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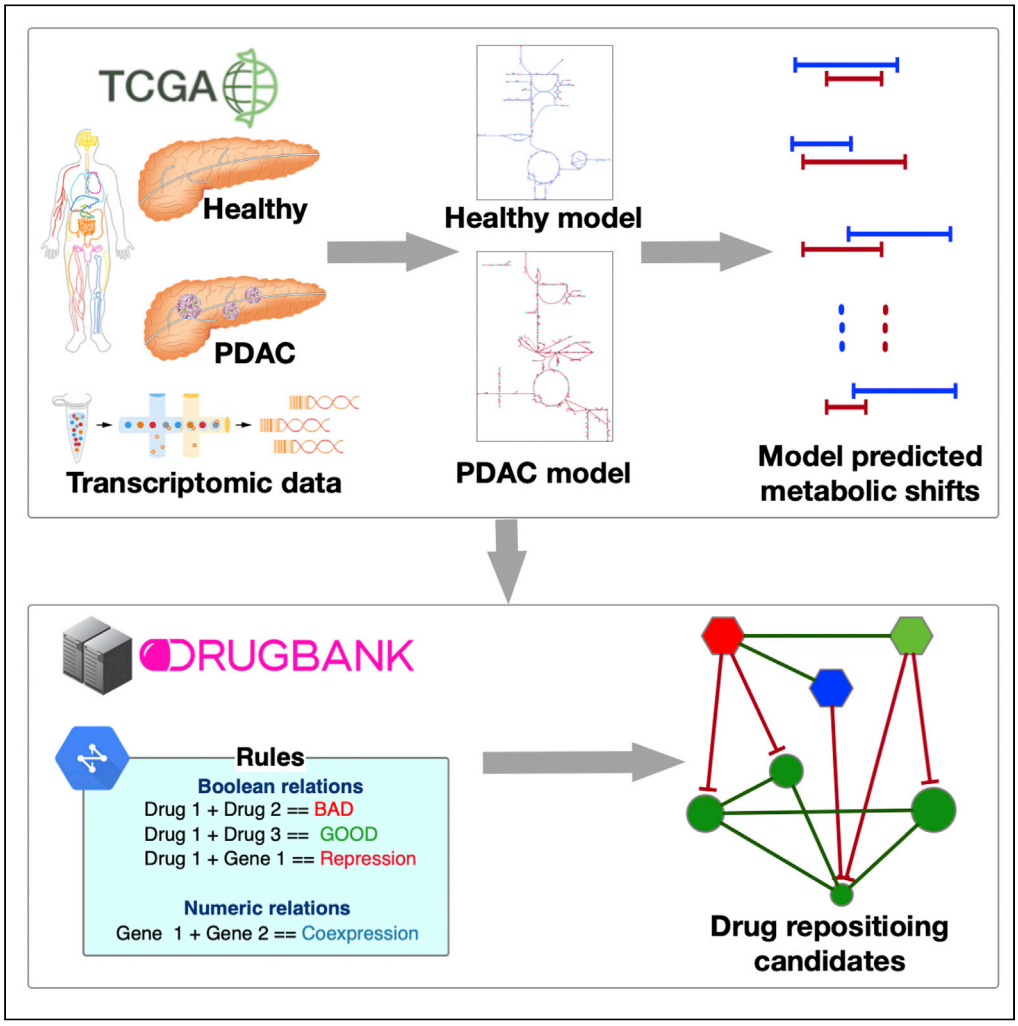

Mohammad Mazharul Islam, Andrea Goertzen, Pankaj K. Singh, Rajib Saha

rsaha2@unl.edu

Highlights

Omics-integrated metabolic models of healthy and PDAC cells were reconstructed

Potential therapeutic targets were explored using model-predicted flux modulations

Potential drug combinations and repositioning strategies were suggested

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Exploring the metabolic landscape of pancreatic ductal adenocarcinoma cells using genome-scale metabolic modeling

Mohammad Mazharul Islam,[1] Andrea Goertzen,[1] Pankaj K. Singh,[2,3] and Rajib Saha[1,4,*]

SUMMARY

Pancreatic ductal adenocarcinoma (PDAC) is a major research focus because of its poor therapy response and dismal prognosis. PDAC cells adapt their metabolism to the surrounding environment, often relying on diverse nutrient sources. Because traditional experimental techniques appear exhaustive to find a viable therapeutic strategy, a highly curated and omics-informed PDAC genome-scale metabolic model was reconstructed using patient-specific transcriptomics data. From the model-predictions, several new metabolic functions were explored as potential therapeutic targets in addition to the known metabolic hallmarks of PDAC. Significant downregulation in the peroxisomal beta oxidation pathway, flux modulation in the carnitine shuttle system, and upregulation in the reactive oxygen species detoxification pathway reactions were observed. These unique metabolic traits of PDAC were correlated with potential drug combinations targeting genes with poor prognosis in PDAC. Overall, this study provides a better understanding of the metabolic vulnerabilities in PDAC and will lead to novel effective therapeutic strategies.

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC), with poor prognosis, resistance to radio- and chemotherapy, and a five-year survival rate of only 8.2% is the most prevalent form of pancreatic cancer and the third-leading cause of cancer-related morbidity in the USA (Abrego et al., 2017). Its poor prognosis can be attributed to its complicated and multifactorial nature, especially the lack of early diagnostic markers as well as its ability to quickly metastasize to surrounding organs (Sarantis et al., 2020; Das and Batra, 2015; Hezel et al., 2006). In addition, high rates of glycolysis and lactate secretion are observed in PDAC cells, fulfilling the biosynthetic demands for rapid tumor growth (Abrego et al., 2017). The combined action of regulatory T cells (Treg), myeloid-derived suppressor cells (MDSCs), and macrophages blocks the $CD8^+$ T cell duties in tumor recognition and clearance and, ultimately, results in PDAC cells manifesting extensive immune suppression (Sarantis et al., 2020).

The PDAC microenvironment is greatly dominated by the presence of dense fibroblast stromal cells. In addition to creating an acidic extracellular environment, the dense stroma surrounding the tumor reduces oxygen diffusion into pancreas cells, resulting in hypoxia. In response to the reduced oxygen uptake, the tumor cells undergo metabolic reprogramming to favor Warburg effect metabolism[12], which involves increased rates of glycolysis. Because cancer cells are characterized by unregulated growth, much of the cellular metabolism is hijacked to maximize the potential to generate biomass. Because PDAC cells are forced to live within a particularly severe microenvironment characterized by relative hypovascularity, hypoxia, and nutrient deprivation, these must possess biochemical flexibility to adapt to austere conditions. Rewired glucose, amino acid, and lipid metabolism and metabolic crosstalk within the tumor microenvironment contribute to unlimited pancreatic tumor progression. The metabolic alterations of pancreatic cancer are mediated by multiple factors. These cells survive and thrive mainly in three ways: (1) Reprogramming intracellular energy metabolism of nutrients, including glucose, amino acids, and lipids; (2) Improving nutrient acquisition by scavenging and recycling; (3) Conducting metabolic crosstalk with other components within the microenvironment (Koppenol et al., 2011). In addition, the metabolic reprogramming involved in pancreatic cancer resistance is also closely related to chemotherapy, radiotherapy and immunotherapy, and results in a poor prognosis. Thus, investigations of metabolism not only benefit the understanding of carcinogenesis and cancer progression but also provide new insights for treatments against

[1]Department of Chemical and Biomolecular Engineering, University of Nebraska-Lincoln, Lincoln, NE 68588, USA

[2]Fred & Pamela Buffett Cancer Center, University of Nebraska Medical Center, Omaha, NE 68198, USA

[3]Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, Omaha, NE 68198, USA

[4]Lead contact

*Correspondence: rsaha2@unl.edu

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pancreatic cancer. A better understanding of the metabolic dependencies required by PDAC to survive and thrive within a harsh metabolic milieu could reveal specific metabolic vulnerabilities.

Systemic chemotherapy is presently the most frequently adopted treatment strategy for PDAC. However, chemotherapy treatments often show limited success due to intrinsic and acquired chemoresistance (Teague et al., 2015; Grasso et al., 2017). Although many previous studies have predicted potential biomarkers for therapeutic purposes, including the *ribonucleotide reductase catalytic subunits M1/2 (RRM1/2)*, an enzyme catalyzing the reduction of ribonucleotides, or the *human equilibrative nucleoside transporter 1 (hENT1)*, a transmembrane protein, the treatment with drugs (i.e., gemcitabine and other combinatorial drugs) often failed (Nakano et al., 2007; Nakahira et al., 2007; Kurata et al., 2011; Valsecchi et al., 2012; Duxbury et al., 2005). The hypoxic microenvironment is also resistive to radiation dosage, reducing the efficacy of radiotherapy. In addition, the overexpression of key regulators of the DNA damage response (e.g., *RAD51* in PDAC) has been reported to contribute to the accelerated repair of DNA damage [128, 129]. Several genes have been reported to be frequently mutated in PDAC (*i.e., KRAS, CDKN2A, TP53*, and *SMAD4*) (Waddell et al., 2015; Cancer Genome Atlas Research Network. Electronic address and Cancer Genome Atlas Research Network, 2017) and, therefore, received increased attention as potential drug targets (Kastenhuber and Lowe, 2017; Cox et al., 2014; Moore et al., 2007; Ruess et al., 2018; Eser et al., 2013). However, successful therapeutic strategies are yet to be developed (Fiskus et al., 2015; Wolpin et al., 2009; Javle et al., 2010). The downstream events of metabolic reprogramming are considered as prominent hallmarks of PDAC (Orth et al., 2019). Therefore, tackling this aggressive cancer through establishing a clear understanding of its metabolism has been a critical challenge to the scientific and medical communities. Because the underlying mechanism of these drug-resistive metabolic traits are only poorly understood, it warrants the use of novel computational techniques to understand the metabolic landscape of tumor progression and further complement the going experimental efforts.

