

Challenges of drug resistance in the management of pancreatic cancer

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Abstract

The current treatment of choice for metastatic pancreatic cancer involves single agent gemcitabine or combination of gemcitabine with capecitabine and erlotinib (tyrosine kinase inhibitor). Only 25-30% of patients respond to this treatment and patients who do respond initially ultimately exhibit disease progression. Median survival for pancreatic cancer patients has reached a plateau due to inherent and acquired resistance to these agents. Key molecular factors implicated in this resistance include: deficiencies in drug uptake, alteration of drug targets, activations of DNA repair pathways, resistance to apoptosis, and the contribution of the tumor microenvironment. Moreover, for newer agents including tyrosine kinase inhibitors, over expression of signaling proteins, mutations in kinase domains, activation of alternative pathways, mutations of genes downstream of the target, and/or amplification of the target represent key challenges for treatment efficacy. Here we will review the contribution of known mechanisms and markers of resistance to key pancreatic cancer drug treatments.

Key words: Pancreatic cancer, Drug resistance, anticancer therapy, Capecitabine, Gemcitabine, Erlotinib, Tyrosine Kinase Inhibitor

Introduction

Pancreatic ductal adenocarcinoma is a devastating disease with an extremely poor prognosis. It is characterized by invasiveness, rapid progression and marked resistance to treatment. Surgery offers the prospect of cure of disease confined to pancreas but has a 5-year survival of < 20% [1]. Due to the limitations of early detection, radiological procedures and lack of unambiguous diagnostic markers, most patients present with locally advanced or metastatic disease.

The treatment of locally advanced pancreatic cancer generally utilizes chemoradiation or chemotherapy alone. The chemotherapeutic agents used for radiosensitization primarily are 5-fluorouracil[2] and gemcitabine[3]. As far as treatment of metastatic pancreatic cancer is concerned, single agent gemcitabine [4], gemcitabine with erlotinib [5] and gemcitabine with capecitabine [6] are three reasonable choices supported by literature and clinical-based evidence. Despite extensive research in the last decade into pancreatic cancer, the overall survival of patients has not significantly improved. Although there has been a slight increase in overall survival with the addition of erlotinib to gemcitabine or capecitabine to gemcitabine, the median survival still remains approx 6 months [5]. However, only a minority (25-30%) of patients respond to gemcitabine based treatments. Patients, who do respond initially, ultimately exhibit disease progression owing to acquired resistance to these agents. Several combinations of gemcitabine have been evaluated but very few showed additional benefits. This has led to the evaluation of gemcitabine free regimen for pancreatic cancer. Recently, a randomized phase III trial comparing FOLFIRINOX (5FU/leucovorin, irinotecan and oxaliplatin) to gemcitabine as first-line treatment for metastatic pancreatic adenocarcinoma showed significantly longer overall survival (10.5 vs 6.9 months), progression-free survival (6.4 vs 3.4 months) and higher response rate (27.6% vs 10.9%) than gemcitabine alone. This study suggests FOLFIRINOX may emerge as the standard treatment for metastatic pancreatic cancer (Conroy *et al.*, 2010 – ASCO presentation, unpublished data)

In order to overcome resistance phenomena we need to understand pancreatic cancer biology and its different mechanism of resistance.

Extensive research in the last two decades has revealed risk factors associated with pancreatic cancer, such as cigarette smoking, environmental factors and genetic mutations. Oncogenes, tumor suppressor genes and DNA mismatch genes are altered leading to cancer development, progression and chemoresistance. Common oncogenes whose expression is altered in pancreatic cancer include k-ras, Akt and A1B1. Tumor suppressor genes associated with pancreatic cancer include MAP2K4, TGFBR2, p53 and CDKN2A. Inactivation of several DNA mismatch repair genes including human mutL homolog1 (MLH1) and breast cancer type 2 susceptibility protein (BRCA2), which code for the proteins that correct errors made randomly during DNA replication, have also been associated with pancreatic cancer. Table 1 summarizes the observed incidence of alterations in these genes in sporadic pancreatic cancer and table 2 summarizes the gene altered in familial pancreatic cancer syndromes.

