Chemoresistance in pancreatic cancer: Emerging concepts (Review)

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Received March 23, 2016; Accepted January 17, 2017

DOI: 10.3892/ol.2017.5777

Abstract. Pancreatic cancer is one of the most lethal types of cancer in the world. The incidence of pancreatic cancer increases each year with no significant decrease in mortality. Pancreatic cancer is a complex disease, and this complexity is partly attributed to late diagnosis, an aggressive phenotype, environmental factors and lack of effective treatment options. Surgical resection followed by adjuvant chemotherapy is the treatment of choice for early stage cancer, whereas gemcitabine is the standard first line therapy for patients with advanced stage disease. Treatment regimens comprising folinic acid, 5-fluorouracil, irinotecan, oxaliplatin and nab-paclitaxel have demonstrated modest effects in improving median survival rates. A number of other chemotherapeutics are currently undergoing clinical trials as components of combination therapies with gemcitabine. An increasing number of novel molecular targets and cellular pathways are being identified, which highlights the complexity of this disease. The development of chemoresistance to gemcitabine is multifactorial and there exists an interplay between pancreatic cancer cells, the tumor microenvironment and cancer stem cells. These components appear to be governed by a complex network of non-coding RNAs such as micro RNAs and long non-coding RNAs. In the present study, studies describing previous research on the understanding of the factors associated with the development of chemoresistance to gemcitabine in pancreatic cancer are reviewed. A comprehensive understanding of the multiple pathways of chemoresistance is key to develop next generation therapeutics to pancreatic cancer.

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Key words: pancreatic cancer, gemcitabine, chemoresistance, cancer stem cells, tumor microenvironment

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1. Introduction

Pancreatic cancer is the fourth leading cause of cancer-associated mortality in the United States, and predicted to be the second leading cause of cancer related mortality by 2030 (1). The mortality rate is almost equal to the incidence rate and it was estimated that in 2015, almost 49,000 incident cases were diagnosed and there were almost 40,000 mortalities (2). Pancreatic cancer exhibits a 5-year survival rate of 7% for all stages (2). An increased life span, smoking, family history of cancer, obesity, chronic pancreatitis, diabetes and occupational hazards are some of the well-established risk factors for pancreatic cancer.

Almost 90% of pancreatic malignancies are pancreatic ductal adenocarcinomas (PDACs). The disease progresses asymptomatically in 80% of patients, and is usually detected in the advanced stages by which time it is non-resectable. Amongst the 10-15% of patients who present with resectable disease, 80% develop relapse within 2-3 years. Several factors such as a delay in diagnosis, the aggressiveness of the established tumors, lack of proper therapy and the development of drug resistance are attributed to the low survival rate. Despite numerous studies having been carried out, no significant progress has occurred in the previous two decades.

2. Therapeutic management of pancreatic cancer

Surgery followed by adjuvant therapy is the treatment of choice for patients who present with early stage disease. However, majority of patients present with locally advanced disease or metastatic disease and exhibit poor prognoses. Without any treatment, these patients only survive for 12-14 weeks (3). Gemcitabine is a standard chemotherapeutic drug and has

been widely used as a first-line drug for patients with advanced staged pancreatic cancer. Patients treated with gemcitabine exhibited a significant improvement in the median overall survival rates (5.65 vs. 4.41 months) and 1-year survival rates (18 vs. 2%) compared with 5-fluorouracil (5-FU) (4). In the previous decade, several phase III trials performed to examine the efficacy of various drugs either alone or in combination with gemcitabine resulted in modest successes (5). Gemcitabine in combination with nab-paclitaxel increased the median survival from 6.7 to 8.7 months and when administered with FOLFIRINOX (folinic acid, 5-FU, irinotecan, and oxaliplatin) treatment, the median survival rate increased from 6.8 to 11.2 months (5).

