Novel discoveries targeting gemcitabine-based chemoresistance and new therapies in pancreatic cancer: How far are we from the destination?

Wenhao Luo | Gang Yang | Jiangdong Qiu | Jingyang Luan | Ying Zhang | Lei You | Mengyu Feng | Fangyu Zhao | Yueze Liu | Zhe Cao | Lianfang Zheng | Taiping Zhang | Yupei Zhao

1Department of General Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China
2Department of Vascular Surgery, Zhongshan Hospital, Institute of Vascular Surgery, Fudan University, Shanghai, China
3Department of Oncology, The Second Xiangya Hospital, Center South University, Changsha, China
4Department of Nuclear Medicine, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China
5Clinical Immunology Center, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

Abstract
Pancreatic cancer (PC) remains one of the deadliest malignancies worldwide. Chemoresistance is a significant clinical problem in pancreatic ductal adenocarcinoma (PDAC) and numerous potential mechanisms have been demonstrated but much remains to be understood. To overcome the existing limitations in PC treatment, newer approaches targeting intrinsic or acquired mechanisms have been found to improve drug therapeutic effectiveness in PC patients. Here, we provide an update of the most recent findings and their implications for clinicians, and attempt to summarize the various aspects of different individualized novel therapies for PC that could most benefit metastatic PDAC patients.

KEYWORDS
chemistry, drug therapy, metabolism, pancreatic cancer, prevention

1 | INTRODUCTION
Pancreatic cancer (PC) is the fourth leading cause of cancer deaths worldwide. The most effective treatment is surgical resection with neoadjuvant therapy. In the early stages of the disease, the few clear clinical signs or symptoms make it difficult to diagnose. Therefore, only 10%–15% of patients can undergo surgical resection of tumor tissue. Nowadays, most patients receive chemotherapy. Several major chemotherapy regimens include gemcitabine (GEM) mono-therapy, the FOLFIRINOX scheme (oxaliplatin, irinotecan, fluorouracil, and leucovorin), GTX (GEM,
membrane proteins hENTs through a different signaling pathway. Therefore, they may be a novel agent in combination with GEM for the treatment of GEM-resistant pancreatic cancer.\textsuperscript{12,13}

Liang et al reported that combination of LY2603618 (CHK1 inhibitor) with GEM synergistically overcomes chemoresistance of PDAC by downregulating RRM1/2 and promoting CDK-dependent DNA damage.\textsuperscript{14}

3 | DRUG TRANSPORT

The effects of GEM are dose-/concentration-dependent and are mediated by transporters to cross extracellular or intracellular membranes. The two main transporter superfamilies are the solute carrier (SLC) superfamily and the ATP-binding cassette (ABC) superfamily. Upregulation of drug uptake SLC transporters or downregulation of drug efflux ABC transporters are promising targets in GEM treatment of pancreatic cancer, but the later is more common strategy to overcome drug resistance.

Dauer et al\textsuperscript{15} showed that IT-139 (inhibitor of glucose regulatory protein78) can not only decrease ABC transporters but also increase reactive oxygen species (ROS) by inhibiting antioxidant responses in PDAC.

Hsu et al reported that arginine methyltransferase 3 (PRMT3) binds to ABCG2 mRNA to upregulate ABCG2, which increases PDAC resistance to GEM. Thus, PRMT3 inhibitors and GEM could be used as a cotreatment for pancreatic cancer in the future.\textsuperscript{16}

Previous studies have reported that histone deacetylases (HDACs) are overexpressed in PC, and inhibiting HDACs could induce apoptosis and curb metastasis.\textsuperscript{17} A promising novel HDAC inhibitor, CG200745, can inhibit pancreatic cancer cell proliferation by increasing tumor suppressor genes and decreasing ABCB3/ABCC4 genes.\textsuperscript{18} Paradoxically, HDAC inhibitors can enhance ABC transporters, thereby attenuating certain drugs.\textsuperscript{19} Accordingly, HDAC inhibitors could be combined with inhibitors of ABC transporters in PDAC.

ABC transporter inhibitor may be a novel target to overcome chemoresistance by increasing the concentration of the conventional anticancer drugs in cancer cells without producing additional toxicity.

4 | PDAC SIGNALING PATHWAYS

Chemoresistance of PDAC are largely attributable to numerous signaling pathways, such as the JAK/STAT, Hedgehog, PI3K, RAS, nuclear factor (NF)-kB, c-Met, WNT-β-catenin, Notch, TGF-β, SMAD, epidermal growth factor receptor (EGFR), mitogen-activated protein kinases (MAPK), and SDF-1/CXCR4 pathways (Table 1).
RAS, a frequently mutated G-quadruplex forming proto-oncogene, is a typical well-known oncogene, and down-regulation of KRAS has been proven to be associated with regulating the chemoresistance of pancreatic cancer. Notably, there are four downstream pathways of KRAS: PI3K/AKT, MAPK, RAL/GEF, and RIN1/ABL pathways. Growth-factor receptors, such as EGFR, which stimulates the RAS/MAPK and PI3K/AKT signaling pathways, and VEGFR, can be overexpressed in PDACs, and signaling via these receptors activate RAS proteins by increasing receptor tyrosine kinase (RTK). RTK is upstream of the RAS/MAPK pathway (RAS/RAF/MEK/ERK/AP-1).20

There are numerous, novel activating or inhibitory mechanisms that regulate the K-RAS signaling cascade, decreasing chemoresistance as a result. Pattanayak et al21 demonstrated that porphyrins (Tetrakis and Octaacetyl) have a high affinity toward the G-quadruplex of KRAS, exhibiting a significant ability to inhibit metastasis by blocking EMT and halting PDAC progression as a result. Cytokine tissue inhibitor of matrix metalloproteases (TIMP1) can bind to its receptor CD63 and then activate PI3K/AKT signaling. Inhibition of TIMP1 could improve the chemosensitivity of PDAC to GEM.22 Furthermore, Mao et al23 demonstrated that combined inhibition of the PI3K/AKT pathway (eg, LY294002) and inhibitors of Polo-like kinase 1 (PLK1) (eg, BI2536) can enhance the GEM-chemosensitivity of PDAC by inducing apoptosis in a nude mice model.