The increase in knowledge of macromolecular structures, availability of numerous biochemical database resources, advances in high-throughput genome sequencing, and increase in computational efficiency have accelerated the use of *in silico* methods for metabolic model development and analysis, biomarkers/therapeutic target discovery, and drug development (Raskevicius et al., 2018; Bordel, 2018; Zhang and Hua, 2015; Dunphy and Papin, 2017; Agren et al., 2014; Mienda et al., 2018). These models provide a systems-level approach to studying the metabolism of tumor cells based on conservation of mass under pseudo-steady state conditions. Because genome-scale metabolic models are capable of efficient mapping of the genotype to the phenotype (Bernstein et al., 2021; Cardoso et al., 2015; Castillo, Patil and Jouhten, 2019; Lewis et al., 2012; Matthews and Marshall-Colón, 2021; O'Brien et al., 2015), integrating multi-level omics data with these models enhances their predictive power and allows for a systems-level study of the metabolic reprogramming happening in living organisms under various genetic and environmental perturbations or diseases. Applications of the genome-scale metabolic modeling to cancer includes network comparison between healthy and cancerous cells, gene essentiality and robustness studies, integrative analysis of omics data, and identifying reporter pathways and reporter metabolites (Zhang et al., 2019; Turanli et al., 2019; Nilsson and Nielsen, 2017; Ghaffari et al., 2015; Jerby and Ruppin, 2012). For example, Turanli et al. used metabolic modeling to pinpoint drugs that could effectively hinder growth of prostate cancer (Turanli et al., 2019). Similarly, Katzir, et al. mapped the reactions and pathways in breast cancer cells using a human metabolic model and various "omics" datasets (Katzir et al., 2019). Pancreatic cell and pancreatic cancer metabolism have been modeled before as a part of reconstructing draft models of several human cell types aimed at identification of anticancer drugs through personalized genome-scale metabolic models (Agren et al., 2012; 2014). Although a pan-cancer analysis of the metabolic reconstructions of ~4000 tumors were attempted recently (Gatto et al., 2020), the models generated were tasked with only finding the origin of the cancer-specific genes and reactions, and were not essentially curated and refined to achieve a high level of predictability. Kinetic modeling of the pancreatic tumor proliferation was also attempted, by modeling the glycolysis, glutaminolysis, tricarboxylic acid cycle, and the pentose phosphate pathway to find enzyme knockout or metabolic inhibitions suppressing the tumor growth (Roy and Finley, 2017). Although these studies have advanced our understanding of the metabolic landscape of pancreatic ductal adenocarcinoma or cancer in general, there is still the necessity of a highly curated and predictive genome-scale metabolic model in order to have a system-level understanding of the metabolic changes.

To understand the PDAC-associated metabolic reprogramming that involves changes in the metabolic reaction fluxes and metabolite levels, genome-scale metabolic reconstructions of the healthy human pancreas

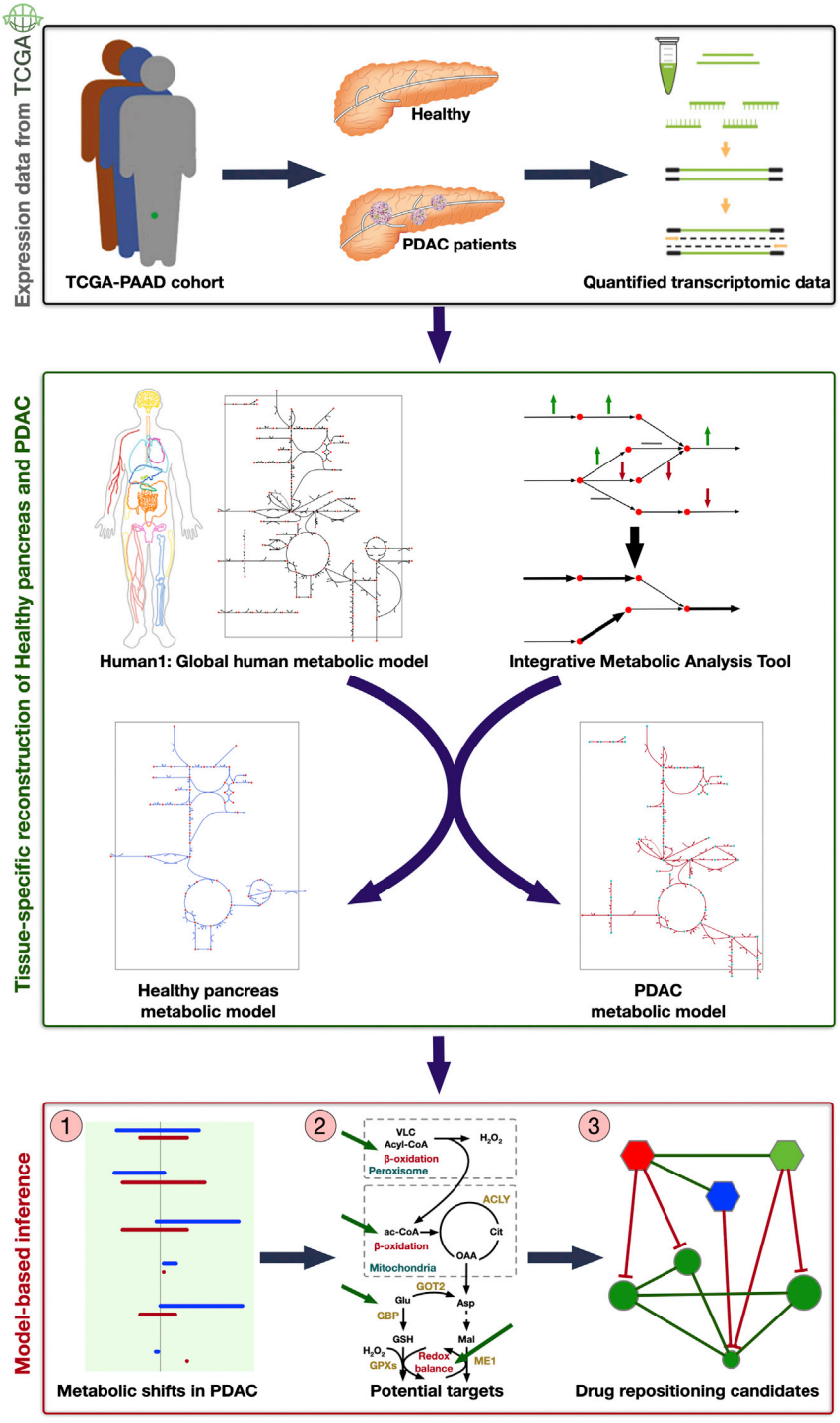

Figure 1. Schematic of the workflow for generating healthy pancreas and PDAC model and elucidating the metabolic divergence in PDAC

and the PDAC cells encompassing the genes, metabolites, and reactions, were developed. This reconstruction process utilized a patient transcriptomic dataset from the Cancer Genome Atlas (https://www.cancer.gov/tcga). The models were used to elucidate the altered metabolism of PDAC cells compared to the healthy pancreas. A concise schematic of the workflow in this study is presented in Figure 1. Upon incorporation of

the transcriptomic data, the shifts in reaction flux spaces were observed across the metabolic network, notably in glycolysis, pentose phosphate pathway, TCA cycle, fatty acid biosynthesis, Arachidonic acid metabolism, carnitine metabolism, cholesterol biosynthesis, and ROS detoxification metabolism. Many of the observed metabolic shifts are in accordance with previously identified cancer hallmarks in omics-based studies. In addition, unique metabolic behavior was observed in mitochondrial and peroxisomal fatty acid beta oxidation, various parts of lipid biosynthesis and degradation, and ROS detoxification, which are discussed as potential for prognostic biomarkers. Significant downregulation in the peroxisomal fatty acid beta oxidation pathway reactions was observed in this study, which explains the shifts in cellular energy production and storage preference during pancreatic tumor proliferation. Furthermore, flux modulation in the carnitine shuttle system and the upregulation in the reactive oxygen species detoxification pathway reactions that was observed in this study indicate the unique strategies the PDAC cells adopt for survival. Potential drug repositioning and synergistic interaction between existing drugs that repressed the differentially expressed genes with poor prognosis in PDAC were identified. These findings manifest the predictive capabilities of genome-scale metabolic models at the reactome-level and can potentially direct new therapeutic approaches.