Table 1: Pancreatic cancer-associated gene alterations

Gene	Frequency (%)	Ref.
Oncogenes		
k-ras	90	[108]
Akt2	20	[109]
A1B1	65	[110]
HER2	10-60	[91]
Tumor suppressor genes		

p53	30-80	[111]
CDKN2A/p16 ^{INK4}	60-80	[112]
DPC4	50-55	[113]
PTEN	70	[114]
LKB/STK11	4	[115]
DNA mismatch repair genes		
MLH1	3-15	[116]
BRCA2	7	[116]

DPC4: Depleted in pancreatic cancer locus 4; HER2: Human epidermal growth factor receptor 2.

Advances in pathological and molecular characterizations have improved our understanding of this disease; however, important aspects of pancreatic cancer biology remain poorly understood. We still do not understand the exact contribution of specific gene mutations in the pathogenesis of pancreatic cancer or the role of stroma in the overall pathogenesis and progression of the disease

Table 2. Familial pancreatic cancer syndromes and associated gene alterations

Familial pancreatic cancer syndromes	Mutated gene
Ataxia telangiectasia	ATM
Hereditary pancreatitis	Cationic trypsinogen gene (PRSS1)
Familial atypical multiple mole melanoma syndrome	CDK2NA, CDK4
Peutz-Jeghers syndrome	STK11
Hereditary nonpolyposis colorectal cancer syndrome	MLH1, MSH2

Data taken from [117]

Resistance to chemotherapeutic drugs is caused by a variety of factors, as shown in Figure 1. Inherent resistance is frequently observed in tumors, but as therapy becomes more effective with improved efficacy, acquired resistance is common. Acquisition of resistance to a range of anticancer drugs is commonly due to over expression of energy-dependent transporters that efflux anticancer drugs from cells (e.g., multi-drug resistance pumps), but other mechanisms of resistance including insensitivity to drug-induced apoptosis and induction of drug-detoxifying mechanisms, also play a key role in acquired resistance. Effective treatment or circumvention of the inherent resistance of pancreatic cancer can only come about through a therapeutically focused understanding the pancreatic cancer biology and the underlying mechanisms of resistance.

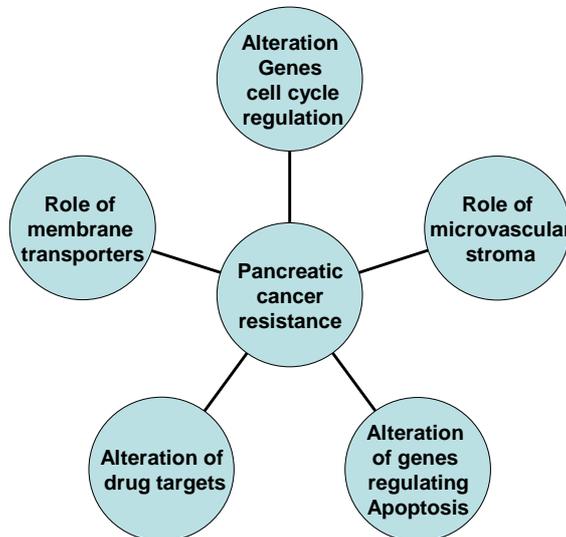


Figure 1. Cellular alteration which can contribute to pancreatic cancer resistance

Cellular alteration which can contribute to pancreatic cancer resistance

Here we will review our current knowledge of the different mechanisms of inherent and acquired resistance to three commonly used anti-cancer agents used in pancreatic cancer; gemcitabine, capecitabine and erlotinib.

