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*Review Article*

# Signaling pathways in pancreatic ductile adenocarcinoma and potential therapeutic targets

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## ABSTRACT

Pancreatic ductile adenocarcinoma (PDAC) has a dismal prognosis, with an overall 5-year survival of <10%. At present, PDAC is treated using systemic chemotherapeutic regimens, which have shown survival benefit in clinical trials. Unfortunately, the survival benefit offered by the current standards do not greatly impact the 5-year overall survival statistics with the disease and are associated with toxicity. The large majority of PDACs are associated with a mutation in Kirsten Ras (KRAS), which results in constative activation of downstream signaling resulting in oncogenesis, tumor progression, cellular survival, and metastasis. Due to the lack of druggable sites, designing direct KRAS inhibitors have proven difficult and extensive effort has been placed in finding upstream or downstream targets as potential therapeutic avenues. The epidermal growth factor receptor (EGFR), hedgehog (HH), and mTOR signaling pathways have all gained recent attention as potential candidates for targeted PDAC therapies. Erlotinib, an EGFR small-molecule inhibitor, has shown promise in preclinical studies against PDAC. It is currently the only Food and Drug Administration (FDA) approved targeted therapy for PDAC when used in conjunction with gemcitabine. However, clinical trials comparing erlotinib plus gemcitabine to gemcitabine alone have demonstrated only modest statistical significance in overall survival. Due to the unique hypovascular microenvironment in PDAC, designated by the term desmoplasia, the HH signaling pathway has also gained recent research interest. Recent studies have shown lithium, a divalent cation originally FDA approved for bipolar disorder, to inhibit PDAC progression through its mechanism of glycogen synthase 3 inhibition in the HH pathway. Metformin, a biguanide medication used in type II diabetes mellitus, has been shown to inhibit mammalian target of rapamycin complex 1 (mTORC1) signaling indirectly through its activation of AMPK. Preclinical studies have demonstrated tumor regression, induction of apoptosis, and effects on the microenvironment in PDAC through the inhibition of mTORC1 by metformin. We present compelling scientific rationale, based on unique signal transduction pathways, tumor pathophysiology, and therapeutics potential for the combination of erlotinib, lithium, and metformin for the treatment of PDAC.

**Keywords:** Heat shock proteins, Metformin, Microenvironment, Pancreatic cancer, Signaling pathways, Therapeutic targets

## INTRODUCTION

Despite extensive research efforts, pancreatic cancer remains highly resistant to targeted as well as traditional therapies. As of 2018, some epidemiological studies have ranked pancreatic cancer as the 14th most common, while the GLOBICON estimates are as high as 11th with 458,918 new cases and 432,232 deaths worldwide.[1,2] With the incidence continuing to rise, pancreatic cancer is projected to become the second leading cause of cancer-related death by the year 2020.[3] At 90% of all pancreatic cancers reported, pancreatic ductile adenocarcinoma

(PDAC) accounts for the overwhelming majority.[4] PDAC has a dismal prognosis, with 24% of people living 1 year and 5-year survival of <10%.[1,5] Unfortunately, successful screening methods are lacking, and most people with PDAC are diagnosed at more advanced stages of the disease. Although not guaranteed to be a curative measure, surgical resection remains a mainstay of PDAC treatment. However, only 15–20% of patients are eligible for immediate surgical resection, and tumor relapse rates are extremely high post-surgery with a median survival rate of only 8–10 months.[6] A significant effort over the past few decades has been put forth to develop adjuvant therapies which extend the overall survival for PDAC patients. The current status of adjuvant therapy for PDAC involves systemic chemotherapies. In patients deemed physically capable, the current standard of care is a combination of modified folinic acid, 5-fluorouracil, irinotecan, and oxaliplatin, which has been given the name modified FOLFIRINOX (mFOLFIRINOX).[7] Patients who are unable to tolerate the adjuvant mFOLFIRINOX regimen are treated using the pyrimidine analog gemcitabine in combination with capecitabine.[6,7]

Extensive efforts have been made to understand the genetic driver mutations and signaling pathways involved in PDAC. Among the gene mutations studied, the four most prominent are Kirsten Ras (KRAS), CDKN2A, SMAD4, and TP53. Of the four most prominent mutations, KRAS is mutated in 90% of PDACs.[8] Mutations in KRAS result in the transcription of a constitutively activated GTPase which, when locked in a GTP-bound conformation, results in uncontrolled activation of downstream signaling pathways which ultimately promote oncogenesis, metastasis, and tumor progression.[4,8,9] Because KRAS mutations are represented in the majority of PDACs, a significant amount of work has been done to find clinically relevant targeted therapies. Unfortunately, attempts at designing direct KRAS inhibitors have been disappointing as RAS proteins lack significant druggable sites which would allow small-molecule inhibitors to be efficacious.[9] Therefore, most of the research effort has been spent developing upstream and downstream targets in the KRAS pathway. Of note, there has been some relative preclinical and clinical success with the EGFR small-molecule tyrosine kinase inhibitor erlotinib used in combination with gemcitabine against PDAC. The erlotinib and gemcitabine combination is currently Food and Drug Administration (FDA) approved in the treatment of PDAC.[8,10,11] Other aspects of KRAS signaling pathways have also been studied as potential druggable targets, some of which already have FDA approved drugs on the market. Examples explored in this article have been studied with the purpose of potentially repurposing drugs, which are already FDA approved for other pathologies that could act synergistically with erlotinib to inhibit KRAS-related signaling pathways in PDAC. Metformin, a biguanide drug used in the first-line treatment of type II diabetes mellitus, has been heavily researched for the drug's role in mammalian target of rapamycin complex 1 (mTORC1) inhibition through AMPK activation.[12-15] Several studies have demonstrated metformin's efficacy in prohibiting PDAC cell growth and proliferation, while other studies have demonstrated synergism between metformin and erlotinib in other cancer cell types.[12-15] Lithium, a divalent cation FDA approved in the clinical treatment of bipolar disorder, has also been studied as a potential repurposed drug for PDAC due to the inhibition of glycogen synthase (GSK)-3β, an important regulator of many signaling pathways.[16-19]