RESULTS AND DISCUSSION

Transcriptomic analyses of the gene expression dataset from TCGA

The transcriptomic data used to customize the global human model to a pancreatic reconstruction was obtained from the Cancer Genome Atlas (TCGA) database (https://www.cancer.gov/tcga). The Cancer Genome Atlas contains genomic, epigenomic, transcriptomic, and proteomic data on 33 cancer types in humans, and is publicly available for the scientific research community. To obtain a representative set of transcriptomic data on both healthy and cancerous pancreas cells, 18 samples from the TCGA-PAAD project that contained quantified RNASeq transcriptomic data, were used. These samples contained Fragments Per Kilobase of transcript per Million mapped reads (FPKM) data of individuals from different ethnic backgrounds, ages, and sexes. Because the dataset accounted for a numerical expression value of every single of the 60,483 genes across all the samples without any unique genes in the samples, the dataset was filtered for genes with no read count across samples. After that, 50,392 genes remained, out of which 3628 metabolic genes overlapped with the genes in the Human1 metabolic reconstruction (Robinson et al., 2020). Differential gene expression analysis of the metabolic genes within the transcriptomic dataset from TCGA revealed 102 significantly differentially expressed genes, among which 53 showed significant upregulation and 49 showed repressions in PDAC cells compared to healthy pancreatic cells (see details in STAR Methods). Genes involved in glycolysis/gluconeogenesis, fatty acid and cholesterol biosynthesis, tRNA synthesis, Arachidonic acid metabolism, protein kinases, glutathione metabolism, RNA polymerase, DNA repair, mitochondrial beta oxidation, cytosolic carnitine metabolism, leukotriene and linoleate metabolism, and estrogen metabolism were consistently upregulated in all PDAC samples. On the other hand, genes related acylgylyceride metabolism, peroxisomal beta oxidation, mitochondrial and peroxisomal carnitine metabolism, several peroxidases, chondroitin, keratan, and heparan sulfate biosynthesis, glycerolipid metabolism, and different types of vitamin metabolism, including vitamins B_{12}, D, and E, showed significant downregulation in PDAC. The complete results of differential gene expression analysis are presented in Table S1.

Lipogenic enzymes, especially ATP citrate lyase (ACLY) was overexpressed in PDAC, which have been reported in many previous studies as well (Swierczynski et al., 2014; Menendez and Lupu, 2007). The growth of PDAC cells was previously shown to be inhibited by the interference of ACLY activity in a xenograft tumor model (Hatzivassiliou et al., 2005). At the initial step of *de novo* lipid synthesis, ATP-citrate lyase (ACLY) converts citrate to acetyl-CoA, which is then channeled to cytoplasm. Acetyl-CoA and malonyl-CoA are coupled to acyl-carrier protein (ACP) domain of fatty acid synthase (FASN) and the downstream genes to synthesize monounsaturated and polyunsaturated as well as saturated fatty acids (Baenke et al., 2013). Acetyl-CoA is also converted to cholesterol and cholesterol ester. This observation agrees with the elevated expression of HMG-CoA (3-hydroxy-3-methylglutaryl-Coenzyme-A) reductase and LDLR (low-density lipoprotein receptor) in a mouse model with PDAC (Guillaumond et al., 2015).

Reduction in Reaction Oxygen Species (ROS) levels by superoxide dismutase (SOD1) and peroxidases (GPX2, GPX3, TPO, and MPO) is partly responsible for acquired chemoresistance of PDAC cells. In this study, the expression of *SOD1, GPX2, GPX3, TPO,* and *MPO* was found to be upregulated in PDAC cells. ROS stimulates other pro-growth pathways early on in cancer progression (e.g., PI3K signaling) (Sullivan

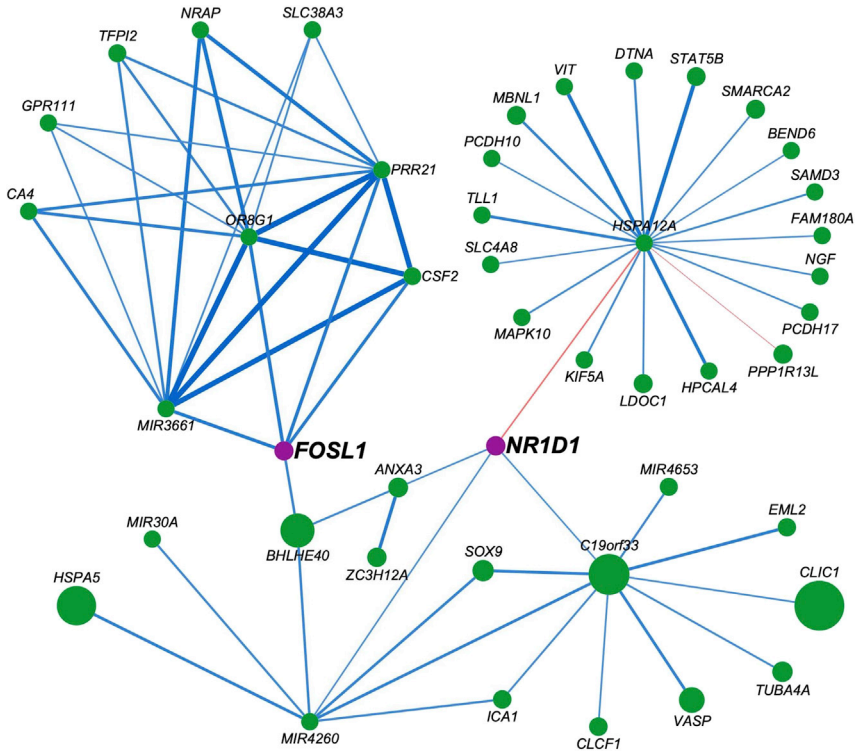

Figure 2. Co-expression network for differentially expressed regulatory genes NR1D1 and FOSL1 (colored purple)

The size of the green circle for the co-expressed gene denotes the fold change in PDAC compared to healthy pancreas. The thickness of the lines corresponds to the Pearson correlation coefficient ($p < 0.05$) between each gene pair.

and Chandel, 2014). The generation of genetic mutations by ROS may also play a tumor-promoting role in cancer development. Chemotherapy adds to ROS levels in the PDA microenvironment, which further compounds the oxidative perils routinely faced by these malignant cells (Sangeetha et al., 1990). Thus, as the dangers of ROS mount, adverse consequences and toxicities associated with ROS start to exceed any pro-survival benefits favoring tumor growth. Therefore, enhanced antioxidant defense mechanisms though these genes become paramount to PDAC cells for survival (Izuishi et al., 2000).

Co-expression analysis based on the differentially expressed regulatory genes

Of the 102 differentially expressed genes, two overexpressed genes (*NR1D1* and *FOSL1*) were identified as regulatory genes using the Human Protein Atlas. These genes have been shown to influence a number of important biological processes in pancreatic cells, including cellular regulation and cell cycle, metabolite transport, and carcinogenesis. A list of the genes regulated by each of these genes was obtained from RegNetwork (Liu et al., 2015). The expression patterns of the two regulatory genes and their targets were examined to develop gene co-expression networks with the goal to identify highly co-expressed genes that could be considered regulators for genes expressed in PDAC. Figure 2 shows the co-expression network for the two regulatory genes.

NR1D1 regulates circadian rhythm and metabolic pathways including lipid and bile acid metabolism, adipogenesis, and gluconeogenesis. Hydrophobic bile acids are carcinogens, and increased levels of bile acids in the cellular environment has been linked with PDAC (Feng and Chen, 2016; Martín-Blázquez et al., 2020). NR1D1 *positively regulates* bile acid synthesis. Circadian clock regulator genes, including NR1D1 have previously been identified as therapeutic targets for cancer treatment (García-Costela et al., 2020). The circadian clock is important for its role in the cell cycle, cell proliferation, and apoptosis. Disruptions of the circadian rhythm interrupt homeostasis, which has been linked to several diseases, including cancer (Masri and Sassone-Corsi, 2018). In our co-expression study, NR1D1 expression was found

to be correlated with ANXA3 expression, which is reported to be highly expressed in lung, liver, and ovarian cancer, and is correlated with poor prognosis of patients (Pan et al., 2015; Yan et al., 2010; Liu et al., 2009). Another gene whose expression was highly correlated with NR1D1 is SLC1A5, which codes for an amino acid transporter. A variant of this transporter transports glutamine into the mitochondria, allowing for glutamine-induced ATP production in PDAC cells (Yoo et al., 2020). It is well-known that cancer cells rely increasingly on glutamine for ATP production (Li and Le, 2018).