Gemcitabine

Gemcitabine (2', 2'-difluoro-2'-deoxycytidine, dFdC, Gemzar®, [Eli Lilly, IN, USA]) a deoxycytidine analogue, is now considered the cornerstone of pancreatic cancer treatment as a single agent and in combination with other agents. However, most pancreatic cancer patients will exhibit disease progression and treatment provides only a slight survival advantage. Gemcitabine also has activity against other cancers including lymphomas and solid tumors such as lung, bladder and ovarian cancer [7-10].

Cellular metabolic scheme for gemcitabine activation in the cell

As outlined in Figure 2, gemcitabine is a pro-drug that is phosphorylated by dCK to its mononucleotide in a rate limiting step of its cellular anabolism. Subsequent action by nucleotide kinases convert gemcitabine monophosphate to its active metabolites, gemcitabine diphosphate and gemcitabine triphosphate. Thymidine synthase is inhibited by the deaminated form of gemcitabine monophosphate. The *de novo* DNA synthesis

pathway is blocked by gemcitabine diphosphate through inhibition of ribonucleotide reductase (RNR) and gemcitabine triphosphate which incorporates itself into DNA and RNA, thereby preventing cellular growth and initiating apoptosis [11].

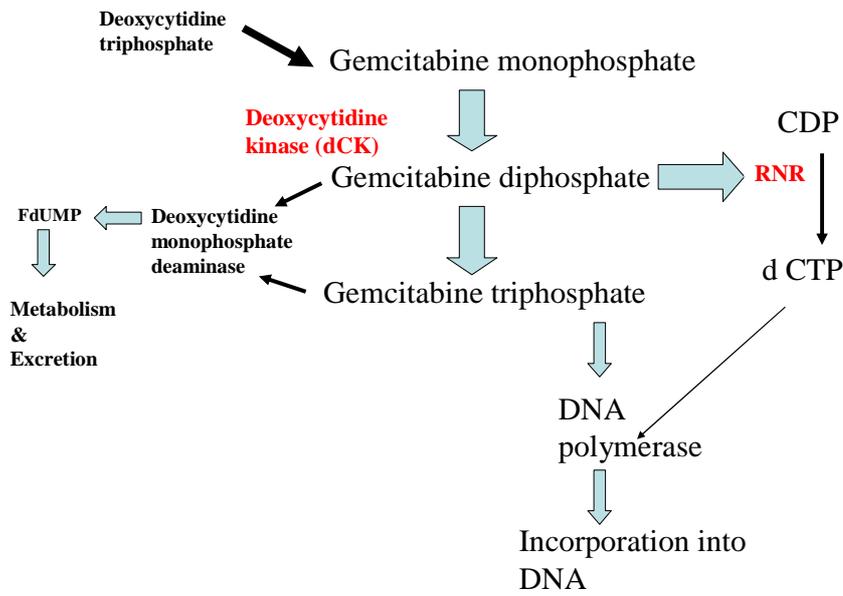


Figure 2. Cellular metabolic scheme for gemcitabine activation in the cell.

Downregulation of dCK and upregulation of RNR (red font) are two common alteration in gemcitabine metabolism associated with its resistance.

dCK: Deoxycytidine kinase; FdUMP: Fluorodeoxyuridine monophosphate; RNR: ribonucleotide reductase.

Mechanisms of gemcitabine resistance

The sensitivity/resistance of cancer cells to gemcitabine cannot be predicted by a single factor but may be determined by the balance of several factors. Molecular factors associated with this process include: deficiencies in gemcitabine uptake through altered nucleoside transporters, limitations of gemcitabine cellular activation/phosphorylation enzymes, activation of DNA repair pathways, resistance to apoptosis, altered cell cycle and proliferation pathways as well as a transition to a more epithelial to mesenchymal transition (EMT) like phenotype. Table 3 summarizes the primary factors which have been demonstrated to contribute to gemcitabine resistance.

Table 3. Summary of factors known to be involved in gemcitabine-resistance.