AKT is downstream of the Bitter taste receptor T2R10.24 Stern et al25 found that caffeine inhibited AKT phosphorylation and then reduced ABCG2 expression by triggering T2R10, which renders PDAC chemosensitive. Selumetinib and trametinib are superior MEK inhibitors that can reduce initial pancreatic volume.26 Inhibition of one mechanism may activate other compensatory protumor mechanisms that induce the chemoresistance of GEM. Therefore, inhibition of two mutually compensated chemoresistance mechanisms can dramatically improve chemosensitivity to GEM.

Mitogen-activated protein kinases (MAPKs, also called extracellular signal-regulated protein kinases (ERKs)) are downstream of the RTK/RAS/MAPK pathway.27 Ji et al demonstrated that CUDC-101, a multitargeted inhibitor of HDAC, EGFR, and HER2, applied with GEM can not only induce apoptosis via PI3K/mTOR signaling, but also reduce GEM resistance of PC by inhibiting cell proliferation and EMT in PC via the ERK/Snail signaling pathway.28

The nuclear transcription factor kappa B (NF-KB) pathway, which is a family of pleiotropic transcription factors associated with the regulation of immune and inflammatory responses, has a vital role in the development of PDAC.29 Inhibition of the NF-KB signaling pathway can downregulate antiapoptosis downstream genes, representing a novel target for antiresistance in pancreatic cancer.30 Previous studies have proven that cyclooxygenase inhibitors such as sulindac and celecoxib can inactivate NF-κB, which as a result, reduces chemoresistance of PC.31 Recently, Pastorelli et al32 reinforced previous clinical evidence that curcumin (an inhibitor of NF-κB) can potentiate the anticancer activity of GEM in PC cells with low toxicity. They suggested that curcumin can be used as the first line therapy of advanced PC. Horiuchi et al found that a potent NF-κB inhibitor, nafamostat mesilate enhances the antitumor effect of GEM/nPTX chemotherapy for PC.33 Moreover, inhibition of tripartite motif containing 31 (TRIM31) could reduce NF-κB signaling and subsequently inactivate various antiapoptosis genes, which can enhance GEM efficiency in PDAC.30

Notch signaling has much more significance in PDAC by activating KRAS to promote EMT and tumor growth. Cui et al34 found that SNHG1 long noncoding RNA might reinforce the chemoresistance of PDAC by decreasing its

**Table 1** Novel drugs target signaling pathways and RNAs

| Novel therapies | Characteristic | Mechanism | Function |
|-----------------|---------------|-----------|----------|
| LY294002 and BI2536 | PI3K/Akt and Polo-like kinase 1 inhibitors | Signaling pathway | Enhance Chemosensitivity to Gemcitabine in PC |
| CUDC-101 | HDAC inhibitor | Signaling pathway | Reduce GEM-resistance of PC |
| IONP-LPrA2/DAPT | Leptin/notch inhibitor | Signaling pathway | Reduce chemoresistance of PC |
| Tetrakis and Octaacetyl | KRAS inhibitor | Signaling pathway | Reduce Epithelial to mesenchymal transition |
| Nafamostat mesilate | NF-κB inhibitor | Signaling pathway | Enhance the antitumor effect of GEM/nPTX chemotherapy |
| Curcumin | NF-κB inhibitor | Signaling pathway | Enhance the anticancer activity of GEM in PC cells |
| SP600125 | JNK inhibitor | Signaling pathway | Reduce chemoresistance on PC stem cells |
| AZD1480 | JAK/STAT3 inhibitor | Signaling pathway | Enhance GEM delivery in PC |
| Caffeine | Akt phosphorylation inhibitor | Signaling pathway | Enhance chemo-sensitivity of PC |
| Circ-IARS | sponges miR-122 (reduces its level) | Noncoding RNA | Enhance endothelial permeability and Inhibit chemo-resistance |
apoptosis in cancer through the activation of the NOTCH signaling pathway. Inhibitors of SNHG1 could be an effective approach to chemotherapy. Leptin is vital for the activation of NOTCH signaling and induces the chemoresistance of PDAC. Recent clinical data showed that inhibition of leptin (eg, iron oxide nanoparticle-leptin peptide receptor antagonist 2, IONP-LPrA2/notch (eg, gamma-secretase inhibitor, DAPT) signaling can be used as a novel method to overcome the chemoresistance of PDAC.35

In conclusion, we found that different signaling pathways can interact with each other or share common pathways. This may be because they share a common effector. For example, MET and NOTCH can both activate the NF-KB signaling pathway, and PI3K/AKT can be activated by RAS and MET. Therefore, we concluded that blocking downstream signaling pathways or downstream common pathways may be more effective than directly blocking upstream pathways. At present, there is insufficient experimental data to support this hypothesis, but it provides new ideas for future clinical trials.

5 | APOPTOSIS AND CHEMORESISTANCE

Many studies of PC have demonstrated that chemoresistance can be explained by disturbances in apoptotic signaling pathways. There are two main apoptosis pathways. One is the extrinsic pathway, which is triggered by the Fas death receptor, the other is the intrinsic pathway, which leads to the release of cytochrome-c from the mitochondria.

5.1 | Novel therapy in the intrinsic pathway

One of the most important regulators of the apoptosis pathway is the BCL-2 family, which includes proapoptotic members (BAX, BAK, BAD, and BID) and antiapoptotic members (BCL-2, BCL-XL, and BCL-W).36

BCL-XL is much more effective at preventing apoptosis than the other BCL-2 family members. BCL-XL is overexpressed in 90% of PDAC cases, and its expression level increases with the progression from PanIN-1 to PDAC.37 On the contrary, overexpression of BAX can improve sensitivity to GEM.38 Therefore, the transcriptional regulator Yin Yang-1 (YY1) becomes a potential druggable target for the development of pancreatic cancer treatments by directly activating BAX gene transcription.39

Lu et al found that verticillium A, a selective histone methyltransferase (HMTase) inhibitor, significantly increased the GEM sensitivity of human PDAC by increasing apoptosis (downregulating BCL-X, FLIP, and MCL-1, while enhancing BAK, BAX, and BIM). Moreover, melatonin and its metabolite N1-acetyl-N2-formyl-5-methoxykynuramine enhanced the cytotoxic and tumor cancer cell proliferation inhibition effects of GEM in pancreatic cancer with upregulation of the BAX/BCL-2 ratio and increased expression of active caspase.41

5.2 | The extrinsic pathway

TRAIL, also called Apo-2 ligand (Apo-2L), belongs to the tumor necrosis factor family that preferentially triggers apoptosis in various tumor cells.42 It has drawn major attention for its potential utilization in cancer therapy as an effective anticancer tool that causes almost no cytotoxicity to normal cells. Combinations of TRAIL and certain DNA-damaging drugs may have synergistic antitumor therapeutic effectiveness.