FOSL1 is a regulatory gene responsible for upregulation of cell population proliferation, the cell cycle, and the apoptotic process (Tsuchiya et al., 1993). FOSL1 has been identified as an oncogene. It promotes KRAS-driven cancer (a cancer caused by a mutation in a cell cycle protein in a normal cell) in lung and pancreatic cancer (Elangovan et al., 2018). It also plays a role in the Th17 pathway, which activates immune cells. For example, the network shows a correlation between FOSL1 and CSF2, which is a gene that controls the production, differentiation, and function of granulocytes and macrophages (Fagerberg et al., 2014). The Th17 pathway can have either a positive or negative impact on carcinomas. Although Th17 cells can provide an immune response to attack tumors, under certain conditions they can also drive angiogenesis and suppress antitumor immunity (Bailey et al., 2014). Another gene that strongly correlates with FOSL1 is BHLHE40, which regulates the circadian rhythm by negatively regulating the activity of clock genes (Nakashima et al., 2008; Honma et al., 2002). As mentioned earlier, there is a connection between disruption of the circadian rhythm and carcinogenesis.

Tissue-specific consensus pancreas metabolic reconstruction using transcriptomics data

A metabolic model describes reaction stoichiometry and directionality, gene-protein-reaction associations (GPRs), organelle-specific reaction localization, transporter/exchange reaction information, transcriptional/translational regulation, and biomass composition (Kumar et al., 2012). By defining the metabolic space, genome-scale metabolic models can assess allowable cellular phenotypes and explore the metabolic potential and restrictions under specific disease conditions (Saha et al., 2014). The latest global human metabolic reconstruction, Human1 (Robinson et al., 2020), is an extensively curated, genome-scale model of human metabolism. It unified two previous and parallel model reconstruction lineages by the Systems Biology community, namely the Recon (Brunk et al., 2018; Duarte et al., 2007; Thiele et al., 2013) and the Human Metabolic Reaction (HMR) (Mardinoglu et al., 2013; Mardinoglu et al., 2014) series using an open-source version-controlled repository. In addition to curating the aggregated reconstruction, Human1 addressed issues with duplication, reaction reversibility, mass and energy conservation, imbalance, and constructed a new generic human biomass reaction based on various tissue and cell composition data sources. This standardized model allowed us to conveniently integrate omics data to develop a pancreas-specific metabolic reconstruction.

The preliminary pancreas metabolic reconstruction was obtained using the FPKM values for the 3628 metabolic genes in the TCGA dataset by iMAT (Zur et al., 2010) (details in the STAR Methods section). It contained 3,628 genes, catalyzing 7,076 reactions, involving 4,415 metabolites located in 8 intracellular compartments (Cytosol, Mitochondria, Inner mitochondria, Golgi apparatus, Lysosome, Nucleus, Peroxisome, and Endoplasmic reticulum). The reactions are distributed across 133 different pathways, the largest of which include transport reactions, exchange/demand reactions, fatty acid oxidation, and peptide metabolism. Flux Variability Analysis (Mahadevan and Schilling, 2003) found that the 1444 reactions across 54 pathways could occur at an unreasonably high rate not supported by thermodynamics, which are named unbounded reactions. The pathways contributing the largest number of unbounded reactions were transport, fatty acid oxidation, nucleotide metabolism, and drug metabolism. After the model had been refined by rectifying reaction imbalances and identifying and fixing infeasible cycles using Optfill (Schroeder and Saha, 2020) (see a complete list in Table S2), a thermodynamically feasible intermediate metabolic reconstruction of the pancreas encompassing all the reactions in both healthy and cancerous pancreas cells was obtained. This reconstruction was used as a baseline for generating the healthy and cancerous genome-scale pancreas metabolic model.

Metabolic models of PDAC and healthy pancreas cell

The healthy pancreas and PDAC models were reconstructed from the consensus metabolic reconstruction of the pancreas. The Integrative Metabolic Analysis tool (iMAT) (Zur et al., 2010) was used to customize the model according to the gene expression values and corresponding ranking of the reactions (see STAR Methods section for details) in both healthy and PDAC cells. The healthy cell model contains 3,628 genes,

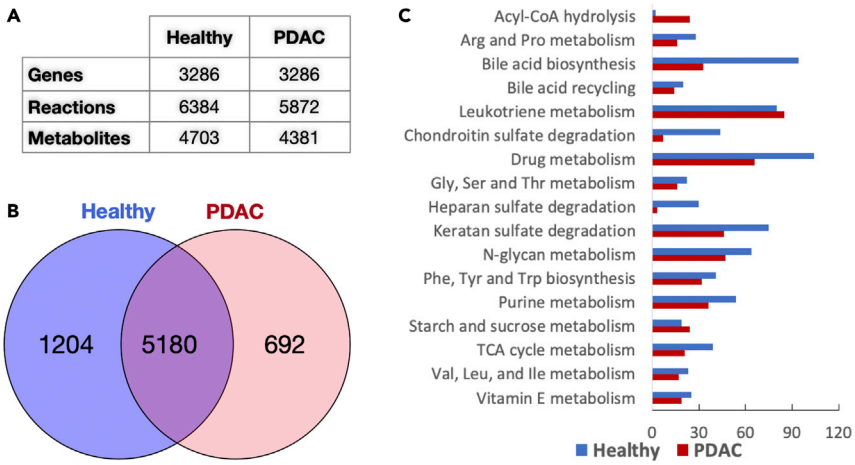

Figure 3. Model statistics for the healthy pancreas and the PDAC models

(A) Numbers of Genes, Reactions, and Metabolites, (B) overlap and uniqueness of metabolic reactions (Blue: Healthy, Red: PDAC), and (C) Most divergent pathways between the two models.

catalyzing 6,384 reactions, across 129 pathways, involving 4,703 metabolites, whereas the PDAC cell model contains 3,628 genes, catalyzing 5,872 reactions, across 127 pathways, involving 4,381 metabolites. In both models, the pathways involving the largest number of internal reactions include fatty acid oxidation, cholesterol formation, peptide metabolism, and transport reactions. Datas S1 and S2 contain the genome-scale metabolic model of the healthy and cancerous pancreas cells in Systems Biology markup Language level 3 version 1, respectively.

Figure 3 shows further details of the two models. Although there are 5180 reactions overlap between the healthy and PDAC models, they have 1204 and 692 unique metabolic reactions, respectively (see Figures 3A and 3B). The unique reactions are distributed across divergent pathways in these two models (Figure 3C). The PDAC model distinctly shows better completeness of the Acyl-CoA hydrolysis, leukotriene metabolism, and starch and sucrose metabolism. On the other hand, many pathways have a more complete presence in the healthy cell model, including amino acid metabolism, structural carbohydrates (heparan and keratan sulfate) degradation, glycan metabolism, bile acid synthesis, and TCA cycle. Although the more complete Acyl-CoA hydrolysis and sugar metabolism have been known to be associated with cancer cells, particularly interesting are the more complete leukotriene metabolism and lack of structural carbohydrate degradation pathways in the PDAC cell. It has been reported that the leukotrienes derived from membrane phospholipids play an important role in carcinogenesis (Heukamp et al., 2006; Roebuck, 1992). Furthermore, glycosaminoglycans (e.g., keratan sulfate, heparan sulfate, and chondroitin sulfate) degradation in lysosomes are part of the normal homeostasis of glycoproteins. These molecules must be completely degraded to avoid undigested fragments building up and causing a variety of lysosomal storage diseases (Aronson and Kuranda, 1989). Lack of these degradation pathways in the PDAC indicates an increased accumulation of glycosaminoglycans in the tumor cell, which have previously been associated with cancer metastasis (Ishiwata et al., 2007; Caterson and Melrose, 2018).

Unique metabolic traits in PDAC

The mathematically feasible flux ranges of the reactions in the healthy and PDAC models were assessed (see details in STAR Methods sections) to explore the distinct shifts in PDAC cell metabolism. In Figure 4, the pathways with the biggest fraction of reaction fluxes (>20% of all the reactions in the pathway) significantly upregulated and downregulated are shown (a more detailed version is presented in Figure S1, and detailed flux results are in Table S3). Although the observed metabolic shifts agree with the differential gene expression results discussed before, they also reveal some unique metabolic traits in PDAC. The model simulation results capture the most well-known metabolic hallmarks of pancreatic ductal adenocarcinoma. For example, the expansion of the flux space of the reactions in glycolytic pathways, bile acid biosynthesis, nucleotide metabolism, pentose phosphate pathway, and arachidonic acid metabolism is consistent with many studies (Eser et al., 2013; Heukamp et al., 2006; Orth et al., 2019) on pancreatic cancer in recent years.