There are other recent reviews that describe, in detail, the current drugs undergoing clinical trials (5,6). The present review will focus on the significant recent advances over the previous year in the development of chemoresistance, and the potential pathways and molecules that may be targeted to effectively improve the efficacy of therapy for pancreatic cancer.

3. Chemoresistance

The majority of studies examining chemoresistance in advanced pancreatic cancer focus on gemcitabine, as the data on the action of other drugs remain preliminary. It is uncertain why pancreatic cancer cells are more susceptible to gemcitabine compared with other anticancer drugs. Despite of this sensitivity, the majority of patients with pancreatic cancer develop resistance to gemcitabine, which means that the delineation of the mechanisms of gemcitabine chemoresistance is important. Several cellular factors such as human equilibrative nucleoside transporter 1, human concentrative nucleoside transporter 1 and deoxycytidine kinase are involved in gemcitabine resistance mechanisms and have been investigated extensively (6,7). The present review will focus on the gemcitabine resistance mechanisms that have been identified recently to augment the pre-existing data.

4. Mechanism of action of gemcitabine in cells

Gemcitabine, also termed 2',2'-difluoro-2'-deoxycytidine (dFdC), is a deoxycytidine analog. Entry of gemcitabine into cells is mediated by human nucleoside transporters (7). Inside the cells, the pro-form of gemcitabine is phosphorylated to form the active triphosphate form of gemcitabine, dFdC triphosphate (dFdCTP) (7). The main mechanism of action of gemcitabine occurs through the direct inhibition of DNA synthesis (8): dFdCTP is incorporated into growing DNA strands by DNA polymerase, which results in the termination of DNA synthesis by a process termed 'masked chain termination' (7). Another mechanism of gemcitabine action is the inhibition of the enzymes required for deoxynucleotide metabolism (7). Gemcitabine has also demonstrated the ability to trigger apoptosis through caspase signaling (7).

5. Gemcitabine resistance mechanisms

The tumor protein (p)53 gene is frequently mutated in patients with PDAC. Gemcitabine stabilizes the expression of mutant p53 and the corresponding downstream targets cyclin-dependent kinase 1 and cyclin B1, resulting in hyperproliferation and chemoresistance (9) thus, mutant p53 contributes to gemcitabine resistance. The concomitant treatment of gemcitabine with the p53-reactivating molecules CP-31398 and recombining binding protein suppressor of hairless-interacting and tubulin associated protein resulted in an increased level of apoptosis and reduced growth rates (9). Hypoxia is another critical factor in tumor development and chemoresistance. Hypoxic cancer cells stabilize the transcription factor hypoxia-inducible factor-1α (HIF-1α) and activate nuclear factor (NF)-κB leading to an epithelial-mesenchymal transition (EMT) phenotype characterized by an overexpression of vimentin and neural-cadherin (10). Hypoxia also results in the accumulation of lactate dehydrogenase-A, which assists in the maintenance of the hypoxic phenotype and increasing chemoresistance (11). Novel molecular targets and molecules that have been identified include: Protein tyrosine kinase-6 (12); vitamin D receptor (VDR) (13); mucin-1 (MUC1) (14); ormeloxifene that targets the sonic hedgehog pathway (15); sodium metaarsenite (KML001) that targets the epidermal growth factor receptor (EGFR) and matrix metalloproteinase (MMP) 2 (16); the quinazolinedione-based redox modulator QD232 that targets proto-oncogene tyrosine-protein kinase/focal adhesion kinase (FAK) and signal transducer and activator of transcription 3 phosphorylation (17); aspirin, which was revealed to reduce tumor growth and sensitize cells to gemcitabine (18); the curaxin CBL0137, which targets NF-κB and ribonucleotide reductase (19) and sepantronium bromide (YM155) (20-22), which inhibits the action of inhibitor of apoptosis protein family members (20), as summarized in Table I.

The development of chemoresistance in pancreatic cancer is multifactorial and may be attributed to the interplay between the tumor microenvironment, cancer stem cells (CSCs) and non-coding RNAs, as illustrated in Fig. 1.