Spano et al used gene-modified adipose mesenchymal stromal/stem cells (AD-MSC) to stably secrete soluble TRAIL and induce rapid apoptosis, to demonstrate effective gene therapy for pancreatic cancer.43

Rossignoli et al further found that PTX can restore PC sensitivity to MSC-delivered TRAIL and that the two compounds show improved cytotoxicity in pancreatic tumor cells, thus acting as a tool to improve treatment efficacy.44

In conclusion, the activation of proapoptotic genes and the blockade of antiapoptotic genes might enhance the chemosensitivity of pancreatic cancer.

6 | AUTOPHAGY AND CHEMORESISTANCE

Autophagy is characterized as an intracellular self-digestion process that is involved in the recognition and degradation of damaged proteins and organelles. It plays an important role in maintaining cellular homeostasis in response to stress. Autophagy has either prosurvival or prodeath functions in pancreatic cancer. For example, Onconase, a highly cytotoxic member of the pancreatic-type ribonuclease (RNase) super-family, strongly inhibits PDAC cell proliferation by triggering Beclin1-mediated autophagic cell death and sensitizing cancer cells to GEM.45

Experimental evidence suggested that promotion of autophagy may sensitize cells to a cytotoxic effect, which leads to a better outcome for patients with pancreatic cancer.46 However, many studies also demonstrated autophagy provides resistance to treatment. One study reported that autophagy associates with the activity of pancreatic cancer stem cells and blockade of autophagy reduces pancreatic cancer stem cell activity and enhances the effect of GEM.47

Autophagy inhibitors include genetic inhibition (RNAi) and pharmacologic inhibition (chloroquine). Researchers reported that miR-29a inhibits autophagy by blocking autophagy flux and downregulating autophagy proteins (TFEB and ATG9A),
and contributes to reducing the invasive potential of pancreatic cells and sensitizing chemoresistant cancer cells to GEM.48

Recently, several experiments showed that many novel drugs focusing on autophagy regulate chemoresistance. 3-MA has been reported to inhibit the activity of PI3-Kinase and block the formation of preautophagosomes and autophagosomes.49 Linc-RNA ROR (linc-ROR) has been identified as an oncogenic IncRNA in pancreatic cancer, which confers GEM resistance to pancreatic cancer cells partly by inducing autophagy. And Linc-ROR siRNA showed similar effect as 3-MA in enhancing GEM sensitivity.50

Chloroquine (CQ) are commonly used in the treatment of malaria, but also are antiautophagy drugs in cancer treatment that raise intralysosomal pH and interfere with autophagosome degradation in the lysosomes.51 CQ can also inactivate pancreatic stellate cells (PSCs) by keeping them in a quiescent state and altering the tumor stroma, which reduces pancreatic tumor invasiveness.52 However, recent studies showed that CQ is not effective enough when applied alone. As above mentioned, gambogic acid sensitizes GEM efficacy but induces the autophagic process and promotes the survival of pancreatic cancer cells. Recent studies proved that CQ combined with gambogic acid can suppress pancreatic cancer and inhibit gambogic acid-induced autophagic cancer survival.53

Thakur et al54 attempted to determine the combination effects of inhibitors of endoplasmic reticulum stress and autophagy applied with GEM in animal models. They concluded that triplet combination of chemotherapy (GEM + paclitaxel) + sunitinib + chloroquine showed the highest survival rate, indicating that the combination of autophagy inhibitors with GEM-associated chemotherapy would be a novel therapy in PDAC.

Ubiquitin specific peptidase 9X (USP9X) is correlated with GEM resistance. Moreover, an inhibitor of USP9X, WP1130, can inhibit GEM-induced autophagy to sensitize pancreatic cancer cells to GEM.55

In conclusion, autophagy has dual roles in pancreatic cancer progression, acting as a tumor suppressor or tumor protector. Targeting autophagy by promoting cell death in tumors and avoiding its survival mechanisms are novel therapies to overcome chemoresistance.

7 | TUMOR MICROENVIRONMENT

The tumor microenvironment (TME) consists of both cellular and noncellular components, containing nonquiescent PSCs, immune cells, endothelial cells, fibroblasts, and extracellular matrix (ECM).56 These components provide pathological barriers hindering the delivery of drugs to cause chemoresistance.

However, the complex mechanisms of each component in promoting chemoresistance have been implied. Although TME hinders the delivery of drugs, some components can also contribute to tumor inhibition. Blindly depleting TME to increase the concentration of GEM in the tumor tissue may produce unpredictable consequences. Therefore, the current research direction is to specifically remove those components that can promote tumors. In other words, we can decrease pathological barriers using more selective strategies to preserve the tumor-inhibiting components. An alternative approach is to inhibit the tumor-promoting effect caused by TME depletion via drug combinations (Table 2).

7.1 | Noncellular components

Extracellular matrix, mainly consists of collagen, noncollagen glycoprotein, heparan sulfate, and glycosaminoglycans. In PDAC, ECM is desmoplastic with abnormal accumulation of collagen fibers, leading to decreased delivery of drugs. However, previous studies depleting collagen fibers have had disappointing outcomes.

In recent years, hyaluronic acid (HA), another important component of the TME, has gradually attracted the attention of scientists. As HA within tumor tissue can bind water molecules, researchers speculated HA may exert pressure on surrounding structures and cause pathological barriers. Provenzano et al57 combined GEM with PEGPH20, a drug-depleting HA, and observed significantly prolonged median survival (median overall survival of GEM- (55.5 days) and GEM + PEGPH20 (91.5 days)-treated mice were significantly different (P = 0.004); treatment with PEGPH20 alone showed a trend toward increased survival (median = 63 days) that did not reach statistical significance (P = 0.1). Importantly, PEGPH20 combined with GEM had significant therapeutic benefit on PDAC.