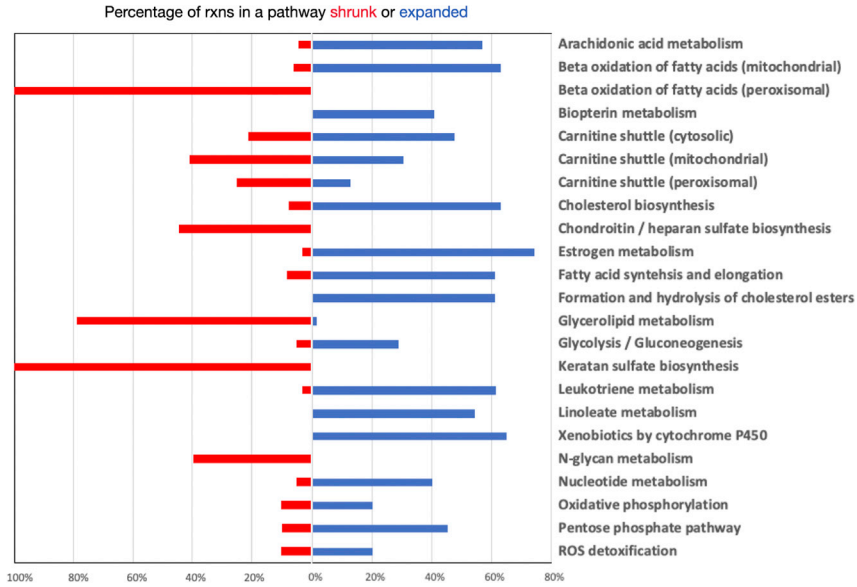

Figure 4. Significantly upregulated and downregulated pathways in PDAC cell metabolism

The bars (red: downregulated, blue: upregulated) represent the percentage of the total number of reactions in the respective pathway that changed their flux ranges.

This major metabolic reprogramming in pancreatic ductal adenocarcinoma arises from the well-known Warburg effect (Warburg, 1956) because of constitutive activation of KRAS oncogene (Feldmann et al., 2007; Gaglio et al., 2011). KRAS activation in PDAC cells upregulates the uptake of glucose and enhance the glycolytic flux, including the production of lactate through lactate dehydrogenase (which demonstrates expanded flux ranges in PDAC) and channels carbon flux into the hexosamine biosynthetic pathway and pentose phosphate pathway. Both primary and metastatic PDAC tumors demonstrate increased glycolytic gene expression (Chaika et al., 2012b). Notably, upregulation of pentose phosphate pathway and the downstream nucleotide biosynthesis pathway has been implicated in PDAC progression and therapy resistance (Vernucci et al., 2019; Olou et al., 2020; Gunda et al., 2017; Shukla et al., 2017; Shukla et al., 2015; Chaika et al., 2012a; Gebregiworgis et al., 2018). Increased bile acid secretion has previously been identified in PDAC patients, which is indicative of tumor expansion into the bile duct (Martín-Blázquez et al., 2020) and may result in bile acid reflux into the pancreatic duct and acinar cells, from which PDAC is derived (Feng and Chen, 2016). *NR1D1*, one of the two differentially expressed regulator genes, positively regulates bile acid synthesis (Duez et al., 2008), indicating a possible link between overexpression of that gene and PDAC carcinogenesis through increased bile acid synthesis. In addition, glutamine metabolism is vastly reprogrammed to balance the cellular redox homeostasis. Glutamine is sequentially converted to glutamate and aspartate in the mitochondria, which is shuttled into cytoplasm and eventually generates NADPH after a series of reactions to maintain redox homeostasis. The regeneration of NAD + as an upstream substrate of NADH production is, therefore, an absolute requirement for PDAC cell survival, particularly when mitochondrial demands escalate. Alterations in glucose and glutamine metabolism have also been linked with poor response to chemotherapy in PDAC (Gebregiworgis et al., 2018; Mehla and Singh, 2020; Shukla et al., 2017).

Reactions in the arachidonic acid metabolism and leukotriene metabolism were observed to expand their flux space in PDAC by increasing their maximum flux by 1.5-fold compared to the healthy model. The two distinct branches of arachidonic acid metabolism, mainly driven by cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX), both carried higher levels of fluxes in the PDAC model. Several studies have reported that eicosanoid metabolism, especially arachidonic acid (AA) metabolizing enzymes including prostaglandins and leukotrienes (LT), play an important role in carcinogenesis (Heukamp et al., 2006; Roebuck, 1992). Specifically, the eicosanoids formed via COX-2 and 5-LOX metabolism directly contribute to pancreatic cancer cell proliferation in humans (Knab et al., 2014). Leukotrienes are also known to initiate inflammation and mount adaptive immune responses for host defense (Peters-Golden and Henderson, 2007). Prostaglandins have also been shown to regulate tumorigenesis in PDAC (DelGiorno et al., 2020).

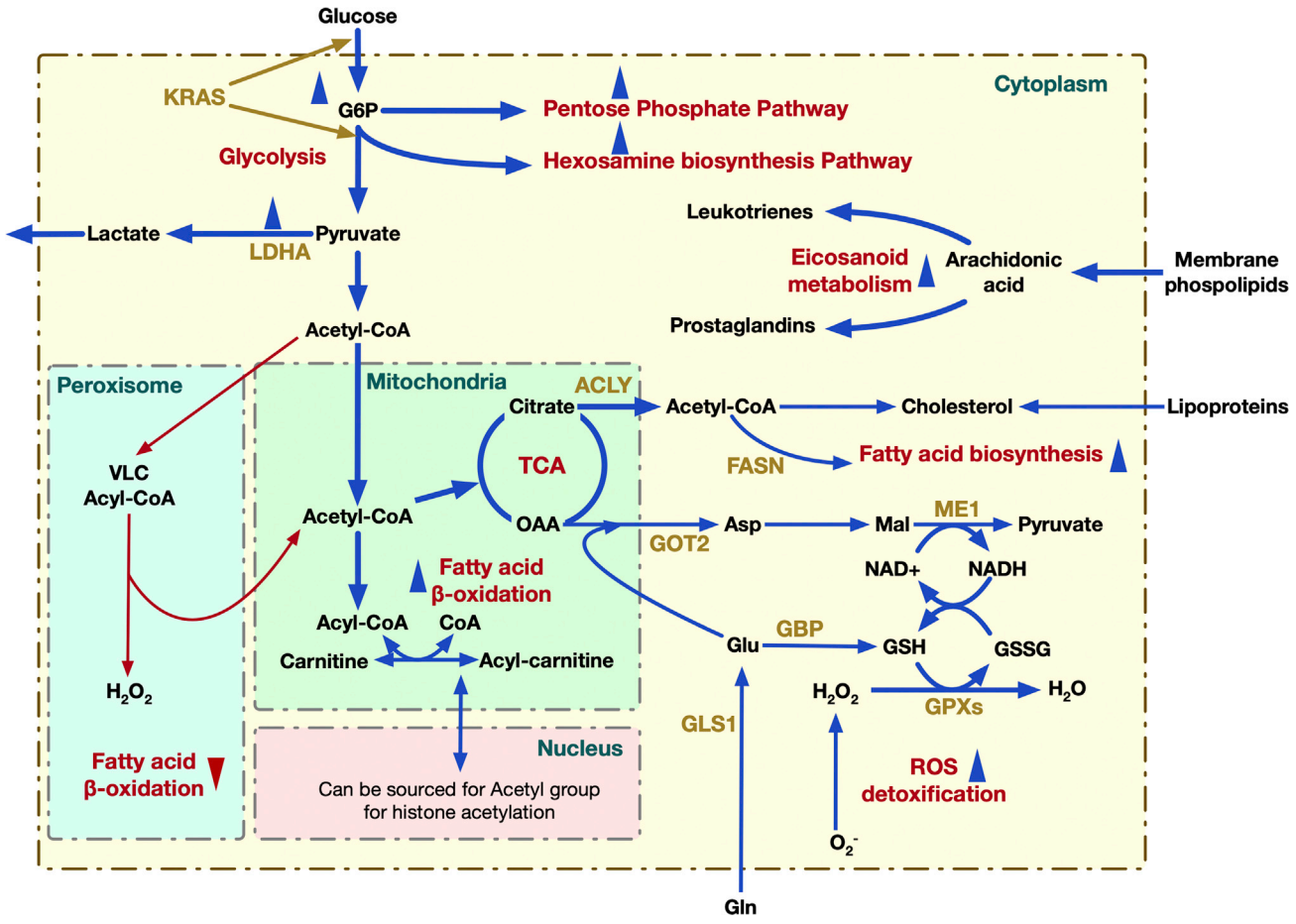

Figure 5. Distinct metabolic features of PDAC cells

Blue and red pathway lines denote expanded and shrunk reaction flux in PDAC compared to the healthy pancreas model, respectively. The lines thicknesses approximate the magnitude of the fold change of reaction flux in PDAC. ACLY: ATP-citrate lyase; Asp: aspartate; FASN: fatty acid synthase; Gln: glutamine; GLS1: glutaminase; Glu: glutamate; GOT: glutamic-oxaloacetic transaminase; GPX: glutathione peroxidase; GSH: glutathione reduced; GSSG: glutathione oxidized; LDHA: lactate dehydrogenase A; ME: malic enzyme; OAA: oxaloacetic acid; TCA: tricarboxylic acid; VLC: very long chain.