Mechanism	Factors	Ref.
Membrane transport mechanisms	hENT-1 hCNT1 and hCNT3	[16] [18]
Enzymes in important in DNA/RNA synthesis	dCK RNR	[19] [118]
Genes involved in cell cycle regulation, proliferation and apoptosis	Loss of p53 function P13K-Akt pathway modification Src phosphorylation changes Reduced BNIP-3 expression Reduced S100A2, S100A4 expression Increased ILK expression Increased CEACM6 expression Epithelial to Mesenchymal transition	[25] [31] [26] [31] [32] [41] [119] [45,120]

BNIP-3: Bcl-2/adenovirus E1B 19kDa protein interacting protein; CEACM6: Carcinoembryonic antigen-related adhesion molecule 6; hCNT: Human concentrative nucleoside transporter; hENT1: Human equilibrative nucleoside transporter; ILK: Integrin-linked kinase; RNR: Ribonucleotide reductase.

Transport mechanism across the cell membrane

Nucleosides are hydrophilic and cannot traverse cell membranes by passive diffusion; therefore, specialized transport systems are required for the passage of nucleoside analogs in or out of the cell. Two groups of nucleoside transporters have been identified. These include human equilibrative nucleoside transporters hENT1 and -2 [12, 13] and human concentrative type nucleoside transporters (hCNT) 1, -2 and -3 [14].

Several investigators have demonstrated the important role of nucleoside transporters in gemcitabine entry into the cells. Using gemcitabine in combination with BIBW22BS, a potent inhibitor of facilitated diffusion-mediated nucleoside transport, Jansen *et al.* found a 30-100 fold decrease in the activity of gemcitabine in various human cancer cell lines [15]. Similarly Mackey *et al.* found that cells with a nucleoside transporter deficiency were highly resistant to gemcitabine [16]. This study also suggests that the type of

nucleoside transporter in a cell may help determine sensitivity/resistance to gemcitabine. Several studies have been undertaken in pancreatic cancer to determine the impact of these nucleoside transporters in the prognosis of patients. Spratlin *et al.* have suggested that the presence of abundant hENT1 staining within pancreatic tumor cells may be a prognostic marker of survival [17]. Patients with uniformly detectable hENT1 had a significantly longer survival with gemcitabine treatment (median survival: 13 months) than patients with tumors lacking detectable hENT1 (median survival: 4 months).

Marechal *et al.* found that not only hENT1, but also hCNT1 and hCNT3 are responsible for gemcitabine uptake into cancer cells [18]. In a study of 45 pancreatic adenocarcinoma patients treated with gemcitabine as an adjuvant treatment after curative resection, they found that patients with high hENT1 expression had significantly longer disease free survival and overall survival than patients with low expression. They also found that high hCNT3 expression was associated with longer overall survival. This indicates that hENT1 and hCNT1 and hCNT3 all play a role in the transport of gemcitabine, and deficiency of these transporters may cause innate resistance to this agent. Only a minority of patients respond to gemcitabine. These data suggests that it may be possible to select patients by determining hENT1 and hCNT1 and hCNT3 expression in tumors. However, this hypothesis needs careful examination in prospective randomized studies before adoption.

Alterations in sub-cellular targets.

Alterations in the cellular targets of gemcitabine result in decreased sensitivity to the agent. Research in this area has focused on two key enzymes, dCK and RNR.

Role of dCK in gemcitabine resistance

In the cell, gemcitabine is phosphorylated by dCK to its monophosphate form. This first step in phosphorylation is the rate limiting step for further phosphorylation to active metabolites and thus essential for the activation of gemcitabine. Gemcitabine is also phosphorylated by TK2 [11].

Several authors have described a relationship between dCK activity and acquired resistance to gemcitabine. Most of these studies were performed in ovarian cancer cell lines and acquired resistance was associated with dCK deficiency. Van der Wilt *et al.* studied the effect of *dCK* gene transfection in *dCK* deficient gemcitabine-resistant human ovarian AG6000 cells [19]. Recovery of dCK activity in these cells by transfection with the *dCK* gene not only increased *in vitro* sensitivity to gemcitabine, but evaluation of same cells cultured as a xenograft *in vivo* by growth in nude mice also supported the important role of *dCK* in tumor cell sensitivity to gemcitabine.