Another promising strategy is to inhibit HA synthesis. 4-Methylumbelliferone (4-MU), a competitive substrate for UDP-glucuronosyltransferase (UGT), can inhibit HA synthesis by depleting cellular UDP-GlcUA, and also downregulate HAS2 and/or HAS3.58 Hyaluronidase (HYAL) has also been demonstrated to be related to HA synthesis. Therefore, hyaluroycin, a HYAL inhibitor, was developed to decrease the concentration of LMW-HA, and good results have been observed.59 Minnelide, which can also decrease the synthesis of HA, has been proved to result in better drug delivery in multiple animal models of pancreatic cancer. Notably, Minnelide is currently undergoing phase I clinical trials.60

Moreover, Chauhan et al61 demonstrated that HA-induced vascular dysfunction is largely correlated with collagen. Therefore, they used losartan, a dual inhibitor of stromal collagen I and hyaluronan production by downstream inactivation TGF-β1 to reduce solid stress, resulting
in reduced tumor vessels. Increased drug and oxygen delivery can be observed with significantly improved chemotherapy outcomes.

7.2 | Cellular components

CAFs (Cancer-associated fibroblasts) produce type I collagen. Recently, Biffi et al.\textsuperscript{62} demonstrated two CAF subtypes: myofibroblastic phenotypes (myCAFs) and inflammatory phenotypes (iCAFs). iCAFs have been shown to have potential tumor-promoting components, whereas myCAFs can directly modulate EMT (Epithelial–mesenchymal transition) of PDAC cells, further restraining tumor progression.\textsuperscript{63}

Previously, Özdemir et al.\textsuperscript{64} employed a genetic strategy to deplete αSMA + myofibroblasts, and a significantly decreased tumor collagen content was observed. However, in this model, GEM therapy did not result in improved overall survival, because increased tumor invasion associated with myofibroblast depletion was reported.

Considering the heterogeneity of CAFs, more selective new therapeutic strategies targeting tumor-promoting mechanisms may open the door to overcome CAF-related chemoresistance.\textsuperscript{65} Richards et al.\textsuperscript{66} reported that GEM-exposed CAFs release exosomes to increase the chemoresistance-inducing factor, Snail. They adopted GW4869 to inhibit exosome release, leading to significantly reduced survival of cocultured tumor cells. Duluc et al.\textsuperscript{67} reported CAFs promote chemoresistance by secreting proteins such as IL-6. They combined SOM230 analogue (Pasireotide) with GEM treatment to reduce IL-6 production by inhibiting the protein synthesis mTOR/4E-BP1 regulatory pathway, and with resulting reduced tumor growth and chemoresistance. As IL-6 can lead to chemoresistance by activating JAK-mediated STAT3 signaling, Nagathihalli et al.\textsuperscript{65} combined GEM with AZD1480, a JAK/STAT3 inhibitor. This therapy enhanced drug delivery to the tumor without depletion of stromal collagen or hyaluronan, further improving therapeutic efficiency.

8 | CANCER STEM CELLS

Recently, cancer stem cells (CSCs) have become a potential therapeutic target in PDAC. CSCs, which mainly remain in the G0 phase of the cell cycle, are naturally GEM-resistant. Meanwhile, high expression of several important multidrug resistance transporter efflux chemotherapy drugs, such as ABCG2, has been reported on the surface of CSCs.\textsuperscript{58} A novel

| Novel therapies | Characteristic | Mechanism | Function |
|-----------------|---------------|-----------|----------|
| PX-478 | HIF-1α inhibitor | CSCs | Enhance gemcitabine treatment in PC |
| Bethanechol | Muscarinic agonist | CSCs | Reduce pancreatic tumorigenesis and cancer stemness |
| Metformin | Oxidative metabolism modulator | CSCs | Cause fatal energy crisis in CSCs |
| 4-MU | UGT competitor | TME | Reduce hyaluronan synthesis and cell migration in PC |
| Hyaluromycin | HYAL inhibitor | TME | Reduce hyaluronan synthesis and cell migration in PC |
| Minnellite | Prodrug of triptolide | TME | Reduce hyaluronan synthesis and cell migration in PC |
| GW4869 | Exosome release inhibitor | TME | Reduced survival of PC |
| Pasireotide | Somatostatin analogue | TME | Reduce PC chemoresistance |
| LB-MSNP | GEM/PTX codelieverer/CDA inhibitor | Metabolism | Enhance PC chemosensitivity |
| Gambogic acid | RRM2 inhibitor | Metabolism | Enhance the efficacy of gemcitabine in PC |
| miR-608 or miR-101-3p | RRM1 inhibitor | Metabolism | Enhance the efficacy of gemcitabine in PC |
| Astaxanthin and Scareolide | RR and hENTs inhibitors | Metabolism | Enhance the efficacy of gemcitabine in PC |
| LY2603618 | RRM1/2 inhibitor | Metabolism | Enhance the efficacy of gemcitabine in PC |
ABC G2 nonsubstrate antitumor molecule (FL118) reverses resistance to GEM in pancreatic cancer via complex mechanisms, one of which is reducing stem-like pancreatic cancer cell populations.60

Moreover, the activation of CSC-associated signaling pathways including MYC, JNK, AKT1, WNT/β-catenin, NOTCH, and Shh, are also related to chemoresistance.70 Therefore, inhibiting these pathways is a promising strategy to increase the efficacy of chemotherapeutics. Suzuki et al71 reported that after treatment with JNK inhibitor SP600125, the effects of GEM on pancreatic CSCs were significantly enhanced with an increased proportion of dead cells.

Recently, researchers have relied on some characteristics of CSCs to design more-targeted treatment drugs.