While the Warburg model explains these shifts to a great extent, especially in increased uptake of glucose and subsequent increased oxidative phosphorylation, recent studies have shown that the balance between glycolysis and oxidative phosphorylation may not always be homeostatic. Rather, the metabolic reprogramming happening in PDAC is highly dynamic and dependent on the harsh tumor microenvironment (Jose et al., 2011). Therefore, it is imperative to investigate other less suspected sources of unique metabolic traits of PDAC cells. Simulating the flux space of the PDAC cell model and comparing that with the healthy pancreas model allows us to examine the distinct changes in metabolism in the pathway level. These observations are concisely presented in Figure 5.

Increased abundance of acetyl-CoA and upregulated cytosolic carnitine metabolism (see Figures 4 and 5) result in more carnitine and acyl-carnitine (mostly acetyl-carnitine) transport between the mitochondria and cytosol. Carnitine can also be transported to the cytosol and accumulated in biomass. Recent findings have suggested that carnitine shuttle could be considered as a gridlock to trigger the metabolic flexibility of cancer cells (Melone et al., 2018; Muoio, 2014). Carnitine shuttle system is involved in the bidirectional transport of acyl moieties between cytosol to mitochondria, thus playing a fundamental role in tuning the switch between the glucose and fatty acid metabolism. This is crucial for the mitochondrial fatty acid beta-oxidation and maintaining normal mitochondrial function (balancing the conjugated and free CoA ratio) (Sharma and Black, 2009). Higher burning of long-chain fatty acids produces increased energy for the cell to survive (Longo et al., 2016). The available acetyl-CoA can be fed into the TCA cycle to produce more energy or acetyl moieties can be repurposed in the nucleus to recycle acetyl group for histone acetylation (Melone

et al., 2018). Thus, the carnitine shuttle system plays a significant role in tumors by supplying both energetic and biosynthetic demand for cancer cells (Melone et al., 2018).

The majority (>63%) of the mitochondrial fatty acid beta oxidation pathway reactions showed significant flux expansion (with an average 2-fold increase and maximum 6-fold increase in the maximum flux value) compared to the healthy pancreas model. Although the mitochondrial beta oxidation pathway reactions primarily showed an expansion in flux space, 100% the reactions in the peroxisomal beta oxidation pathway shrunk their flux space (by decreasing their maximum flux values by 50% of that of the healthy pancreas model). This is an interesting feature of pancreatic ductal adenocarcinoma because peroxisomal beta oxidation pathway was found to be upregulated in some cancer types (Zha et al., 2005) and downregulated in others (Litwin et al., 1999; Lauer et al., 1999; Keller et al., 1993). The primary differences between fatty acid beta oxidation in mitochondria and peroxisome is the chain length at which fatty acids are synthesized and the associated product. Mitochondria catalyze the beta oxidation of the majority of the short to long-chain fatty acids, and primarily generate energy, whereas peroxisomes are involved in the beta oxidation of very-long-chain fatty acids and generate H_2O_2 in the process (Reddy and Hashimoto, 2001). This means that while mitochondrial beta-oxidation is governed by the energy demands of the cells, peroxisomal beta-oxidation does not. Peroxisomal beta-oxidation is mostly involved in biosynthesis of very-long-chain fatty acids and do not produce energy, while the mitochondrial pathway is related to mostly catabolism and is coupled to ATP production (Demarquoy and Le Borgne, 2015). Therefore, it is expected that the rapidly proliferating and energy-demanding tumor cells will favor the more energy-efficient mitochondrial pathways instead of the less required very-long-chain fatty acid-producing peroxisomal pathways. Furthermore, the reduction of the peroxide byproduct by downregulating the peroxisomal beta oxidation pathways reduce the oxidative stress, which helps the cancer cell to survive.

Lipid metabolism is essential for cancer progression, because it provides the necessary building blocks for cell membrane formation and produces signaling molecules and substrates for the posttranslational modification of proteins. However, the role of fatty acids in pancreatic cancer is complicated and still not very well understood. In PDAC, we observe that >62% of the reactions participating in *de novo* fatty acid biosynthesis, fatty acids elongation, and cholesterol biosynthesis pathways are upregulated on an average of 1.5-fold, including citrate synthase, ATP citrate lyase, fatty acid synthase, and coenzyme A reductase. Overexpression of these lipogenic enzymes in PDAC have been reported in some previous studies as well (Swierczynski et al., 2014; Menendez and Lupu, 2007; Tadros et al., 2017). Of note, increased fatty acid biosynthesis has been shown to impart poor chemotherapy responsiveness (Tadros et al., 2017). At the initial step of *de novo* lipid synthesis, ATP-citrate lyase (ACLY) converts citrate to acetyl-CoA, which is then channeled to cytoplasm. Acetyl-CoA and malonyl-CoA are coupled to acyl-carrier protein domain of fatty acid synthase (FASN) and the downstream genes to synthesize monounsaturated and polyunsaturated as well as saturated fatty acids (Baenke et al., 2013). Acetyl-CoA is also converted to cholesterol and cholesterol ester. This observation agrees with the elevated expression of HMG-CoA (3-hydroxy-3-methylglutaryl-Coenzyme-A) reductase and LDLR (low-density lipoprotein receptor) in a mouse model with PDAC (Guillaumond et al., 2015) as well as metabolomic studies confirming strong lipid activity in human PDAC cells (Martín-Blázquez et al., 2020; Wang et al., 2021). In addition to the observed higher intercellular lipid synthesis, uptake of extracellular lipids is also increased in PDAC. This indicates an increased demand of nutrients for rapid proliferation that the PDAC cells must meet for *survival*. This enhanced rate of phospholipid degradation to supply energy demand for expansion and dissemination of rapidly proliferating PDAC cells have also been observed in metabolomics studies such as by Martín-Blázquez et al. (Martín-Blázquez et al., 2020).

Lactate dehydrogenase (LDHA) enzyme has shown a 300% increase in flux space in PDAC compared to the healthy pancreas cell model, accelerating lactate production. The overexpression of LDHA in pancreatic cancer and its ability to induce pancreatic cancer cell growth have been reported by Rong et al., in 2013 (Rong et al., 2013). In addition, they showed that knocking down the LDHA in the pancreatic cancer cells significantly inhibited the cell growth revealing the oncogenic trait of LDHA and its association with poor prognosis (Rong et al., 2013). LDHA overexpression and its association with the poor survival outcome have also been reported (Mohammad et al., 2016). This finding is also coherent with higher levels of lactate in PDAC cells compared to healthy cells observed through NMR-based metabolomic experiments (Napoli et al., 2012). Although a complete mechanistic insight behind the causal effect of upregulation of LDHA could not be established yet, it potentially serves as an independent prognostic marker of PDAC.

Potential drug repurposing

The uniqueness in gene expression and metabolic profile in PDAC cells allows for an extended search for potential drug-gene interactions. In addition to that, the ever-increasing challenges associated with the therapy-resistance of PDAC have necessitated the repurposing of old drugs. Leveraging the development in the various data-driven approaches, drug repurposing is becoming an efficient way of drug discovery which is cost effective. We identified 25 genes associated with poor prognosis in pancreatic cancer which had an overexpression in PDAC (see Table S4 for a complete list). In Figure 6, these genes are shown to be associated with several drugs currently in use in humans, which are at different stages of the approval process. The edges connecting the drug to the genes indicated the evidence of repressive effects on the genes, according to the DrugBank Pharmaco-transcriptomic database (Wishart et al., 2018). Several of these drugs have potential synergistic association between each other, as shown in Figure 6. These non-oncology drugs can potentially target not only known but also hitherto unknown vulnerabilities in pancreatic cancer. Although many of the drugs are either approved (*e.g.*, Ofloxacin, Ciprofloxacin) or at the investigational stage (*e.g.*, Puromycin) for treating other diseases in the human body, some of these drugs (*e.g.*, troglitazone) has been withdrawn from the market because of risk of severe liver failure that can be fatal (Funk et al., 2001; Gale, 2006). Nonetheless, they are still included in this association study, because newer studies have revealed anti-proliferative activities of the derivatives of this drug in other cancer types (Salamone et al., 2012; Mazerbourg et al., 2016; Bordessa et al., 2014; Colin et al., 2010), which can result in an improved benefit-to-risk ratio for these drugs as well as suggest new drug combinations for reduced hepatotoxicity (Saha et al., 2012).

