three biweekly cycles of systemically delivered bi-shRNA$^{PDX1}$ nanoplexes, which ablated human PDAC tumors [26, 28]. Intravenous infusions of bi-shRNA$^{PDX1}$ nanoplexes in a large bio-relevant Yucatan mini-pig model was associated minimal toxicity, thus demonstrating safety[44]. Therefore, preclinical studies suggest that PDX1 RNAi therapy is a promising adjuvant, targeted therapy for PDAC (Figure 1).

## Gene therapy platform targeting PDX1 in PDAC

The field of gene therapy holds great promise in serving as adjuvant therapy for cancer, however has not yet fulfilled its potential and reached the stage of routine clinical care for patients with cancer. While there are a myriad of reasons limiting its potential, with one of the main limitations being the lack of an ideal gene delivery system, the field continues to move forward to develop effective targeted therapies for cancer. In this spirit, our team pursued a strategy of delivering a synthetic cytotoxic gene that would only be activated in

the PDAC cell delivered by DOTAP:cholesterol liposome system encapsulating adenoviral vectors, thus limiting toxicity [45, 46]. To do so, a synthetic insulin promoter, which was shown to be activated only by PDX1 in the PDAC cells, was used to drive the cytotoxic viral thymidine kinase gene (TK) [47, 48]. Once expressed in the cancer cell, TK is coupled with its prodrug, ganciclovir, to induce cancer cell death. Liposomal delivery of the IP-TK gene construct allowed repeated intravenous infusions, which significantly ablated human PDAC tumors in mice with minimal toxicity *in vivo* [45, 46, 49]. These preclinical studies suggest that gene therapy targeting PDX1 could potentially serve as adjuvant, targeted therapy for PDAC.

## Small Molecules targeting PDX1-targeted in PDAC

We then sought to identify small molecules that inhibit PDX1 in human PDAC cells using high-throughput drug screening technology. To achieve this goal, a powerful synthetic human insulin promoter (SHIP) was designed with excellent dynamic range and sensitivity to drive firefly luciferase reporter gene to reflect inhibition of the PDX1 transcription factor-Insulin promoter complex within PDAC cells. Small-molecule libraries were tested in 384-well plates with PDAC cells to search for small molecules that suppress PDX1 within the PDAC cell. We chose to study an FDA approved drug library, which consists of 2100 drugs from the NIH clinical collection (I and II; version 5) that could be repurposed for PDAC therapy. Repurposing of drugs is of great interest, as it reduces costs of drug research and development and saving years of drug development time. A panel of FDA approved drugs that effectively targeting PDX1 were identified, .one of which was metformin, the most widely prescribed diabetes drug worldwide. Metformin has been associated with a substantially lower risk of cancer mortality in multiple studies and meta-epidemiological analyses and is emerging as a promising anticancer drug in clinical trials at various stages [50-52]. There are currently ten clinical trials in progress to determine whether metformin might prolong life for patients with pancreatic cancer [53, 54]. For these reasons, we chose to study and metformin and demonstrated that it suppresses growth of PDAC cells via inhibition of PDX1 via a variety of adenosine monophosphate (AMP)-activated protein kinase (AMPK)-dependent and/or AMPK-independent mechanisms [55]. (Figure 1). Thus, these preclinical studies suggest that FDA approved drugs targeting PDAC actionable genes, such as PDX1, can be identified through high-throughput screening technology which in turn could be repurposed for PDAC adjuvant, targeted therapy.

## Conclusion

While remarkable discoveries have been made through next generation omics approaches, the gap between genomic information its translation into clinical care represents a great clinical challenge for the 21$^{st}$ century. We as surgeons need to incorporate genomic information into the practice of surgery to enhance our outcomes and prolong survival of our patients. There is no greater example of this need that of pancreatic surgery for PDAC. While survival is greatest following surgery, which is the only chance of cure, there remains an urgent need for more effective and less toxic adjuvant therapy gleaned from the genomic information of each patient's PDAC to prolong survival after surgery. In this spirit, our team is pursuing an actionable genomic platform which proposes to match actionable genes of

PDAC with effective adjuvant targeted therapies, such a RNAi, gene therapy and/or metformin. Preclinical studies support the hypothesis that identification of PDAC actionable genes could permit translation of a patient's genomic information into precision targeted adjuvant therapy for PDAC, thus usher in a new era of precision medicine and personalized surgery.

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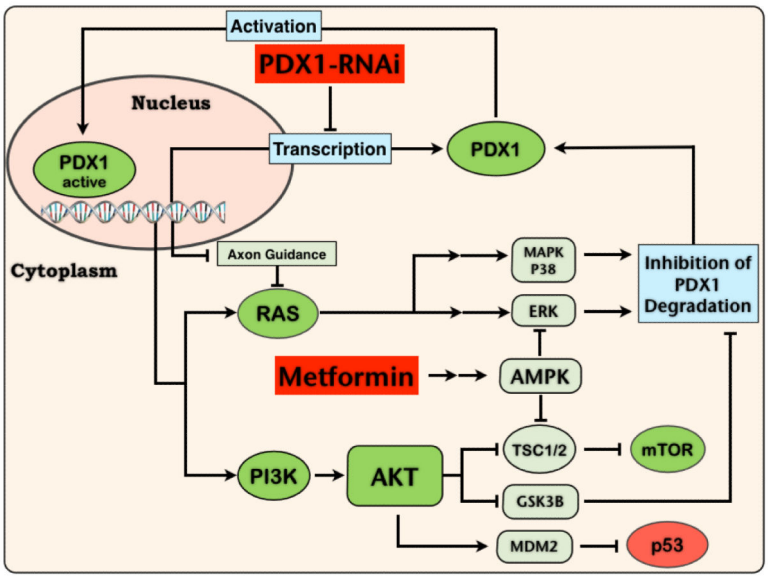


**Figure.1.**

PDX1 is an oncogenic transcription factor in an amplification loop model. PDX1 enhances the RAS and PI3K pathways, which, in turn, inhibit PDX1 degradation resulting in PDX1 overexpression. PDX1-RNAi and metformin were demonstrated to inhibit the amplification loop and PDX1 levels in cancer cells.