Sebastiani *et al.* explored genetic alterations of the *dCK* gene in pancreatic cancer patients who progressed on gemcitabine and in 17 pancreatic cancer cell lines and found

no mutation in any of the patients or the cell lines, indicating that mutation in the *dCK* gene may not be a frequent mechanism of gemcitabine resistance in human pancreatic cancer [20]. However, pre-treatment immunostaining of dCK in pancreatic cancer tissue strongly correlated with progression free and overall survival, proving the importance of the level of this enzyme in cells in gemcitabine sensitivity.

Nakano *et al.* analyzed by RT-PCR the importance of the ratio of mRNA expression of 4 proteins, dCK, hENT1 and ribonucleotide reductase M subunit 1 and 2 (RRM1 and RRM2), in gemcitabine sensitivity by reverse transcription PCR (RT-PCR) [21]. Through an examination of six pancreatic cancer cell lines, they found that the ratio of mRNA expression of dCK x hENT1/RRM1 x RRM2 was more indicative of drug sensitivity and the higher the ratio the more sensitive the cell line was to gemcitabine and *vice versa*.

Role of RNR in gemcitabine resistance

Inhibition of RNR is one of the self-potentiating mechanisms of gemcitabine action. Gemcitabine diphosphate inhibits RNR which results in a decrease in deoxyribonucleotide pools, including dCTP, leading to decreased feedback inhibition of dCK [11]. As dCTP competes with gemcitabine triphosphate for incorporation into DNA, decreased dCTP levels will increase gemcitabine incorporation into DNA. Several investigators have described the link between RNR activity and gemcitabine resistance. Nakahira *et al.* found up regulation of *RRM1* in the gemcitabine-resistant pancreatic cancer cell line, MiaPaCa2-RG, by microarray analysis [22]. Knockdown of *RRM1* by siRNA reduced MiaPaCa2-RG gemcitabine resistance to that of the parental cell line. Furthermore, this observation translated clinically, as patients with high levels of RRM1 had significantly poorer survival outcome after gemcitabine treatment compared to those with low RRM1 levels. Resistance to gemcitabine has also been observed in cells over expressing a second sub unit of the RNR complex, RRM2. Duxbury *et al.* found increased expression of RRM2 in gemcitabine-resistant PANC-1 cells [23]. Knockdown of the M2 subunit gene in pancreatic cancer cells by siRNA enhanced the chemosensitivity to gemcitabine. In one study, pretreatment biopsies from unresectable pancreatic cancer patients showed that patients with low tumor *RRM2* mRNA levels have a better overall outcome compared to patients with high *RRM2* mRNA levels [24].

Gemcitabine resistance: role of cell cycle regulation, apoptosis and EMT genes

Genes involved in cell cycle regulation, survival and proliferation play a key role in pancreatic chemoresistance to anticancer agents, including gemcitabine. *p53* is an important gene in regulating the cell cycle and functions as a tumor suppressor involved in regulating cell growth. Galmarini *et al.* developed two breast cancer cell lines, MN-1I and MDD-2 with MN-1 cells containing wild type *p53* and the MDD-2 cell line containing a mutant *p53* [25]. Exposure to gemcitabine induced a higher degree of apoptosis in MN-1 than in MDD-2 cells. This corresponded with suppression of Bcl-2 and Bcl-x_L expression in the wild type *p53* cells exposed to gemcitabine, whereas Bcl-2 levels remained stable and Bcl-x_L levels increased in the mutant *p53* cells exposed to the drug. The authors suggested that loss of *p53* function leads to loss of cell cycle control

and alterations in the apoptotic cascade, conferring resistance to gemcitabine in cancer cell lines displaying the mutant *p53* phenotype.