Because these drug-gene associations are predicted in different tissue or disease systems and are a result of text mining through literature, we furthered our analysis of these associations by validating their effect on the fitness of the pancreatic cancer cell. To this end, we checked the inhibition effect on PDAC biomass when each of these genes are knocked out. The strongest growth inhibiting effect was observed when *SLC2A1* was knocked out, resulting in a no-growth phenotype during out model simulations. *SLC2A1* encodes major small sugar transporters across cellular membranes and between cellular organelles (Kapoor et al., 2016; Klepper et al., 1999; Lee et al., 2015; Mueckler and Makepeace, 2008; 2009). With its broad substrate specificity, *SLC2A1* can transport a wide range of aldoses including both pentoses and hexoses (Mueckler and Makepeace, 2009). This is not only a rate limiting factor in sugar transport (Klepper et al., 1999; Koch et al., 2015), promoting aggressive tumor proliferation but also have been observed to be deregulated in pancreatic ductal adenocarcinoma (Nagarajan et al., 2017). Therefore, *SLC2A1* appears to be a high-confidence target for repositioning of the drugs repressing its expression, including fluoroquinolone-based antibiotics Ofloxacin and Ciprofloxacin. Other moderately growth-inhibiting genetic perturbations include monocarboxylate transporter *(SLC16A1)*, which is responsible for catalyzing the proton-linked transport of monocarboxylates such as L-lactate, pyruvate, and the ketone bodies (Vijay and Morris, 2014); Methylenetetrahydrofolate Dehydrogenase (*MTHFD1)*, which is closely coupled with nuclear *de novo* thymidylate biosynthesis (Field et al., 2015); and Cytochrome *c* Oxidase Subunit 6B2 (*COX6B2*), which accelerates oxidative phosphorylation, NAD + generation, and cell proliferation (Cheng et al., 2020). The growth inhibition study points out the limitations that arise from the uncertainties of genome-scale metabolic networks as well as the gaps in our knowledge about drug interactions.

Pancreatic ductal adenocarcinoma (PDAC) is the most common type of pancreatic cancer, with late diagnosis, early metastasis, insufficient therapy response, and very low survival rates. Owing to these challenges associated with the diagnosis and treatment of PDAC, it has been a research area of great interest. With the goal of understanding the metabolic reprogramming in proliferating PDAC cells, we reconstructed contextualized healthy and PDAC models by incorporating patient transcriptomic data into a genome-scale global human metabolic model. Comparing the metabolic flux space for the reactions in the two context-specific models, we identified significantly divergent pathways in PDAC. These results allowed us to further investigate signatory genes in PDAC and identify potential drug combinations that can be repositioned for treatment of PDAC.

Limitations of the study

In this study, we attempted to identify a few poorly explored metabolic traits of PDAC cells, which can potentially complement the ongoing effort of finding novel therapeutic targets against pancreatic cancer. Although many aspects of the pancreatic tumor progression have been studied with help of transcriptomics, proteomics, and metabolomics, this metabolic model-based study helps unravel the reactome layer of biochemical features that are associated with PDAC. It is to be noted that this systems-level metabolic

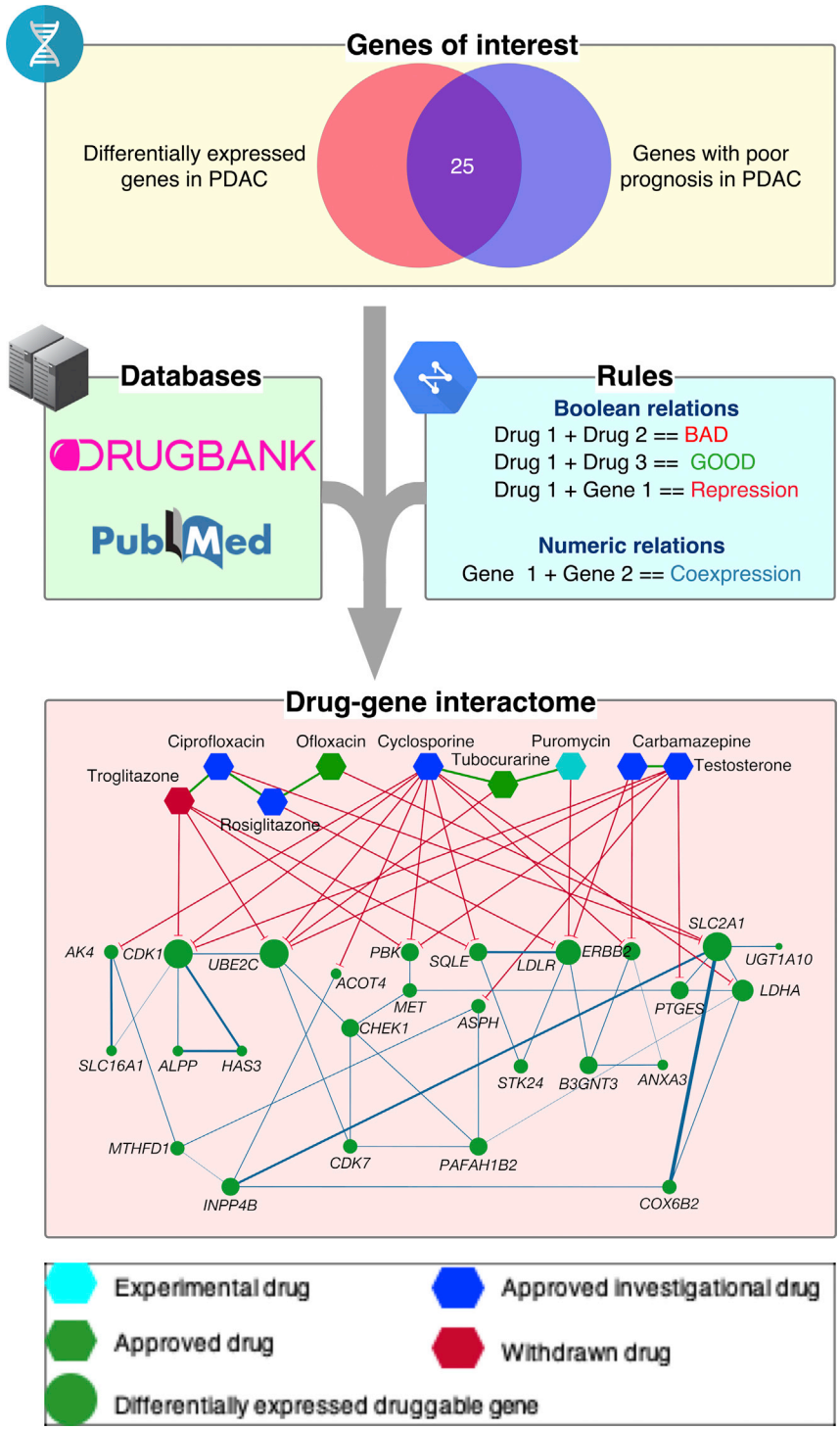

Figure 6. Potential drug interactions with upregulated genes in PDAC with poor prognosis

Edge thickness between the genes denotes the correlation coefficient and the size of the nodes denote the magnitude of the gene expression fold change value in PDAC.

analysis incorporates a relatively small sample size of clinical data. Although this study allows us to assess the genome-scale changes in metabolism under tumor progression and therefore can unravel previously unknown mechanistic insights into cancer cell proliferation as well as identify potential drug associations

and synergistic drug combinations that can be repurposed, our results require further experimental validation and if proven useful, potential future clinical studies to be undertaken. A better understanding of the metabolic dependencies needed to survive harsh conditions will uncover metabolic vulnerabilities and guide alternative therapeutic strategies.

DATA AVAILABILITY

All data generated or analyzed during this study are included in this published article and its supplemental information files.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2022.104483.

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AUTHOR CONTRIBUTIONS

Conceptualization, R.S. and P. K. S.; Methodology, M.M.I. and A.G.; Software, M.M.I. and A.G.; Validation, M.M.I. and A.G.; Formal Analysis, M.M.I. and A.G.; Investigation, M.M.I. and A.G.; Resources, R. S.; Writing – Original Draft, M.M.I. and A.G.; Writing – Review & Editing, M.M.I., R. S., and A.G.; Visualization, M.M.I. and A.G.; Supervision, R. S.; Project Administration, R. S. and P. K. S.; Funding Acquisition, R. S. and P. K. S.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Transcriptomic dataset for healthy and PDAC cells	National Cancer Institute GDC data portal	https://bit.ly/3L54KPf
Software and algorithms		
iMAT	Zur et al. (2010)	PMID: 21081510

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Rajib Saha (rsaha2@unl.edu).

Materials availability

This study did not generate any new unique reagent.

Data and code availability

This paper analyzes existing, publicly available data. These accession numbers for the datasets are listed in the key resources table. This paper does not report original code. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Study species

We studied pancreatic ductal adenocarcinoma (PDAC) and healthy pancreatic cells upon gathering publicly available gene expression data (as mentioned below).