A recent survey demonstrated that EMT and the CSC phenotype are closely correlated because CSC generation is attributed to the activation of EMT. Indeed, the treatment of PDAC cells that have entered the CSC state with most conventional chemotherapeutics is rendered inefficient by activating EMT programme, promoting CSC-dependent disease relapse as a result.72

8.1  |  EMT largely associated with CSCs

EMT can be defined as the phenotypic transition from an epithelial to a mesenchymal state. Previous studies demonstrated an inverse correlation between CSCs and EMT, which was largely associated with ZEB1 (zinc finger E-box-binding homeobox 1). Smigiel et al73 adopted shRNA-mediated knockdown to silence ZEB1, resulting in an transition from mesenchymal/CSCs to epithelial/non-CSCs. We can speculate that EMT has an important role in chemotherapeutic resistance, but the underlying mechanisms have yet to be elucidated. Zheng et al74 reported that suppression of EMT enhanced the expression of nucleoside transporters, which may be attributable to miRNAs in PDAC such as miR‐21, miR‐155, miR‐210, miR‐221, and miR‐222 and so forth.80 miRNAs degrade downstream mRNAs by binding to their 3′-untranslated region (UTR). This mechanism influences the TME and contributes to EMT.81

An et al proved that human ovarian cancer-specific transcript 2 (HOST2), a long noncoding RNA, could promote GEM resistance in human pancreatic cancer cells and that downregulated HOST2 could inhibit cancer cell proliferation and induce apoptosis of pancreatic cancer cells (Table 3).82 Moreover, You et al demonstrated that GEM decreases PVT1 (oncogenic long noncoding RNA) levels but increases its encoded miRNAs (miR-1207-5p/miR-1207-3p). Recently, Moschovis D et al conducted a case–control study to investigate the contribution of two lncRNAs polymorphisms of PVT1 and HOTAIR, respectively, in PC susceptibility and demonstrated that the PVT1 polymorphism was significantly overrepresented in PDAC patients compared with the controls.83 These findings implied that chemoresistance to GEM may partly be induced by lncRNA processing to miRNAs.84 However, Li et al reported that other lncRNAs such as Inc-HOTTOP were associated with GEM sensitivity in PDAC.85

Notably, special lncRNAs such as circular RNA (circRNAs) can act as miRNA “sponges” to modulate targeted mRNA; circ-RNAs are usually viewed as miRNA sponges as they include numerous conserved miRNA target sites.86 It has been demonstrated that circRNAs may be involved in the tumor development and GEM resistance of PDAC. For example, Xu et al found that circ_102747 could potentially
bind miR-21, a significant modulator in GEM resistance to PDAC through regulation of apoptosis by directly regulating BCL2 and PTEN expression. Moreover, Hao et al demonstrated that circ_0007534 regulates pancreatic cell proliferation, apoptosis, and invasion by sponging miR-625 and miR-892b. In addition, Qu et al showed that circRHOT1 could bind miR-26b, miR-125a, miR-330, and miR-382 to regulate multiple tumor-associated pathways especially in PC.

A promising mechanism of circular RNAs to decrease chemoresistance is physiologically increasing endothelial permeability. In human microvascular vein endothelial cells (HUVECs), activation of RAS homolog gene family member A (RhoA) signaling results in increased endothelial permeability, while miR-122 decreases RhoA signaling activity. Li et al revealed that circ-IARS can enter HUVECs and sponges miR-122 to reduce its levels, leading to the activation of RhoA. This signaling pathway represents a novel, physiologically relevant mechanism to inhibit chemoresistance.

Above all, current studies have shown that noncoding RNAs participate in chemosensitivity and chemoresistance in pancreatic cancer. Notably, circRNAs were recently identified as a novel strategy for decreasing chemoresistance in patients with pancreatic cancer.

### 10 | CONCLUSION

Hitherto, numerous potential chemoresistance mechanisms of PDAC have been elucidated. However, which mechanism has the dominant impact on PDAC chemoresistance and how to balance the effects of chemotherapy and toxicity remains unclear. In this article, we conclude that a reasonable combination of drugs that target different mechanisms generally improves chemosensitivity to GEM compared with monotherapy. In conclusion, our research aims should be focused on how to improve its efficiency and reduce toxicity. Combining those novel therapies with existing first-line chemotherapy drugs may bring new hope for improving the survival of patients with pancreatic cancer.

### ACKNOWLEDGMENTS

We acknowledge all the participants in searching, analyzing, and concluding those studies that contributed to this piece of work and all the collaborators who make such studies possible. We acknowledge the clinic staff and managers of Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College for their valuable contributions to this research. We sincerely thank Luo Wentao for his constant support and encouragement to Luo Wenhao. Luo Wentao contributed a lot to this article without asking for anything. We thank H. Nikki March, PhD for editing the English text of a draft of this manuscript.
AUTHORS’ CONTRIBUTIONS

Study design: LWH; Literature search: LWH, LJY, and ZY; Study selection: LWH, LJY, and ZY; Study draft and revision: LWH, LJY, ZY, and YG; Article guarantor: Dr. ZHANG Tai-ping. The main contribution is completed by LWH.

ETHICAL APPROVAL

Not required.

DATA SHARING

No additional data available.

ORCID

Wenhao Luo https://orcid.org/0000-0001-9914-5338
Taiping Zhang https://orcid.org/0000-0003-1689-6908