An increasing amount of interest has been placed on the unique microenvironment of PDAC. Pancreatic stellate cells (PSCs) have been shown to be a key regulator in orchestrating the accumulation of collagens, fibronectin, and laminin to form a dense stroma in a process termed desmoplasia.[15,20] The thick stroma created by desmoplasia creates a hypovascular environment, which has been implicated in PDAC resistance to current treatments. The PDAC tumor microenvironment displays immunosuppressive qualities and several studies have linked the regulatory T-cells role in the release of transforming growth factor-beta (TGF-β) via the hedgehog (HH) signaling pathway.[15,21,22] Heat shock proteins (HSPs) have been implicated in many cancers. HSP is a family of chaperone proteins, which are activated in times of cellular stress and act to prevent cellular damage and protect function.[23] Several studies have demonstrated how HSP may play a role in PDAC resistance to treatment with gemcitabine.[24,25] Other HSPs play a role in cellular survival signaling, including NF-κβ. It has been hypothesized how PDAC, among other cancers, can hijack these protective mechanisms to avoid apoptosis.[26,27] In this article, we review the brief history of adjuvant therapy in PDAC as well as the current standards, and KRAS signaling pathways with a specific focus on potential candidates for targeted therapies. We will also review the PDAC tumor microenvironment, the process of desmoplasia, the connection to HH signaling, and the role of HSP in PDAC.

## CURRENT STANDARDS

As discussed by Klaiber *et al*., clinical trials for the use of adjuvant therapy in PDAC were performed as early as the 1970s. From 1974 until 1982, the first randomized trial to compare chemoradiotherapy and observation took place. The results showed prolonged survival in the treatment cohort (21.0 vs. 10.9 months), but the trial was terminated prematurely due to poor accrual[6,28] [Table 1]. Although several subsequent trials were performed to analyze the use of chemotherapy as an adjuvant treatment, the first high impact study which compared gemcitabine against 5-fluorouracil was published in 1997.[7] In this study, 126 patients were

**Table 1:** Major clinical trials in the development of current PDAC standards.

| References | Year published | Drugs used | Median OS (months) |
|---|---|---|---|
| Kaiser *et al*.[6,28] | 1985 | Chemoradiotherapy+5- Fluorouracil+Maintenance 5-Fluorouracil; Observation | 21; 10.9 |
| Burris *et al*.[29] | 1997 | Gemcitabine; 5-Fluorouracil | 5.65; 4.41 |
| Neoptolemos *et al*.[30] | 2010 | 5-Fluorouracil/folic acid; gemcitabine | 23; 23.6 |
| Neoptolemos *et al*.[31] | 2017 | Gemcitabine; gemcitabine/capecitabine | 25.5; 28.0 |
| Conroy *et al*.[34] | 2011 | Irinotecan+Oxaliplatin+Leucovorin+5-Fluorouracil (FOLFIRINOX); Gemcitabine | 11.1; 6.8 |
| Conroy *et al*.[35] | 2018 | mFOLFIRINOX; gemcitabine | 54.4; 35 |

randomized to either receive weekly gemcitabine at a dosage of 1000 mg/m$^2$, followed by a week of rest, then subsequent gemcitabine treatments every 3 out of 4 weeks or 5-fluorouracil at a weekly dose of 600 mg/m$^2$.[29] As described in the study, the results demonstrated a clinical benefit of 23.8% in the patients treated with gemcitabine compared to 4.8% in those treated with 5-fluorouracil (5.65–4.41 months of median overall survival)[7,29] [Table 1].

The 1997 trial marked the beginning of increased interest in gemcitabine as adjuvant therapy in PDAC. However, it was not until the second version of the ESPAC-3 clinical trial, which occurred during the recruitment period of 2000 through 2007, when gemcitabine became the treatment of choice for PDAC following surgical resection.[6] At the time of the trial, ESPAC-3 (v.2) was the largest adjuvant therapy study for PDAC ever conducted, in which 1088 patients from 16 different countries were randomized into two different treatment groups with either 5-fluorouracil/folinic acid or gemcitabine.[30,31] Interestingly, the results of the ESPAC-3 trial showed no significant difference in survival between patients treated with gemcitabine compared with 5-fluorouracil/folinic acid (23.0 months for 5-FU/FA vs. 23.6 months for gemcitabine) [Table 1]. However, the grade ¾ toxicities were significantly higher in the 5-fluorouracil/folinic acid treatment group.[6,30] The ESPAC-4 trial was conducted from 2008 until 2014. In this trial, 730 post-PDAC resection patients were treated with either a combination of gemcitabine plus oral capecitabine or gemcitabine alone.[32] The median survival was significantly improved in the patients taking the combination therapy with a median overall survival of 28.0 months compared with gemcitabine only treatment[6,32] [Table 1]. In 2013, a nanoparticle-bound taxane in combination with gemcitabine was shown to enhance overall survival by 2 months when compared to gemcitabine monotherapy.[33] The results of the clinical trials, further, justified the use of gemcitabine as adjuvant therapy for PDAC.