METHOD DETAILS

Transcriptomic data processing

Transcriptomic data of 18 individuals (16 PDAC, 2 healthy normal) was obtained from the Cancer genome atlas (https://www.cancer.gov/tcga). The Fragments Per Kilobase of transcript per Million mapped reads were used as the input of differential gene expression analysis. The transcriptomic data included FPKM information for 60,483 genes for each of the samples. The FPKM values were filtered to exclude the genes with zero expression values throughout samples. The DESeq algorithm in R software package "Bioconductor" was used for differential gene expression analysis (Anders and Huber, 2010). DESeq employs negative binomial distribution and a shrinkage estimator for the distribution's variance methods to test for differential expression (Anders and Huber, 2010). Genes with a $\log_2$ (foldchange) value of 2 or higher were considered overexpressed and genes with a $\log_2$ (foldchange) value of -2 or lower were considered underexpressed, while satisfying an adjusted p-value of <0.05 (Rodriguez-Esteban and Jiang, 2017). Heatmap was generated using Morpheus (https://software.broadinstitute.org/morpheus) from the Broad Institute.

Co-expression analysis with regulatory genes

A threshold of >0.7 was used on Pearson's correlation coefficient with a p value of <0.05 for the development of the co-expression networks. Correlation clusters were developed grouping highly correlated genes to produce the co-expression networks. Network visualization was performed in Cytoscape (Shannon et al., 2003) version 3.8.2 with manual repositioning. Gene expression data was visualized with varying node sizes, and correlation coefficients between genes were visualized with edge color and thickness.

Preliminary pancreas metabolic reconstruction

A genome-scale metabolic model of a pancreatic cell describing reaction stoichiometry, directionality, and gene-protein-reaction (GPR) association was built by mapping these transcriptomic datasets to the latest

global human metabolic model, Human1 (Robinson et al., 2020). This global human model contains 13,417 reactions, 10,135 metabolites, and 3,628 genes, as of the github repository down in December 2020. This tissue-specific pancreas metabolic reconstruction was obtained from the Human1 model using the Integrative Metabolic Analysis Tool (iMAT) (Zur et al., 2010). iMAT is optimization-based too that enables the integration of transcriptomic and proteomic data with genome-scale metabolic network models to predict the metabolic fluxes based on gene expression and user-defined objective functions. While many other methods have been developed for contextualizing genome-scale metabolic reconstructions, no one method outperforms others in every situation. We compared iMAT metabolic reconstructions with another popular method, GIMME, and the overall findings of the study did not change significantly (numerical results are included in Table S5). iMat is readily available and user-friendly package in the COBRA toolbox that has been used in this study. First, the reactions from the Human1 model were assigned artificial "expression values" (see Zur et al., 2010 (Zur et al., 2010) for details) based on their associated gene and its corresponding expression values in the TCGA data. These expression values were then grouped into 3 categories: highly expressed, moderately expressed, and lowly expressed. Expression values greater than half a standard deviation above the mean were considered highly expressed and assigned a value of 1. Expression values less than half a standard deviation below the mean were considered lowly expressed and assigned a value of -1. Expression values that fell within a half a standard deviation of the mean were considered moderately expressed and assigned a value of 0. The expression for the Human1 biomass reaction was manually set to 1 so the biomass equation and all the other necessary reactions producing biomass precursors are included in the model. The iMAT algorithm then generated a model using the reaction expression information and reactions in the Human1 model.

Flux Balance Analysis

Flux Balance Analysis (FBA) (Orth, Thiele and Palsson, 2010) was used to analyze the model performance during the different stages of refinement. The model was represented by a stoichiometric matrix, where the columns were representative of metabolites, and the rows representative of reactions. Constraints were imposed on the reactions given by upper and lower bounds for each based on nutrient availability and other conditions. FBA gives the flux value for each reaction in the model according to the following optimization formulation:

$$\begin{aligned} &maximize\ (v_j) \quad v_{biomass} \\ &subject\ to \\ &\quad \sum_{j \in J^k} S_{ij} \bullet v_j = 0 \quad \forall i \in I \\ &\quad LB_j \le v_j \le UB_j \quad \forall j \in J \end{aligned}$$

In this formulation, I is the set of metabolites and J is the set of reactions in the model. S_{ij} is the stoichiometric coefficient matrix representing a model with i metabolites and j reactions, and v_j is the flux value of each reaction. The objective function, $v_{biomass,}$ is representative of the growth rate of an individual cell. LB_j and UB_j are the minimum and maximum flux values allowed for each reaction.

Model curation

The consensus model was curated through the classic design-build-test-refine cycle (Thiele and Palsson, 2010) to accurately reflect the metabolic capabilities of a pancreatic cell. Three reactions contained imbalances either in their stoichiometries or molecular formulas, and these imbalances were rectified. For reactions with imbalances caused by stoichiometric inaccuracies, changes were made to the stoichiometric coefficient matrix of the model. For reactions whose imbalances were due to incorrect molecular formulas, fixes were applied to the metabolic formula section of the model (see details in Table S2).

Thermodynamically infeasible cycles (TICs) are groups of reactions whose products, reactants, and directionality create a loop that allows unlimited flux to pass through each reaction, yielding no net consumption or production of metabolites. The presence of these cycles allows for many reactions in the model to occur at a very high rate even through the nutritional input to the model is negligible (or zero), which is unrealistic. These reactions are called unbounded reactions. It is important to eliminate these cycles to ensure the flux values for each reaction are thermodynamically feasible. Flux Variability Analysis (FVA) was performed on the model to identify mathematically possible flux ranges of the reactions in the model as well as identify the unbounded reactions. Unbounded reactions are characterized by flux distributions that hit the upper and/or lower bounds in FVA when all the metabolic uptake reactions are turned off. This initial analysis

revealed 1444 unbounded reactions in the model, across multiple pathways including transport, fatty acid oxidation, nucleotide metabolism, and drug metabolism. The thermodynamically infeasible cycles comprising these unbounded reactions were identified using OptFill (Schroeder and Saha, 2020). OptFill identifies TICs through iteratively identifying the smallest number of reactions with nonzero flux for which the sum of their fluxes is 0. All uptakes are turned off for OptFill so that all reactions carrying high flux are involved in a TIC. These cycles were eliminated by i) removing duplicate reactions from the model(s), ii) restricting reaction directionality if there is literature evidence of thermodynamic information, iii) removing erroneous reactions, and iv) using correct cofactors in reactions (for example NAD vs NADP) if that information is available (complete details in Table S2). 932 reactions were modified in total. 609 reactions were turned off because they were duplicates of other reactions or lumped reactions. 23 reactions that were initially irreversible were made reversible if there was literature evidence indicating their reversibility. 286 reactions that were initially reversible were made irreversible in the forward direction, and 14 initially reversible directions were made irreversible in the backward direction. When turning reactions off to fix cycles, it was ensured that all essential reactions remained active in the model.

Drug interaction analysis

From the list of differentially expressed genes in the PDAC model, those associated with poor prognosis were identified using the Human Protein Atlas (http://www.proteinatlas.org). The differentially expressed genes associated with poor prognosis were then identified as potential therapeutic targets. For each of these genes, a list of drugs and their activation or repression effects were obtained from the DrugBank Pharmaco-transcriptomic database (Wishart et al., 2018) as well as the published literature in PubMed. The relevant entries on all the drugs associated with these genes the retrieved database based on the co-occurrence between the drugs by using natural language processing in Python. For instance, if a drug *D* represses gene *G1*, and gene *G1* is correlated to another gene *G2*, then drug *D* may have a repressive effect on *G2* as well. Cytoscape (Shannon et al., 2003) version 3.8.2 with manual repositioning was used to create the drug-gene interactome in Figure 6.

Software and hardware resources

The General Algebraic Modeling System (GAMS) (Bussieck and Meeraus, 2004) version 24.7.4 was used to run FBA, FVA, and the OptFill algorithm on the model. GAMS was run on a high-performance cluster computing system at the Holland Computing Center of the University of Nebraska-Lincoln. The COBRA Toolbox (Becker, 2007; Schellenberger et al., 2011) version 3.0 in Matlab version 9.6.0.1174912 (R2019a) was used to run iMAT (Zur, Ruppin and Shlomi, 2010), identify essential reactions and reaction imbalances, and run FBA and FVA on the model.

QUANTIFICATION AND STATISTICAL ANALYSIS

Hypergeometric test for reaction enrichment analysis

Hypergeometric enrichment test was used to identify reaction pathways which are overrepresented in the set of reactions with altered flux space. The list of reactions with changing flux spaces obtained from running flux variability analysis was used to conduct a two-tailed hypergeometric test. This test was used to obtain the pathways showing significant representation in the list of altered reactions.

$$P(X = k) = \frac{\binom{K}{k}\binom{N-K}{n-k}}{\binom{N}{n}}$$

In this equation, $P(X = k)$ is the probability that there are k reactions by chance with altered flux space in a given subsystem. K is the total number of reactions in a given subsystem, N is the total number of reactions in the model, and n is the total number of reactions in the model with altered flux space. The hypergeometric test was conducted for overrepresentation in each pathway in the model. For $P(X = k) < 0.05$, it is likely that the subsystem is over-represented due to a high number of altered reactions in the pathway rather than by chance. The p values were then subjected to multiple-hypothesis correction using Benjamini-Hochberg method (Benjamini and Hochberg, 1995) using False Discovery Rate with $\alpha = 0.05$. From this, a list of pathways in the model most affected by PDAC was obtained.