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin. 2018;68(1):7-30.
2. Patra CR, Bhattacharya R, Mukhopadhyay D, Mukherjee P. Fabrication of gold nanoparticles for targeted therapy in pancreatic cancer. Adv Drug Deliv Rev. 2010;62(3):346-361.
3. Burris HA, Moore MJ, Andersen J, et al. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreatic cancer: a randomized trial. J Clin Oncol. 1997;15(6):2403-2413.
4. Conroy T, Desseigne F, Ychou M, et al. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. N Engl J Med. 2011;364(19):1817-1825.
5. Fine RL, Fogelman DR, Schreibman SM, et al. The gemcitabine, docetaxel, and capcitabine (GTX) regimen for metastatic pancreatic cancer: a retrospective analysis. Cancer Chemother Pharmacol. 2008;61(1):167-175.
6. Von Hoff DD, Ervin T, Arena FP, et al. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. N Engl J Med. 2013;369(18):1691-1703.
7. Mini E, Nobili S, Cacciagli B, Landini I, Mazzetti T. Cellular pharmacology of gemcitabine. Ann Oncol. 2006;17(Suppl 5):v7-12.
8. Meng H, Wang M, Liu H, et al. Use of a lipid-coated mesoporous silica nanoparticle platform for synergistic gemcitabine and paclitaxel delivery to human pancreatic cancer in mice. ACS Nano. 2015;9(4):3540-3557.
9. Xia G, Wang H, Song Z, Meng Q, Huang X, Huang X. Gambogic acid sensitizes gemcitabine efficacy in pancreatic cancer by reducing the expression of ribonucleotide reductase subunit-M2 (RRM2). J Exp Clin Cancer Res. 2017;36(1):107.
10. Rajapour A, Afgar A, Mahmoodzadeh H, Radfar J-e-D, Rajaei F, Teimoori-Toolabi L. MiR-608 regulating the expression of ribonucleotide reductase M1 and cytidine deaminase is repressed through induced gemcitabine chemoresistance in pancreatic cancer cells. Cancer Chemother Pharmacol. 2017;80(4):765-775.
11. Fan P, Liu LI, Yin Y, et al. MicroRNA-101-3p reverses gemcitabine resistance by inhibition of ribonucleotide reductase M1 in pancreatic cancer. Cancer Lett. 2016;373(1):130-137.
12. Yan T, Li H, Wu J, et al. Astaxanthin inhibits gemcitabine-resistant human pancreatic cancer progression through EMT inhibition and gemcitabine resensitization. Oncol Lett. 2017;14(5):5400-5408.
13. Chen S, Wang YE, Zhang W-L, Dong M-S, Zhang J-H. Sclareolide enhances gemcitabine-induced cell death through mediating the NICTD and Gli1 pathways in gemcitabineresistant human pancreatic cancer. Mol Med Rep. 2017;15(4):1461-1470.
14. Liang M, et al. CHK1 inhibition sensitizes pancreatic cancer cells to gemcitabine via promoting CDK-dependent DNA damage and ribonucleotide reductase downregulation. Oncol Rep. 2018;39(3):1322-1330.
15. Dauer P, Sharma NS, Gupta VK, et al. GRP78-mediated antioxidant response and ABC transporter activity confers chemoresistance to pancreatic cancer cells. Mol Oncol. 2018;12(9):1498-1512.
16. Hsu M-C, Pan M-R, Chu P-Y, et al. Protein arginine methyltransferase 3 enhances chemoresistance in pancreatic cancer by methylating hnRNPA1 to increase ABCG2 expression. Cancers (Basel). 2018;11(1):8.
17. Sanaei M, et al. In vitro effect of the histone deacetylase inhibitor valproic acid on viability and apoptosis of the PLC/PRF5 human hepatocellular carcinoma cell line. Asian Pac J Cancer Prev. 2018;19(9):2507-2510.
18. Lee HS, et al. A novel HDAC inhibitor, CG200745, inhibits pancreatic cancer cell growth and overcomes gemcitabine resistance. Sci Rep. 2017;7:41615.
19. Ni X, Li L, Pan G. HDAC inhibitor-induced drug resistance involving ATP-binding cassette transporters (Review). Oncol Lett. 2015;9(2):515-521.
20. Wiktiewicz AK, et al. Whole-exome sequencing of pancreatic cancer defines genetic diversity and therapeutic targets. Nat Commun. 2015;6:6744.
21. Pattanayak R, Barua A, Das A, et al. Porphyrsins to restrict progression of pancreatic cancer by stabilizing KRAS G-quadruplex: in silico, in vitro and in vivo validation of anticancer strategy. Eur J Pharm Sci. 2018;125:39-53.
22. D’Costa Z, Jones K, Azad A, et al. Gemcitabine-induced TIMP1 attenuation therapy response and promotes tumor growth and liver metastasis in pancreatic cancer. Cancer Res. 2017;77(21):5952-5962.
23. Mao Y, Xi L, Li Q, et al. Combination of PI3K/Akt pathway inhibition and Plk1 depletion can enhance chemosensitivity to gemcitabine in pancreatic carcinoma. Transl Oncol. 2018;11(4):852-863.
24. Pan S, Sharma P, Shah SD, Deshpande DA. Bitter taste receptor agonists alter mitochondrial function and induce autophagy in airway smooth muscle cells. Am J Physiol Lung Cell Mol Physiol. 2017;313(1):L154-L165.
25. Stern L, Giese N, Hackert T, et al. Overcoming chemoresistance in pancreatic cancer cells: role of the bitter taste receptor T2R10. J Cancer. 2018;9(4):711-725.
26. Alagesan B, Contino G, Guiramares AR, et al. Combined MEK and PI3K inhibition in a mouse model of pancreatic cancer. Cancer Res. 2015;21(2):396-404.
27. Wada T, Pennington JM. Mitogen-activated protein kinases in apoptosis regulation. Oncogene. 2004;23(16):2838-2849.
28. Ji M, et al. Antitumor activity of the novel HDAC inhibitor CUDC-101 combined with gemcitabine in pancreatic cancer. Am J Cancer Res. 2018;8(12):2402-2418.
29. O'Reilly LA, et al. Loss of NF-kappaB1 causes gastric cancer with aberrant inflammation and expression of immune checkpoint regulators in a STAT-1-DEPENDENT MANNER. *Immunity*. 2018;48(3):570-583.e8.

30. Yu C, et al. Oncogenic TRIM31 confers gemcitabine resistance in pancreatic cancer via activating the NF-kappaB signaling pathway. *Theranostics*. 2018;8(12):3224-3236.

31. Gao S, et al. IGFBP2 activates the NF-kappaB pathway to drive epithelial-mesenchymal transition and invasive character in pancreatic ductal adenocarcinoma. *Cancer Res*. 2016;76(22):6543-6554.

32. Pastorelli D, Fabricio A, Giovanis P, et al. Phytosome complex of curcumin as complementary therapy of advanced pancreatic cancer improves safety and efficacy of gemcitabine: results of a prospective phase II trial. *Pharmacol Res*. 2018;132:72-79.

33. Horiuichi T, et al. New treatment strategy with nuclear factor-kappaB inhibitor for pancreatic cancer. *J Surg Res*. 2016;206(1):1-8.

34. Cui L, Dong Y, Wang X, et al. Downregulation of long noncoding RNA SNHG1 inhibits cell proliferation, metastasis, and invasion by suppressing the Notch-1 signaling pathway in pancreatic cancer. *J Cell Biochem*. 2019;120(4):6106-6112.

35. Harbuzariu A, Rampoldi A, Daley-Brown DS, et al. Leptin-Notch signaling axis is involved in pancreatic cancer progression. *Oncotarget*. 2017;8(5):7740-7752.