In 2011, Conroy *et al*. published the results of a landmark clinical trial, in which 342 patients were either treated with a combination therapy of irinotecan (180 mg/m$^2$), oxaliplatin (85 mg/m$^2$), leucovorin (400 mg/m$^2$), and 5-fluorouracil as an initial 400 mg/m$^2$ bolus, followed by a continuous infusion of 2400 mg/m$^2$ or monotherapy of gemcitabine at 1000 mg/m$^2$ weekly for 7 of 8 weeks and then weekly for 3 of 4 weeks.[7,34] The survival benefit from the combination therapy, termed FOLFIRINOX, was significant with overall survival of 11.1 months compared to 6.8 months in the gemcitabine treatment group[7,34] [Table 1]. Due to the significant hematologic toxicities and diarrhea associated with FOLFIRINOX, Conroy *et al*. followed up ± previously described original ACCORD11/PRODIGE4 study with another clinical trial, in which the bolus of 5-fluorouracil was removed. This mFOLFIRINOX regimen resulted in an overall survival of 54.4 months compared with gemcitabine monotherapy at 35.0 months[6,35] [Table 1]. Several groups have also reported reducing the toxicity of the original FOLFIRINOX regimen by reducing the dosage of irinotecan.[7] The mFOLFIRINOX treatment regimen is currently the standard of care for PDAC adjuvant therapy in patients deemed fit enough, whereas gemcitabine therapies are being used when mFOLFIRINOX is considered too toxic.[6,7]

## TUMOR MICROENVIRONMENT

The microenvironment of PDAC is relatively unique among neoplasms due to the hypovascular nature created by a dense stroma which consequently limits the ability of therapeutic agents to reach specified targets.[20] The cellular components of PDAC that is responsible for the microenvironment include myeloid-derived suppressor cells, tumor-associated macrophages, regulatory T-cells (Tregs), and PSCs.[21] As mentioned in the introduction, studies have indicated how PSCs are largely responsible for the accumulation of extracellular matrix (ECM) constituents.[21,22] As reported by Murakami *et al*. PSCs are one of the types of cancer-related fibroblasts (CAF) represented in the PDAC microenvironment, in which the fibroblastic population can be as high as 90%. The role of CAF, such as PSC, has been shown to contribute to epithelial-mesenchymal transition and metastasis by the secretion of proteoglycans, collagen types I and III, fibronectin, and glycosaminoglycans which are believed to add a mechanical force in the ECM for

cancer cellular migration.[22] The process of ECM growth and hypovascularization is described in the literature as desmoplasia.[15,20,22]

Immunosuppression is also a hallmark of the PDAC microenvironment. As described by Murakami *et al*., the normal immune response against tumors consists of the antigen-presenting cells' antigen containing MHC I molecule interacting with cytotoxic T-cells ($CD8^+$ T-cells). In PDAC, there is a decrease in MHC I expression, which blunts the response of $CD8^+$ T-cells, causing disruption to the induction of apoptosis through the extrinsic cascade through the expression of Fas ligand and subsequent excretion of perforin and granzyme, as well as the $CD8^+$ T-cell release of cell checkpoint inhibitors.[22] The immunosuppressive cytokine TGF-β is also implicated in the lack of normal immune responses in PDAC.[21] TGF-β is secreted by Tregs and in the normal immune response is responsible for preventing autoimmune reactions through the process of self-tolerance.[21] In the PDAC microenvironment, the accumulation of TGF-β secreting Tregs is associated with tumor aggressiveness and a poor prognosis that attributed to the immunosuppressive effects of TGF-β as well as the stimulation of CAF by TGF-β.[21,22]

The HH signaling pathway plays a role in the desmoplastic reaction of PDAC. Originally discovered in *Drosophila*, the HH pathway has been shown to be an important regulator of development in animals by controlling segmental pattern formation.[16,19] As eloquently described by McCubrey *et al*., HH signaling begins with the binding of an HH ligand to 12-pass transmembrane receptor patched which stimulates the internalization and destruction of the G-protein coupled receptor Smoothened (SMO). The degradation of SMO, as also described by McCubrey *et al*., promotes the dissociation of glioma-associated oncogene homologue (GLI) from suppressor of fused (SUFU). Once free from SUFU, GLI can be phosphorylated and subsequently activated by PKA, GSK-3, and CK-1. Once activated, GLI can stimulate the transcription of downstream HH genes which promote cellular growth and survival[16] [Figure 1].

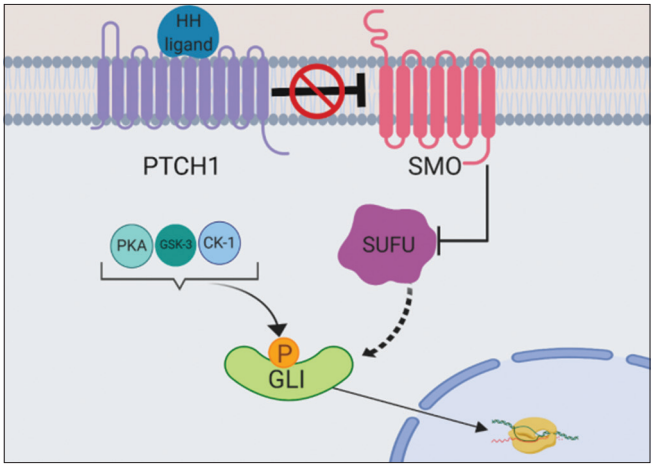


**Figure 1:** HH signaling and the role of GSK-3. When the HH ligand binds the PTCH1 receptor in the HH pathway, SMO is internalized which leads to the release of GLI from SUFU. Once released, PKA, GSK-3, and CK-1 can activate GLI through phosphorylation. Once phosphorylated, GLI can translocate into the nucleus and act as a transcription factor for many downstream targets. Lithium is an inhibitor of GSK-3. HH: Hedgehog, GSK-3: Glycogensynthase 3, PTCH1: Pass transmembrane receptor patched, SMO: Smoothened, GLI: Gliomaassociated oncogene homologue, and SUFU: Suppressor of fused.