Cancer stem cells. CSCs are a small subset of cancer cells hypothesized to be the driving force of tumor development. CSCs possess the ability to self-renew, initiate tumor development at distant sites and develop drug resistance. This has encouraged previous studies to understand the mechanisms that drive the formation of CSCs and to find ways to specifically inhibit them. A recent study suggests that CSCs exhibit elevated expression levels of c-Jun N-terminal kinases (JNK), which are essential for their maintenance and are important in developing chemoresistance to gemcitabine and 5-FU. The knockdown of JNK results in an increase of gemcitabine-induced reactive oxygen species production (23). Another recent study has revealed that extrinsic Wnt signaling confers cancer cell stemness on susceptible cells through the activation of the extracellular signal-regulated kinase (ERK) 1/2 and EMT pathways, and is mediated by the Wnt enhancer R-spondin 2 (24). Retinoic acid (RA) has been demonstrated to reduce the mRNA expression of cancer stem cell-like markers cluster of differentiation (CD) 44, CD24, CD133 and aldehyde dehydrogenase 1 (ALDH1) (25). RA also increases the apoptotic activity of gemcitabine (25). ALDH1 expression is regulated by transforming growth factor (TGF)-β in a SMAD family member 4-dependent manner (26). CSCs also express a high level of CD47, which communicates with signal

Table I. Newly identified molecular targets and pathways for pancreatic cancer sensitization.

Markers/molecules	Targeted pathways, mechanism of action	(Refs.)
PTK6 siRNA	PTK6, Inhibition of cell cycle and induced apoptosis	(12)
Vitamin D receptor knockdown	Vitamin D receptor, disrupting DNA repair	(13)
MUC1 shRNA	MUC1, p42-44 MAPK, Akt, Bcl-2 and MMP13 pathways	(14)
Ormeloxifene	SHH signaling pathway, Inhibition of stromal cell infiltration and invasion of tumor cells	(15)
KML001 (sodium meta-arsenite)	EGFR and MMP2, Inhibition of cell proliferation, migration and invasion	(16)
QD232	Src/FAK and STAT3 phosphorylation, Decreases cell migration, invasion and induction of apoptosis	(17)
Aspirin	Reduced growth, invasion and sensitized cells to gemcitabine	(18)
CBL0137	NF-kB and ribonucleotide reductase, Induces apoptosis and targets cancer stem cells	(19)
YM155	Induced apoptosis by inhibiting IAP family proteins	(20)

PTK6, protein tyrosine kinase-6; siRNA, silencing RNA; MUC1, mucin-1; shRNA, short hairpin RNA; p42-44, tumor proteins 42-44; Akt, protein kinase B; Bcl-2, B-cell lymphoma 2; MMP13, matrix metalloproteinase 13; SHH, sonic hedgehog; EGFR, epidermal growth factor receptor; Src, proto-oncogene tyrosine-protein kinase Src; FAK, focal adhesion kinase; STAT3, signal transducer and activator of transcription 3; NF-kB, nuclear factor kB; IAP, inhibitor of apoptosis.

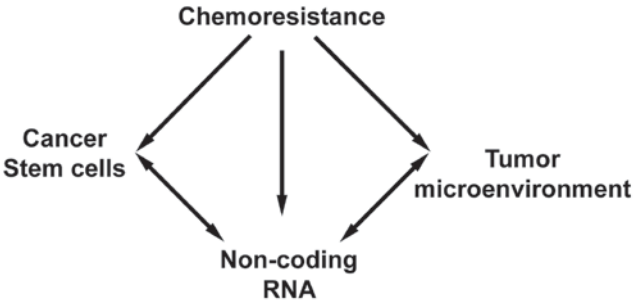

Figure 1. Factors associated with chemoresistance in pancreatic cancer. Chemoresistance in pancreatic cancer results from an interplay between the cancer cells, the tumor microenvironment, cancer stem cells and is intricately controlled by small non-coding RNAs like micro RNAs and long non-coding RNAs.

regulatory protein-α on the tumor associated macrophages of the tumor stroma and results in the evasion of phagocytosis. Blocking the expression of CD47 using anti-CD47 antibodies in combination with gemcitabine or Abraxane induced significant tumor regression (27).