Duxbury *et al.* suggested the role of the nonreceptor tyrosine kinase Src in innate and acquired resistance to gemcitabine [26]. They developed pancreatic cell lines resistant to gemcitabine and found that the resistance was associated with higher Src phosphorylation and activity as compared to gemcitabine-sensitive cell lines.

There is evidence to suggest that activation of antiapoptosis regulating genes may contribute to the chemoresistance of pancreatic cancer cells to gemcitabine. In pancreatic cancer, expression of the anti-apoptosis gene Bcl-x_L leads to shorter patient survival [27]. A study by Bold *et al.* found that cellular overexpression of *Bcl-2*, a potent inhibitor of apoptosis, significantly decreased gemcitabine-induced apoptosis in pancreatic cancer cell lines [28]. Transfection of *Bcl-2* siRNA of the pancreatic cancer cell line, YAP C into nude mice xenograft showed a synergistic interaction with gemcitabine compared to gemcitabine alone. Silencing of the *Bcl-2* gene also restored sensitivity to gemcitabine *in vitro* [29].

In contrast, enhanced expression of the pro-apoptotic *Bax* gene significantly increased the sensitivity of the pancreatic cancer cell line, AsPc-1, to gemcitabine and 5FU. Friess *et al.* found that *Bax* expression in pancreatic tissue was a strong indicator of survival [27]. Further development of this concept suggests that the ratio of Bax to Bcl-2 may have stronger functional significance. Shi *et al.* found that if the ratio of Bax to Bcl-2 tipped towards Bax, that this could promote apoptosis and be predictive of gemcitabine sensitivity [30]. Akada *et al.* identified the Bcl-2/adenovirus E1B 19kDa protein interacting protein (BNIP3), a Bcl-2 family proapoptotic protein as lowly expressed in gemcitabine-resistant pancreatic cancer cell lines [31]. BNIP3 expression was downregulated in drug resistant pancreatic cancer cell lines and its expression was also found to be downregulated by 90% in 21 pancreatic cancer tissue specimens. siRNA-mediated knockdown of BNIP3 in pancreatic cancer cell line reduces gemcitabine-induced cytotoxicity *in vitro*. Mahon *et al.* identified the upstream genes, *S100A2* and *S100A4* as BNIP3 suppressors in PDAC pancreatic cancer cell lines, with the ability to repress exogenous BNIP3 promoter activity *in vitro* [32]. *S100A4* knockdown resulted in an increased sensitivity of the resultant cells to gemcitabine treatment, which was coupled with an increase in apoptosis and cell cycle arrest.

Other antiapoptotic signal transduction pathways linked to the chemoresistance of pancreatic cancer include PI3K-Akt and NF- κ B. The PI3K signaling cascade plays a crucial role in the regulation of apoptosis, acting in part via its downstream target Akt in several cancer cell types including pancreatic cancer. Gene expression cDNA microarray profiling of 15 pancreatic cancer cell lines exposed to gemcitabine revealed alterations in levels of seven genes that encode proteins active in the PI3K-Akt pathway [31].

Recent investigations have shown that reduction of PI3K and Akt activity in pancreatic cancer cell lines correlates with enhanced gemcitabine-induced apoptosis and antitumor activity, suggesting a significant role for these enzymes in mediating drug resistance in