In PDAC, the HH pathway stimulates CAF and is considered to play a large role in desmoplasia.[20,22] There has been increasing interest in targeting the HH pathway, and the pathway's inhibition was one of the first successful preclinical approaches to decrease the CAF population in the PDAC microenvironment which decreased the stromal volume and allowed for better delivery of gemcitabine.[22] A popular targets of the HH pathway are through the regulation of GSK-3-beta, which phosphorylates SUFU after HH pathway stimulation and promotes a disassociation from GLI[16] [Figure 1]. Lithium is a divalent cation which was originally FDA approved for the treatment of mania, bipolar, and other depressive disorders since 1970 and was one of the first direct GSK-3 inhibitors described.[16] In several recent studies, lithium has been shown to decrease tumorigenesis in PDAC through GSK-3's role in HH signaling.[17,19] These results show the promise of repurposing drugs such as lithium through the exploitation of the anti-tumorigenic effects in the HH pathway. Unfortunately, after promising preclinical results, the results of several phase II clinical trials, where specific HH inhibitors were employed, were disappointing as tumor progression was not halted, even though desmoplasia was shown to be reduced.[22] The HH pathway is induced by the KRAS pathway through the activation of nuclear factor-kappa light chain enhancer of activated B-cells (NF-Kb)[21] which establish a link between the two pathways. Perhaps, future experiments can explore this connection by finding drugs, which will act synergistically to inhibit PDAC proliferation as well as address desmoplasia and the microenvironment.

## KRAS SIGNALING PATHWAYS

Kirsten rat sarcoma viral oncogene homolog, known as KRAS, a member of the Ras GTPase family, is mutated in PDAC to be constitutively activated in a GTP bound configuration.[8,33,36] The KRAS gene is reported in the literature as being mutated from 90% to 95% of all PDACs.[8,36] Early in PDAC tumorigenesis, as described by Waters and Der, abnormal morphological changes occur in pancreatic epithelial cells, where a transition from a flat to more cuboidal appearance is observed, and mucin is produced. These

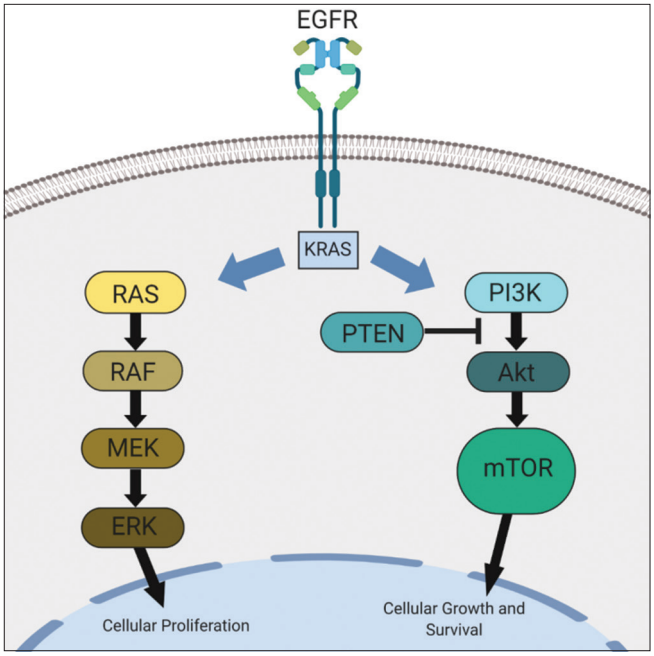


**Figure 2:** KRAS downstream signaling to EGFR. Aberrant KRAS signaling in PDAC can activate downstream targets which stimulate cellular proliferation, growth, and survival. Shown is the well characterized RAS/RAF/MEK/ERk and PI3K/Akt/mTOR pathway. EGFR is a tyrosine kinase transmembrane receptor that has been explored as an upstream target for dysfunctional KRAS signaling using the small-molecule inhibitor erlotinib. KRAS: Kirsten Ras, EGFR: Epidermal growth factor receptor, and PDAC: Pancreatic ductile adenocarcinoma.

Early changes in PDAC morphology are called pancreatic intraepithelial neoplasms (PanINs). KRAS mutations are found in the majority of the early PanINs and are believed to be the driving mutation of most PDACs.[10,33] The downstream effects of KRAS signal transduction dictate cellular growth and survival. RAF-MEK-ERK and phosphatidylinositol-3-kinase (PI3K)/Akt are some of the most well-studied downstream signaling pathways of KRAS[8,9] [Figure 2]. Due to the difficulty in creating a direct KRAS inhibitor, much effort has been put forth to find upstream or downstream targets.[33] In this article, we discuss the EGFR pathway as well as the downstream mTORC1 pathway through AMPK-TSC1 and the potential drugs, which target them.

The EGFR pathway has been well characterized in the literature regarding implications in many solid tumors. EGFR is a membrane-spanning glycoprotein with intrinsic tyrosine kinase activity in the HER (erbB). On binding the ligand extracellularly, EGFR's intracellular domain can undergo homodimerization that results in autophosphorylation and subsequent activation of downstream transduction pathways.[8,11] Some of the most well-studied downstream pathways affected by EGFR include the PI3K-Akt and Ras-Raf-MAPK pathways, which play a large role in cell cycle progression, cellular growth, and survival[8,11] [Figure 2]. As described by Chu *et al*. EGFR has been shown to enhance downstream KRAS signaling. However, inhibition of EGFR is not believed to affect the downstream signaling pathways of KRAS due to constitutive activity, but, interestingly, there has been growing evidence to demonstrate how KRAS driven PDAC tumorigenesis is dependent on EGFR signaling (Ardito *et al*., 2012). A study, by Ardito *et al*., demonstrated how EGFR inhibition effectively eliminated KRAS-dependent PDAC tumorigenesis. The investigators hypothesized how the KRAS dependence on EGFR signaling lies in the control of oncogenic precursors as well as the maintenance of extracellular-signal-regulated kinase (ERK) signaling after tumor initiation.[37] Although not found to be mutated in PDAC, EGFR activity is overexpressed in approximately 90% of all PDAC tumors.[4,9] The relevance of EGFR signaling has led to an increased interest in the possibility of using EGFR inhibitors against PDAC to gain a therapeutic advantage.