Pancreatic CSCs exhibit low expression levels of microRNA (miR)-200c and overexpression of miR-200c with a concurrent increase in the expression levels of the EMT activator zinc finger E-box binding homeobox 1 (ZEB1) (28). ZEB1 regulates stemness and chemoresistance by the epigenetic silencing of miR-203. Treatment with mocetinostat, a class I histone deacetylase inhibitor, restored miR-203 function, which led to chemosensitization and a loss of stem cell characteristics (29). A previous study demonstrated that metastasis-associated lung adenocarcinoma transcript-1 (MALAT-1), a long non-coding (lnc) RNA, may increase proportions of pancreatic cancer stem cells, increase self-renewal and decrease chemosensitivity (30). Another study investigated the difference in microRNA (miRNA/miR) profiles between pancreatic stem-like cells and normal cancer cells, and revealed that miR-21 and miR-221 exhibited higher expression levels in pancreatic cancer stem cells. A combination therapy of antisense nucleotides that targeting miR-21 and miR-221 demonstrated a reduction in the rates of cell proliferation, tumor growth and chemosensitivity to gemcitabine and 5-FU (31).

Tumor microenvironment. The tumor microenvironment is the interstitial tissue surrounding the cancer cells and consists of pancreatic stellate cells, fibroblasts, endothelial cells, inflammatory cells, nerve cells and other non-cellular components such as proteoglycans and fibrous proteins (32). These components of the stroma interact with the cancer cells and result in a desmoplastic reaction that induces migration, invasion and chemoresistance (33). The fibrous stroma surrounding the cancer core constitutes 90% of the tumor volume and impedes the proper delivery of chemotherapeutics to the cancer cells. Previous studies demonstrate that the depletion of stroma promoted cancer growth and progression, suggesting that the stroma possess factors that impede tumor growth (34,35). Fibroblasts in stroma secrete soluble proteins that promote chemoresistance in cancer cells. Duluc *et al* (36) reported that the activation of somatostatin receptor 1 in cancer-associated fibroblasts by the SOM230 analogue pasireotide inhibited protein synthesis through the mechanistic target of rapamycin/4E-BP1 pathway, thereby increasing the efficacy of gemcitabine therapy.

Pancreatic stellate cells (PSCs). PSCs are one of the major constituents of the tumor stroma, and serve an important role in tumor growth. PSCs are present in a quiescent state in normal tissue, but upon activation by inflammatory signals such as TGF-β1, become activated and present a myofibroblast-like

phenotype (32). These activated PSCs secrete extracellular matrix (ECM) proteins including collagen, fibronectin and laminin, leading to the formation of a dense stroma matrix. Stellate cells affect tumor cell growth, maintenance and chemoresistance in pancreatic cancer cells. A previous study carried out using a co-culture of PSCs and pancreatic cancer cells demonstrated an increased resistance to gemcitabine through an increased expression of hairy and enhancer of split-1 (Hes1) through the Notch signaling pathway. The knockdown of either Hes1 or the Notch signaling pathway was revealed to reverse chemoresistance (37). PSCs secrete soluble stromal cell-derived factor-1α, which blocks gemcitabine-induced apoptosis in cancer cells by binding to their receptor C-X-C chemokine receptor type 4 and activating the FAK-protein kinase B (AKT) and ERK 1/2 signaling pathways (38). PSCs also express VDR, and their activation by VDR ligands has been demonstrated to regulate tumor stromal remodeling from an active to a quiescent state (39). A recent study has revealed that activated PSCs exhibit decreased expression levels of miR-29a and miR-29b, which is associated with an increased ECM deposition. An overexpression of either of these miRs reversed the process (40).