36. Martinou JC, Youle RJ. Mitochondria in apoptosis: Bcl-2 family members and mitochondrial dynamics. *Dev Cell*. 2011;21(1):92-101.

37. Ikezawa K, Hikita H, Shigekawa M, et al. Increased Bcl-xL expression in pancreatic neoplasia promotes carcinogenesis by inhibiting senescence and apoptosis. *Cell Mol Gastroenterol Hepatol*. 2017;4(1):185-200.e1.

38. Xu Z-W, Friess H, Büchler M, Solioz M. Overexpression of Bax sensitizes human pancreatic cancer cells to apoptosis induced by chemotherapeutic agents. *Cancer Chemother Pharmacol*. 2002;49(6):504-510.

39. Zhang J-J, Zhu YI, Yang C, et al. Yin Yang-1 increases apoptosis through Bax activation in pancreatic cancer cells. *Oncotarget*. 2016;7(19):28498-28509.

40. Lu C, Yang D, Sabbatini ME, et al. Contrasting roles of H3K4me3 and H3K9me3 in regulation of apoptosis and gemcitabine resistance in human pancreatic cancer cells. *BMC Cancer*. 2018;18(1):149.

41. Leja-Szpak A, Nawrot-Portańska K, Góralksa M, et al. Melatonin and its metabolite N1-acetyl-N2-formyl-5-methoxykynuramine (afmk) enhance chemosensitivity to gemcitabine in pancreatic carcinoma cells (PANC-1). *Pharmacol Rep*. 2018;70(6):1079-1088.

42. Almasan A, Ashkenazi A. Apo2L/TRAIL: apoptosis signaling, biology, and potential for cancer therapy. *Cytokine Growth Factor Rev*. 2003;14(3–4):337-348.

43. Spanel C, Grisendi G, Golinelli G, et al. Soluble TRAIL armed human MSC as gene therapy for pancreatic cancer. *Sci Rep*. 2019;9(1):1788.

44. Rossignoli F, Spanel C, Grisendi G, et al. MSC-delivered soluble TRAIL and paclitaxel as novel combinatorial treatment for pancreatic adenocarcinoma. *Theranostics*. 2019;9(2):436-448.

45. Fiorini C, Cordani M, Gotti E, Picone D, Donadelli M. Onconase induces autophagy sensitizing pancreatic cancer cells to gemcitabine and activates Akt/mTOR pathway in a ROS-dependent manner. *Biochim Biophys Acta*. 2015;1853(3):549-560.

46. Pardo R, Lo Ré A, Archange C, et al. Gemcitabine induces the VMP1-mediated autophagy pathway to promote apoptotic death in human pancreatic cancer cells. *Pancreatology*. 2010;10(1):19-26.

47. Yang MC, et al. Blockade of autophagy reduces pancreatic cancer stem cell activity and potentiates the tumoricidal effect of gemcitabine. *Mol Cancer*. 2015;14:179.

48. Kwon JJ, Willy JA, Quirin KA, et al. Novel role of miR-29a in pancreatic cancer autophagy and its therapeutic potential. *Oncotarget*. 2016;7(44):17635-17650.

49. Petiot A, Ogier-Denis E, Blommaert E, Meijer AJ, Codogno P. Distinct classes of phosphatidylinositol 3-kinases are involved in signaling pathways that control macroautophagy in HT-29 cells. *J Biol Chem*. 2000;275(2):992-998.

50. Li C, Zhao Z, Zhou Z, Liu R. Linc-ROR confers gemcitabine resistance to pancreatic cancer cells via inducing autophagy and modulating the miR-124/PTBP1/PKM2 axis. *Cancer Chemother Pharmacol*. 2017;86(1):1199-1207.

51. Solomon VR, Lee H. Chloroquine and its analogs: a new promise of an old drug for effective and safe cancer therapies. *Eur J Pharmacol*. 2009;625(1–3):220-233.

52. Endo S, Nakata K, Ohuchida K, et al. Autophagy is required for activation of pancreatic stellate cells, associated with pancreatic cancer progression and promotes growth of pancreatic tumors in mice. *Gastroenterology*. 2017;152(6):1492-1506.e24.

53. Wang H, et al. Gambogic acid induces autophagy and combines synergistically with chloroquine to suppress pancreatic cancer by increasing the accumulation of reactive oxygen species. *Cancer Cell Int*. 2019;19:7.

54. Thakur PC, Miller-Ocuin JL, Nguyen K, et al. Inhibition of endoplasmic-reticulum-stress-mediated autophagy enhances the effectiveness of chemotherapeutics on pancreatic cancer. *J Transl Med*. 2018;16(1):190.

55. Ma T, Chen W, Zhi X, et al. USP9X inhibition improves gemcitabine sensitivity in pancreatic cancer by inhibiting autophagy. *Cancer Lett*. 2018;436:129-138.

56. Schorer M, Jesenofsky R, Faissner R, et al. Desmoplasia and chemoresistance in pancreatic cancer. *Cancers*. 2014;6(4):2137-2154.

57. Provenzano P, Cuevas C, Chang A, Goel V, Von Hoff D, Hingorani S. Enzymatic targeting of the stroma ablates physical barriers to treatment of pancreatic ductal adenocarcinoma. *Cancer Cell*. 2012;21(3):418-429.

58. Cheng X, Sato N, Koshi S, Koga A, Hirata K. 4-Methyllumelliflorone inhibits enhanced hyaluronic synthesis and cell migration in pancreatic cancer cells in response to tumor-stromal interactions. *Oncol Lett*. 2018;15(5):6297-6301.

59. Koshi S, Sato N, Koga A, Hirata K, Harunari E, Igarashi Y. Hyaluronic acid, a novel hyaluronidase inhibitor, attenuates pancreatic cancer progression and promotes growth of pancreatic tumors in mice. *Oncotarget*. 2017;8(3):3224-3236.

60. Scherzer TJ, et al. Angiotensin inhibition enhances drug delivery, and survival in pancreatic cancer. *Clin Cancer Res*. 2016;22(2):415-425.

61. Chauhan VP, et al. Angiotensin inhibition enhances drug delivery and potentiates chemotheraphy by decompressing tumour blood vessels. *Nat Commun*. 2013;4:2516.