Erlotinib is a small-molecule inhibitor of the EGFR intracellular tyrosine kinase domain. Mechanistically, Erlotinib competitively inhibits the intracellular ATP binding sites which prevent autophosphorylation and subsequently disrupt downstream signaling.[11] In 2007, after successful preclinical testing and moderate success with clinical trials, Erlotinib was approved for the treatment of PDAC when coupled with gemcitabine.[11] The PA.3 phase III clinical trial which was largely responsible for the FDA approval of erlotinib against PDAC was conducted by the National Cancer Institute of Canada Clinical Trials Group (NCIC-CTG) and it compared the combination erlotinib plus gemcitabine against gemcitabine monotherapy.[11,38] Although statistically significant with 1-year survival increases from 17% to 23% with erlotinib combination therapy, the overall survival of 6.24 months for the erlotinib combined with gemcitabine was relatively moderate compared with 5.91 months for gemcitabine alone.[38] The more recent CONKO-005 stage III clinical trial, conducted in PDAC patients exclusively after R0 resection, did not show a statistically significant benefit to the use of erlotinib combined gemcitabine against PDAC, although the recurrence of metastatic disease was shown to occur in 76% of the combination therapy group compared to 82% in the group treated with gemcitabine monotherapy.[39] After evaluating findings from both preclinical and clinical studies, future work may be beneficial to explore the possible synergistic effects of erlotinib and other inhibitors of KRAS downstream signaling.

The mechanistic target of rapamycin, or mTOR, is a downstream target of KRAS through the PI3K-Akt pathway. As a conserved member of the PI3K family, mTOR acts as a serine/threonine kinase which, when activated by upstream signals, promotes many physiological functions including cell growth and survival.[36,40,41] The mTORC1, a multi-protein complex consisting of mTOR and a scaffolding protein named Raptor, serves a vital

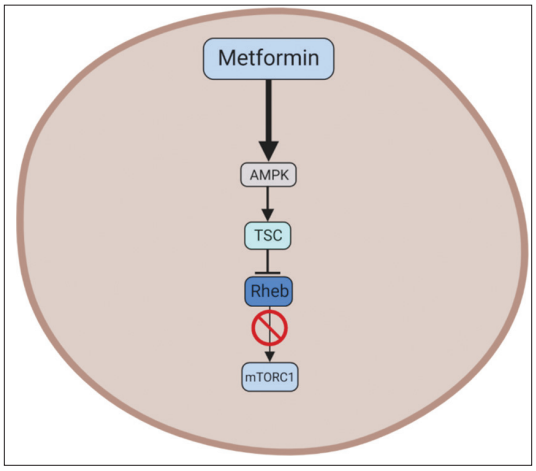


**Figure 3:** Indirect inhibition of mTORC1 by metformin through AMPK. Metformin has a unique mechanism of action through its indirect inhibition of the mTORC1. The stimulation of AMPK leads to the phosphorylation of TSC which inhibits Rheb from activating mTORC1. The disruption of the mTORC1 pathway has been demonstrated to promote tumor regression and induce apoptosis in PDAC cell lines. mTORC1: Mammalian target of rapamycin complex 1, Rheb: Ras homolog enriched in brain, and PDAC: Pancreatic ductile adenocarcinoma.

role in nutrient sensing and regulation of downstream anabolic processes.[42] The upstream regulation of mTORC1 is attributed to the integration of signals by the tuberous sclerosis complex (TSC), a regulator of the mTORC1 activating GTPase Ras homolog enriched in brain (Rheb), through upstream pathways such as PI3K-Akt, RAS-ERK, and the stress response kinase AMPK.[40,41] As discussed by Lu *et al*., targeting of the PI3K/Akt/mTOR pathway using mTOR inhibitors, such as Everolimus, showed efficacy against PDAC tumors *in vivo*. However, a phase II clinical trial showed negligible benefit for patients with gemcitabine resistant PDAC.[36]

Metformin, a biguanide, is the most important drug for treating type II diabetes mellitus worldwide and it has been shown to indirectly stimulate the AMPK pathway through a proposed mechanism of increasing the AMP/ATP ratio through inhibition of complex I of the mitochondrial electron transport chain.[43] Metformin could inhibit mTORC1 through the stimulation of AMPK, which phosphorylates the TSC complex resulting in the inhibition of the mTORC1 activator Rheb[13,43] [Figure 3]. This unique mechanism has led to an increasing epidemiological, preclinical, and clinical support for the use of metformin as an efficacious cancer therapeutic.[43] Metformin has also been shown to inhibit mTORC1 through an AMPK independent mechanism.[12] A retrospective study, by Sadeghi *et al*., reported how PDAC patients with pre-existing diabetes treated with metformin had a statistically significant overall survival benefit of 4.1 months compared to patients not treated with metformin.[44] Another study showed inhibition of DNA synthesis and cell proliferation of PDAC cell lines incubated in a physiological concentration of glucose (5 mM) when treated with metformin, demonstrating the anti-PDAC when treated with therapeutic dosages of metformin.[43] An *in vivo* xenograft study also showed synergism against PDAC when treated with metformin and erlotinib, which resulted in drug target inhibition, tumor regression, and induction of apoptosis.[13] Some studies have even indicated that metformin can suppress desmoplasia in the PDAC microenvironment.[8] Interestingly, a preclinical study demonstrated how metformin-induced AMPK activation could have inhibitory effects on desmoplasia in the PDAC microenvironment by decreasing PDAC cytokine production and subsequent paracrine activation of PSCs.[15]

## HSP IN PANCREATIC CANCER

HSP was discovered in 1962. While working with *Drosophila* in his laboratory, Italian scientist Ferruccio Ritossa noticed how the salivary glands of the *Drosophilia*, which was mistakenly incubated at a high temperature, displayed a characteristic puffing which identified the possibility of higher transcriptional activity.[45,46] Originally, HSP was only thought to function when cells experience stress from high temperatures, but, now, HSP is known to respond to many physiological stresses including hypoxia, ischemia, acidosis, and UV exposure.[45] Recently, an additional role has been ascribed to HSP as danger signals produced and released when cells are under stress and as activators of the immune system. This dual role of HSP as both chaperone and cytokines is now termed chaperokine (read refs below). Once such chaperokine, HSP72, has been demonstrated to enhance the production of cytokines and chemokines as well as to activate and promote maturity of immune cell lines.[47-49] HSP72 binds to receptors with high affinity in natural killer cells, dendritic cells, macrophages, and B-cells which stimulate downstream signal transduction cascades with an increase in intracellular calcium ultimately culminating in the activation of NF-Kb.[49]