Immune cells. Immune cells infiltrate solid tumors and serve either a tumor promoting- or tumor-suppressing role. Immune cells in the stroma such as tissue-associated macrophages (TAMs) secrete an immunomodulatory antimicrobial peptide 18/LL-37 (hCAP-18/LL-37; cathelicidin antimicrobial peptide) that increases the expression levels of the pluripotency associated genes, the rate of self-renewal and tumorigenicity via formyl peptide receptor 2 and P2X purinoceptor 7 receptor-dependent mechanisms (41). TAMs have also been demonstrated to secrete an enzyme termed cytidine deaminase, which degrades the bio-active form of gemcitabine in cancer cells and thereby renders the cells chemoresistant (42). TAMs also secrete interferon (IFN)-stimulated gene 15 in response to IFN-β secreted by CSCs and assists in the self-renewal process and sustained tumorigenicity (43), as summarized in Table II.

Table II. Summary of potential targets to be considered for chemosensitization.

Drug target	With/without gemcitabine	(Refs.)
Cancer stem cells		
JNK	Yes	(23-25,27)
R-Spondin 2/Wnt signaling	No	
Retinoic acid	Yes	
CD47	Yes	
Pancreatic Stellate cells		
Hes1/Notch signaling	With	(36-38)
SDF-1α	With	
VDR ligands	No	
Immune cells		
(Tumor associated macrophages)		
18/LL-37	No	(40-42)
Cytidine deaminase	No	
IFN-stimulated factor ISG15	No	

JNK, c-Jun N-terminal kinase; CD47, cluster of differentiation 47; Hes1, hairy and enhancer of split-1; SDF-1α, stromal cell-derived factor 1α; VDR, vitamin D receptor; IFN, interferon; ISG15, interferon-stimulated gene 15.

Table III. miRNAs that are differentially regulated in pancreatic cancer.

miRNA	Pro/Anti-oncogenic	Chemoresistance	(Refs.)
miR-497	Anti	+	(43)
miR-33a	Anti	+	(43,44)
miR-29a	Anti	+	(45)
miR-330-5p	Anti	+	(45)
miR-21	Anti	+	(46)
miR-17-92	Anti	+	(49)
miR-221	Pro	-	(48)
miR-1246	Pro	-	(47)

miRNA/miR, microRNA.

Non-coding RNAs

miRs. miRs regulate the gene expression in the majority of biological processes in the cell, and therefore have been hypothesized to serve an important role in the chemoresistance to standard treatment regimens in pancreatic cancer. Numerous studies demonstrated that miRs are differentially expressed in a variety of types of cancer (44-50). A study examining the miR profile of gemcitabine-sensitive and resistant pancreatic cancer cell lines reported the presence of 33 differentially regulated miRs (44). Of these miRs, miR-497 was the most downregulated gene, and its upregulation resulted in the sensitization of the pancreatic cancer cells to gemcitabine and erlotinib (44). miR-33a overexpression has been demonstrated to sensitize pancreatic cancer cells to gemcitabine and inhibit tumor growth through the suppression of Pim-3 kinase expression (45). Incidentally, miR-33a was also reported to be downregulated in gemcitabine-resistant cells (44). miR-29a and miR-330-5p have been revealed to serve as tumor suppressors by downregulating MUC1 expression and sensitizing pancreatic cancer cells to gemcitabine (46). Another study demonstrated that miR-21 overexpression results in an increased sensitivity to gemcitabine via a decrease in the expression levels of the p85α subunit of phosphatidylinositol-4,5-biphostphate 3-kinase (47). miR-1246 has been revealed to increase stemness and induce drug resistance in pancreatic cancer cell lines (48). Similarly, miR-221/222 was demonstrated to induce pancreatic cancer progression through the regulation of MMPs (49), indicating that miRs may be pro- and anti-oncogenic. Another study has revealed that chemoresistant pancreatic cancer stem cells exhibit lower expression levels of miR-17-92 compared with chemosensitive cancer

stem cells. The overexpression of this miRNA reduced the CSC self-renewal capacity of the resistant CSCs, and sensitized them to chemotherapy (50), as summarized in Table III.