62. Biffi G, et al. IL-1-induced JAK/STAT signaling is antagonized by TGF-beta to shape CAF heterogeneity in pancreatic ductal adenocarcinoma. *Cancer Discov*. 2018;9(2):282-301.
63. Feig C, Jones JO, Kraman M, et al. Targeting CXCL12 from FAP-expressing carcinoma-associated fibroblasts synergizes with anti-PD-L1 immunotherapy in pancreatic cancer. *Proc Natl Acad Sci*. 2013;110(50):20212-20217.

64. Özdemir B, Pentecheva-Hoang T, Carstens J, et al. Depletion of carcinoma-associated fibroblasts and fibrosis induces immunosuppression and accelerates pancreas cancer with reduced survival. *Cancer Cell*. 2014;25(6):719-734.

65. Nagathihalli NS, Castellanos JA, Shi C, et al. Signal transducer and activator of transcription 3, mediated remodeling of the tumor microenvironment results in enhanced tumor drug delivery in a mouse model of pancreatic cancer. *Gastroenterology*. 2015;149(7):1932-1943.e9.

66. Richards KE, Zeleniak AE, Fishel ML, Wu J, Littlepage LE, Hill R. Cancer-associated fibroblast exosomes regulate survival and proliferation of pancreatic cancer cells. *Oncogene*. 2017;36(13):1770-1778.

67. Duluc C, Moatassim-Billah S, Chalabi-Dchar M, et al. Pharmacological targeting of the protein synthesis mTOR/4E-BP1 pathway in cancer-associated fibroblasts abrogates pancreatic tumour chemoresistance. *EMBO Mol Med*. 2015;7(6):735-753.

68. Zhou S, Morris JJ, Barnes Y, Lan L, Schuetz JD, Sorrentino BP. Bcrp1 gene expression is required for normal numbers of side population stem cells in mice, and confers relative protection to mitoxantrone in hematopoietic cells in vivo. *Proc Natl Acad Sci*. 2002;99(19):12339-12344.

69. Ling X, Wu W, Fan C, et al. An ABCG2 non-substrate anticancer agent FL118 targets drug-resistant cancer stem-like cells and overcomes treatment resistance of human pancreatic cancer. *J Exp Clin Cancer Res*. 2018;37(1):240.

70. Ip C, Li S-S, Tang M, et al. Stemness and chemoresistance in epithelial ovarian carcinoma cells under shear stress. *Sci Rep*. 2016;6(1):26788.

71. Suzuki S, Okada M, Shibuya K, et al. JNK suppression of chemotherapeutic agents-induced ROS confers chemoresistance on pancreatic cancer stem cells. *Oncotarget*. 2015;6(1):458-470.

72. Shibue T, Weinberg RA. EMT, CSCs, and drug resistance: the mechanistic link and clinical implications. *Nat Rev Clin Oncol*. 2017;14(10):611-629.

73. Smigiel JM, Parameswaran N, Jackson MW. Potent EMT and CSC phenotypes are induced by oncostatin-M in pancreatic cancer. *Mol Cancer Res*. 2017;15(4):478-488.

74. Zheng X, Carstens JL, Kim J, et al. Epithelial-to-mesenchymal transition is dispensable for metastasis but induces chemoresistance in pancreatic cancer. *Nature*. 2015;527(7579):525-530.

75. Zhao T, et al. Inhibition of HIF-1α by PX-478 enhances the anti-tumor effect of gemcitabine by inducing immunogenic cell death in pancreatic ductal adenocarcinoma. *Oncotarget*. 2015;6(4):2250-2262.

76. Lonardo E, Cioffi M, Sancho P, et al. Metformin targets the metabolic achilles heel of human pancreatic cancer stem cells. *PLoS ONE*. 2013;8(10):e76518.

77. Lonardo E, Cioffi M, Sancho P, Crusz S, Heeschen C. Studying pancreatic cancer stem cell characteristics for developing new treatment strategies. *J Vis Exp*. 2015;(100):e52801.

78. Zhou C, Qian W, Ma J, et al. Resveratrol enhances the chemotherapeutic response and reverses the stemness induced by gemcitabine in pancreatic cancer cells via targeting SREBP1. *Cell Prolif*. 2018:e12514.

79. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*. 2004;116(2):281-297.

80. Papachristou IG, Manta A, Gazouli M, et al. Expression of microRNAs in patients with pancreatic cancer and its prognostic significance. *Pancreas*. 2013;42(1):67-71.

81. Squadrato ML, Ezziroti M, De Palma M, Pittet MJ. MicroRNA-mediated control of macrophages and its implications for cancer. *Trends Immunol*. 2013;34(7):350-359.

82. An N, Cheng D. The long noncoding RNA HOST2 promotes gemcitabine resistance in Human pancreatic cancer cells. *Pathol Oncol Res*. 2018:1.

83. Moschos D, Vasilaki E, Tzouvala M, et al. Association between genetic polymorphisms in long non-coding RNAs and pancreatic cancer risk. *Cancer Biomark*. 2019;24(1):117-123.

84. You L, Wang H, Yang G, et al. Gemcitabine exhibits a suppressive effect on pancreatic cancer cell growth by regulating processing of PVT1 to miR1207. *Mol Oncol*. 2018;12(12):2147-2164.

85. Li Z, et al. The long non-coding RNA HOTTIP promotes progression and gemcitabine resistance by regulating HOXA13 in pancreatic cancer. *J Transl Med*. 2015;13:84.

86. Thomson DW, Dinger ME. Endogenous microRNA sponges: evidence and controversy. *Nat Rev Genet*. 2016;17(5):272-283.

87. Xu C, Yu Y, Ding F. Microarray analysis of circular RNA expression profiles associated with gemcitabine resistance in pancreatic cancer cells. *Oncol Rep*. 2018;40(1):395-404.

88. Hao L, Rong W, Bai L, et al. Upregulated circular RNA circ_0007534 indicates an unfavorable prognosis in pancreatic cancer. *Oncol Rep*. 2019;34(7):350-359.

89. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*. 2004;116(2):281-297.

90. Luo W, Yang G, Qiu J, et al. Novel discoveries targeting gemcitabine-based chemoresistance and new therapies in pancreatic cancer: How far are we from the destination? *Cancer Med*. 2019;8:6403–6413. https://doi.org/10.1002/cam4.2384