HSP is highly evolutionarily conserved molecular chaperones and is classified into different families based on molecular weight.[50] The HSP families which have been identified to have a large physiological role in mammals are HSP90, HSP70, HSP60, HSP40, and other smaller HSP with HSP70 and HSP90 being implicated in PDAC.[27,45,50,51] In neoplastic cells, the expression of HSP70 is elevated and studies have demonstrated how the elevated levels of HSP70 indicate a poorer prognosis in several cancers that could be due to the ability of HSP70 to inhibit apoptosis by affecting the expression of transcription factors involved with the apoptotic BCL2 family.[26] Interestingly, the cellular effects of HSP70 have been demonstrated to be protective of pancreatic acinar cells in the

setting of acute pancreatitis by counteracting the NF-κB- and trypsin-induced inflammatory response, which could lead to cellular necrotic death.[26] The same protective function of HSP70 prevents apoptosis and promotes tumorigenesis in PDAC.[27,45] In a study performed by Aghdassi *et al*., monoclonal antibodies were used to determine HSP70 expression in PDAC cell lines as well as utilize siRNAs against HSP70 to confirm the protective effects against apoptosis. The results of this study demonstrated the activation of the intrinsic apoptotic cascade when PDAC cells were depleted of HSP70.[50] The higher molecular weight HSP90 is also implicated in PDAC and, as a molecular chaperone, is responsible for the folding of many of the important PDAC proteins already mentioned earlier in this article, including EGFR, PI3K, Akt, and GSK-3β.[51,52] A study, conducted by Belalcazar *et al*., used the HSP 90 inhibitor ganetespib and proteasome inhibitor carfilzomib to demonstrate limited tumor growth in KRAS driven PDAC cell lines by inducing autophagy and reduction in PI3K and Akt signaling, leading to the observation of apoptotic biomarkers in the PDAC cell lines studied. The results of Belalcazar's study correlated with the previous research, which demonstrated how HSP90 inhibition leads to the suppression of survival pathways such as PI3K/Akt/mTOR in other cancers.[52,53]

## DISCUSSION

PDAC currently has a poor prognosis, with <10% of patients living up to 5 years after diagnosis.[1,5] With 458,918 new cases and 432,232 deaths worldwide, PDAC is ranked as high as the 11$^{th}$ most common cancer.[1,2] mFOLFIRINOX is the current standard of adjuvant care for treating PDAC in patients fit enough to withstand the associated toxicities and gemcitabine therapies for those who are not.[6,7] Clinical trials have demonstrated how mFOLFIRINOX has an overall survival benefit of 54.4 months compared with 35.0 with gemcitabine alone.[6,34] Despite the improved overall survival benefit, current standards for adjuvant therapy are associated with toxic side effects and are not curative which reinforces the importance of further research into potential targets of the KRAS signaling pathways.

Erlotinib is a small-molecule inhibitor which competes with the intracellular tyrosine kinase domain of EGFR.[11] EGFR has been shown to be over-expressed in 90% of PDAC.[4] Although currently FDA approved for adjuvant PDAC therapy, and despite success in earlier preclinical studies, clinical trials have demonstrated very moderate to no significant difference in overall survival when using erlotinib in conjunction with gemcitabine versus gemcitabine monotherapy.[11,38,39]

Metformin is an important medication for treating type II diabetes mellitus. Through both an AMPK-dependent and AMPK-independent mechanism, metformin has been demonstrated to have inhibitory effects on the mTORC1 pathway.[12,13,43] Preclinical and epidemiological studies have indicated potential for metformin to be re-purposed in the use against PDAC.[43,44] In addition, some studies have demonstrated how metformin can influence the PDAC microenvironment and desmoplasia.[8,15] A study by Lau *et al*. demonstrated synergistic effects when erlotinib and the mTORC1 inhibitor metformin were used together against PDAC. Further, research could be done to explore the synergistic effects of metformin and erlotinib in combination against PDAC.

Lithium, a divalent cation used in the treatment of bipolar disorder, is an inhibitor of GSK-3 which has promise as a potential target in cancer therapy.[16] Several preclinical studies using lithium have demonstrated an effect on PDAC tumorigenesis as well as desmoplasia, which is linked mechanistically to the inhibition of GSK-3 and HH signaling.[17,19] Unfortunately, several phase II clinical trials using HH inhibitors did not show an effect on tumor progression, even though desmoplasia was reduced.[22]

HSP is molecular chaperones responsible for preventing protein misfolding in times of cellular stress.[45,46] The protective effects of HSP70 have been demonstrated to be hijacked by PDAC to enhance tumorigenesis by prevention of apoptosis.[27,45,50] HSP90 has also been demonstrated to influence PDAC tumorigenesis, and inhibition of HSP90 resulted in a decreased signaling expression of the KRAS-related PI3K/Akt/mTOR pathway.[51-53]

## CONCLUSION

This article has discussed the current clinical standards in PDAC, important signaling pathways with special attention on KRAS-related signaling, HH pathway, potential drug targets and the role of HSP in PDAC. Future studies could be designed to target KRAS-related signal transduction pathways utilizing combination therapies to gain insight into potential synergistic responses against PDAC proliferation and survival as well as the PSC induced desmoplasia observed in the PDAC microenvironment.

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### Ethical approval for studies involving humans

This article does not contain any studies with human participants performed by any of the authors.

### Ethical approval for studies involving animals

This article does not contain any studies with animals performed by any of the authors.

### Declaration of patient consent

Patient's consent not required as there are no patients in this study.

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### Conflicts of interest

There are no conflicts of interest.