lncRNAs. lncRNAs are a group of non-coding RNAs that alter gene expression and also serve as miR sponges. Like 'cleaning sponges' these lncRNAs have been demonstrated to mop up multiple regulatory RNAs and behave as possible epigenetic regulators (51-54). The role of lncRNAs in the development of cancer remains incompletely characterized, yet several studies indicate a role in epigenetic regulatory mechanisms (55). Next generation sequencing technology has identified differential expression levels of several lncRNAs in PDAC samples compared with normal tissues (56). A recent microarray profiling study identified the homeobox A transcript at the distal tip (HOTTIP) as an important lncRNA that is upregulated in PDAC. The knockdown of HOTTIP reduced the rates of cell proliferation and sensitized pancreatic cancer cells to gemcitabine (57).

6. Future directions

The ultimate aim of cancer therapy is to specifically target and destroy tumor cells, and chemoresistance is the major hurdle to achieving this. The poor survival rate of the standard treatment drug gemcitabine has prompted studies investigating combination therapies to increase the efficacy of the drug. A major disadvantage of the previous studies examining drug resistance in patients with pancreatic cancer is the usage of agents targeting single molecules or pathways. A multidirectional approach that targets multiple aspects of the cancer cell such as the tumor cell microenvironment and immune cells is required.

The identification of the novel roles of non-coding RNAs in tumor development has identified avenues for the development of combination drugs. Non-coding RNAs, particularly miRNAs, and their control on the stages of tumor development are well established. Several miRNAs that exhibit a tumor suppressor function are downregulated in advanced tumors, and it is necessary that future drug combinations include miRNA mimics as targets. Although numerous studies investigating the role of miRNAs have been performed, more large scale studies on lncRNAs are necessary. An important goal of future studies is to identify the expression levels of large non-coding RNAs that may serve as potential biomarkers of chemoresistance. The identification of the probable functional role of these lncRNAs in modulating the effect of gemcitabine on target cells is also an important clinical question.

Circular RNAs (circRNA) are another under-studied factor that may serve an important role in the treatment of cancer. Previous studies have identified natural endogenous circRNA that possesses conserved miR target sequences and acts as miR sponges (58,59), which mop up other regulatory RNAs (51-54). Hansen *et al* have identified an endogenous circRNA termed ciRS-7 that has more than 70 selectively conserved miR-7 target sites and have coined the term miR sponges. CiRS-7 has been shown to suppress miR-7 activity (58). circRNAs are also more stable compared with linear miR, and this phenomenon may be exploited to artificially target oncogenic miRs (60).

7. Conclusion

Current chemotherapeutics for the treatment of advanced pancreatic cancer are not successful. The available drugs often result in high toxicity levels and the development of drug resistance. Numerous drug prospects targeting molecular pathways and specific cellular proteins in pancreatic cancer cells and the surrounding pancreatic stellate, and immune cells are being developed to increase the quality of life of patients with pancreatic cancer. Previous studies discussed in the present review have demonstrated the importance of miRNAs as central factors that may serve a vital role in the development of chemoresistance. The role of other non-coding RNAs such as lncRNA and circRNAs remain uncharacterized, and may be important components for understanding the mechanisms of gemcitabine resistance. Drug resistance in pancreatic cancer is multifaceted and future studies targeting different pathways and targets are required to understand and successfully treat pancreatic cancer.

Acknowledgements

The present study was supported by the Department of Internal Medicine, University of Illinois College of Medicine at Peoria, IL; McElroy Foundation (grant no. 2014-06298), Springfield IL and The Theresa Tracy Strive to Survive Foundation, East Peoria, IL 61611.

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