## REFERENCES

1. Rawla P, Sunkara T, Gaduputi V. Epidemiology of pancreatic cancer: Global trends, etiology and risk factors. World J Oncol 2019;10:10-27.
2. McGuigan A, Kelly P, Turkington RC, Jones C, Coleman HG, McCain RS. Pancreatic cancer: A review of clinical diagnosis, epidemiology, treatment and outcomes. World J Gastroenterol 2018;24:4846-61.
3. Santana-Codina N, Roeth AA, Zhang Y, Yang A, Mashadova O, Asara JM, *et al*. Oncogenic KRAS supports pancreatic cancer through regulation of nucleotide synthesis. Nat Commun 2018;9:4945-.
4. Pelosi E, Castelli G, Testa U. Pancreatic cancer: Molecular characterization, clonal evolution and cancer stem cells. Biomedicines 2017;5:65.
5. Balachandran VP, Beatty GL, Dougan SK. Broadening the impact of immunotherapy to pancreatic cancer: Challenges and opportunities. Gastroenterology 2019;156:2056-72.
6. Klaiber U, Hackert T, Neoptolemos JP. Adjuvant treatment for pancreatic cancer. Transl Gastroenterol Hepatol 2019;4:27.
7. Springfeld C, Jager D, Buchler MW, Strobel O, Hackert T, Palmer DH, *et al*. Chemotherapy for pancreatic cancer. Presse Med 2019;48:e159-74.
8. Fitzgerald TL, Lertpiriyapong K, Cocco L, Martelli AM, Libra M, Candido S, *et al*. roles of EGFR and KRAS and their downstream signaling pathways in pancreatic cancer and pancreatic cancer stem cells. Adv Biol Regul 2015;59:65-81.
9. Chu PC, Kulp SK, Bekaii-Saab T, Chen CS. Targeting integrin-linked kinase to suppress oncogenic KRAS signaling in pancreatic cancer. Small GTPases 2018;9:452-6.
10. Felsenstein M, Hruban RH, Wood LD. New developments in the molecular mechanisms of pancreatic tumorigenesis. Adv Anat Pathol 2018;25:131-42.
11. Kelley RK, Ko AH. Erlotinib in the treatment of advanced pancreatic cancer. Biologics 2008;2:83-95.
12. Ikhlas S, Ahmad M. Metformin: Insights into its anticancer potential with special reference to AMPK dependent and independent pathways. Life Sci 2017;185:53-62.
13. Lau YK, Du X, Rayannavar V, Hopkins B, Shaw J, Bessler E, *et al*. Metformin and erlotinib synergize to inhibit basal breast cancer. Oncotarget 2014;5:10503-17.
14. Elmaci İ, Altinoz MA. A metabolic inhibitory cocktail for grave cancers: Metformin, pioglitazone and lithium combination in treatment of pancreatic cancer and glioblastoma multiforme. Biochem Genet 2016;54:573-618.
15. Duan W, Chen K, Jiang Z, Chen X, Sun L, Li J, *et al*. Desmoplasia suppression by metformin-mediated AMPK activation inhibits pancreatic cancer progression. Cancer Lett 2017;385:225-33.
16. McCubrey JA, Steelman LS, Bertrand FE, Davis NM, Sokolosky M, Abrams SL, *et al*. GSK-3 as potential target for therapeutic intervention in cancer. Oncotarget 2014;5:2881-911.
17. Wang X, Fang Z, Wang A, Luo C, Cheng X, Lu M. Lithium suppresses hedgehog signaling via promoting ITCH E3 ligase activity and Gli1-SUFU interaction in PDA cells. Front Pharmacol 2017;8:820.
18. Wang X, Luo C, Cheng X, Lu M. Lithium and an EPAC-specific inhibitor ESI-09 synergistically suppress pancreatic cancer cell proliferation and survival. Acta Biochim Biophys Sin 2017;49:573-80.
19. Peng Z, Ji Z, Mei F, Lu M, Ou Y, Cheng X. Lithium inhibits tumorigenic potential of PDA cells through targeting hedgehog-GLI signaling pathway. PLoS One 2013;8:e61457.
20. Uzunparmak B, Sahin IH. Pancreatic cancer microenvironment: A current dilemma. Clin Transl Med 2019;8:2.
21. Ren B, Cui M, Yang G, Wang H, Feng M, You L, *et al*. Tumor microenvironment participates in metastasis of pancreatic cancer. Mol Cancer 2018;17:108.
22. Murakami T, Hiroshima Y, Matsuyama R, Homma Y, Hoffman RM, Endo I. Role of the tumor microenvironment in pancreatic cancer. Ann Gastroenterol Surg 2019;3:130-7.
23. Santos TG, Martins VR, Hajj GN. Unconventional secretion of heat shock proteins in cancer. Int J Mol Sci 2017;18:946.
24. Okuno M, Yasuda I, Adachi S, Nakashima M, Kawaguchi J, Doi S, *et al*. The significance of phosphorylated heat shock protein 27 on the prognosis of pancreatic cancer. Oncotarget 2016;7:14291-9.
25. Okuno M, Adachi S, Kozawa O, Shimizu M, Yasuda I. The clinical significance of phosphorylated heat shock Protein 27 (HSPB1) in pancreatic cancer. Int J Mol Sci 2016;17:137.
26. Kumar S, Stokes J 3rd, Singh UP, Gunn KS, Acharya A, Manne U, *et al*. Targeting Hsp70: A possible therapy for cancer. Cancer Lett 2016;374:156-66.
27. Saluja A, Dudeja V. Heat shock proteins in pancreatic diseases. J Gastroenterol Hepatol 2008;23:S42-5.
28. Kaiser MH, Ellenberg SS. Pancreatic cancer: Adjuvant combined radiation and chemotherapy following curative resection. JAMA Surg 1985;120:899-903.
29. Burris HA 3rd, Moore MJ, Andersen J, Green MR, Rothenberg ML, Modiano MR, *et al*. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: A randomized trial. J Clin Oncol 1997;15:2403-13.
30. Neoptolemos JP, Stocken DD, Bassi C, Ghaneh P, Cunningham D, Goldstein D, *et al*. Adjuvant chemotherapy with fluorouracil plus folinic acid vs gemcitabine following

pancreatic cancer resection: A randomized controlled trial. JAMA 2010;304:1073-81.
31. Neoptolemos J, Büchler M, Stocken DD, Ghaneh P, Smith D, Bassi C, *et al*. ESPAC-3(v2): A multicenter, international, open-label, randomized, controlled Phase III trial of adjuvant 5-fluorouracil/folinic acid (5-FU/FA) versus gemcitabine (GEM) in patients with resected pancreatic ductal adenocarcinoma. J Clin Oncol 2009;27:LBA4505.
32. Neoptolemos JP, Palmer DH, Ghaneh P, Psarelli EE, Valle JW, Halloran CM, *et al*. Comparison of adjuvant gemcitabine and capecitabine with gemcitabine monotherapy in patients with resected pancreatic cancer (ESPAC-4): A multicentre, open-label, randomised, phase 3 trial. Lancet 2017;389:1011-24.
33. Waters AM, Der CJ. KRAS: The critical driver and therapeutic target for pancreatic cancer. Cold Spring Harb Perspect Med 2018;8:a031435.
34. Conroy T, Desseigne F, Ychou M, Bouché O, Guimbaud R, Bécouarn Y, *et al*. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. N Engl J Med 2011;364:1817-25.
35. Conroy T, Hammel P, Hebbar M, Ben Abdelghani M, Wei AC, Raoul JL, *et al*. FOLFIRINOX or gemcitabine as adjuvant therapy for pancreatic cancer. N Engl J Med 2018;379:2395-406.
36. Lu S, Ahmed T, Du P, Wang Y. Genomic variations in pancreatic cancer and potential opportunities for development of new approaches for diagnosis and treatment. Int J Mol Sci 2017;18:1201.
37. Ardito CM, Grüner BM, Takeuchi KK, Lubeseder-Martellato C, Teichmann N, Mazur PK, *et al*. EGF receptor is required for KRAS-induced pancreatic tumorigenesis. Cancer Cell 2012;22:304-17.
38. Moore MJ, Goldstein D, Hamm J, Figer A, Hecht JR, Gallinger S, *et al*. Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: A Phase III trial of the national cancer institute of Canada clinical trials group. J Clin Oncol 2007;25:1960-6.
39. Sinn M, Bahra M, Liersch T, Gellert K, Messmann H, Bechstein W, *et al*. CONKO-005: Adjuvant chemotherapy with gemcitabine plus erlotinib versus gemcitabine alone in patients after R0 resection of pancreatic cancer: A multicenter randomized Phase III trial. J Clin Oncol 2017;35:3330-7.
40. Han J, Wang Y. mTORC1 signaling in hepatic lipid metabolism. Protein Cell 2018;9:145-51.
41. Ben-Sahra I, Manning BD. mTORC1 signaling and the metabolic control of cell growth. Curr Opin Cell Biol 2017;45:72-82.
42. Howell JJ, Hellberg K, Turner M, Talbott G, Kolar MJ, Ross DS, *et al*. Metformin inhibits hepatic mTORC1 signaling via dose-dependent mechanisms involving AMPK and the TSC complex. Cell Metab 2017;25:463-71.
43. Sinnett-Smith J, Kisfalvi K, Kui R, Rozengurt E. Metformin inhibition of mTORC1 activation, DNA synthesis and proliferation in pancreatic cancer cells: Dependence on glucose concentration and role of AMPK. Biochem Biophys Res Commun 2013;430:352-7.
44. Sadeghi N, Abbruzzese JL, Yeung SC, Hassan M, Li D. Metformin use is associated with better survival of diabetic patients with pancreatic cancer. Clin Cancer Res 2012;18:2905-12.
45. Giri B, Sethi V, Modi S, Garg B, Banerjee S, Saluja A, *et al*. Heat shock protein 70 in pancreatic diseases: Friend or foe. J Surg Oncol 2017;116:114-22.
46. Hoter A, El-Sabban ME, Naim HY. The HSP90 family: Structure, regulation, function, and implications in health and disease. Int J Mol Sci 2018;19:2560.
47. Zheng H, Nagaraja GM, Kaur P, Asea EE, Asea A. Chaperokine function of recombinant Hsp72 produced in insect cells using a baculovirus expression system is retained. J Biol Chem 2010;285:349-56.
48. Asea AA, De Maio A. Heat Shock Proteins: Potent Mediators of Inflammation and Immunity. Berlin: Springer; 2007.
49. Asea A. Initiation of the immune response by extracellular Hsp72: Chaperokine activity of Hsp72. Curr Immunol Rev 2006;2:209-15.
50. Aghdassi A, Phillips P, Dudeja V, Dhaulakhandi D, Sharif R, Dawra R, *et al*. heat shock Protein 70 increases tumorigenicity and inhibits apoptosis in pancreatic adenocarcinoma. Cancer Res 2007;67:616-25.
51. Sarkar S, Dutta D, Samanta SK, Bhattacharya K, Pal BC, Li J, *et al*. Oxidative inhibition of Hsp90 disrupts the super-chaperone complex and attenuates pancreatic adenocarcinoma *in vitro* and *in vivo*. Int J Cancer 2013;132:695-706.
52. Giulino-Roth L, van Besien HJ, Dalton T, Totonchy JE, Rodina A, Taldone T, *et al*. Inhibition of Hsp90 Suppresses PI3K/AKT/mTOR signaling and has antitumor activity in burkitt lymphoma. Mol Cancer Ther 2017;16:1779-90.
53. Belalcazar A, Shaib WL, Farren MR, Zhang C, Chen Z, Yang L, *et al*. Inhibiting heat shock protein 90 and the ubiquitin-proteasome pathway impairs metabolic homeostasis and leads to cell death in human pancreatic cancer cells. Cancer 2017;123:4924-33.

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