pancreatic cancer cells [33]. Inhibition of Akt using a cell-permeable derivative of the proapoptotic peptide and Akt antagonist C-terminal modulator protein (CTMP; termed TAT-CTMP) in combination with gemcitabine or radiation therapy augments the effects of gemcitabine and radiotherapy *in vitro* and *in vivo* [34]. NF- κ B plays an important role in cancer progression and increased expression promotes cellular resistance to anticancer therapy. Arlt *et al.* showed that pancreatic cancer cell lines resistant to gemcitabine-induced apoptosis had high basal NF- κ B activity [35]. In addition, inhibition of NF- κ B reduced gemcitabine resistance in these cells. However, the PI3K-Akt pathway was not involved in the gemcitabine resistance of these cells, as inhibition of PI3K-Akt by LY294002 did not affect gemcitabine-induced apoptosis. Yokoi *et al.* demonstrated that hypoxia can induce gemcitabine resistance in pancreatic cancer cells mainly through the PI3K-Akt-NF- κ B pathways and partially through the MAPK (Erk) signaling pathway [36]. In contrast, other studies have shown that NF- κ B expression does not correlate with gemcitabine sensitivity; however, knockdown of NF- κ B *in vitro* and *in vivo* did potentiate the effects of gemcitabine in sensitive cells but not resistant cells [37]. Disruption of NF- κ B activity by inhibition of glycogen synthase kinase-3 failed to sensitize pancreatic cancer cells to gemcitabine, suggesting that perhaps NF- κ B may play a minor role in gemcitabine resistance [38]. Clearly, the contribution of NF- κ B to pancreatic drug resistance is complex and may depend on other factors. However the role of inhibition of NF- κ B in potentiating gemcitabine induced cytotoxicity is well established [39,40].

Duxbury *et al.* suggested a role for integrin-linked kinase (ILK) in gemcitabine chemoresistance in pancreatic cancer [41]. ILK facilitates signal transduction between extracellular events and important intracellular survival pathways involving protein kinase B/Akt. Overexpression of ILK increased cellular gemcitabine chemoresistance, whereas ILK knockdown induced chemosensitization via increased caspase 3-mediated apoptosis.

An interesting paper by Liau *et al.* pointed towards the role of high mobility group protein (HMGA1) in pancreatic cancer resistance to chemotherapeutic agent especially gemcitabine [42]. HMGA1 is a transcriptional protein complex which forms on chromatin and regulates transcription of numerous genes downstream of Ras/ERK signaling pathway. Utilizing the MiaPaca2 and BxPc3 cell lines, they found silencing of HMGA1 by the use of siRNA increased the chemosensitivity of gemcitabine and forced overexpression of HMGA1 promoted resistance to gemcitabine in MiaPaca2. The same phenomenon was demonstrated in xenograft nude mice where again silencing of HMGA1 promoted gemcitabine-induced cytotoxicity and reduces tumour growth [43]. They also suggested that HMGA1 mainly works through Akt activation. Emerging evidence suggests both molecular and phenotypic associations between gemcitabine resistance and the EMT phenotype, pointing towards an interesting mechanism of pancreatic cancer resistance. EMT is a process initially seen in the development of the embryo, whereby cells lose epithelial characteristics and gain a mesenchymal phenotype. Recent research has shown that the EMT may be an important phenomenon in cancer progression; epithelial-derived tumor cells switch properties to a more mesenchymal phenotype facilitating tumor invasion and metastasis. The epithelial state is characterized by E-

cadherin and cytokeratin (e.g., cytokeratin 18) expression. Using immunohistochemical techniques, Nakajima *et al.* examined the expression of vimentin, and N- and E-cadherin in pancreatic cancer tissue specimens [44]. In epithelial cells, the loss of E-cadherin and the increase in N-cadherin expression was found to be associated with a metastatic phenotype. Vimentin expression was observed in a few cancer cells of pancreatic primary tumors but was substantially expressed in pancreatic cancer liver metastasis. Shah *et al.* developed gemcitabine-resistant pancreatic cancer cell lines displaying spindle-shaped morphology, an appearance of pseudopodia and reduced adhesion with increased invasion and migration potential [45]. The gemcitabine-resistant cells demonstrated increased vimentin and decreased E-cadherin expression. Receptor protein tyrosine kinases and c-MET were activated and there was an increase in the expression of the stem cell markers CD24, CD44 and epithelial-specific antigen (ESA) in the resistant cells. To further elucidate the mechanisms for the acquired EMT phenotype of gemcitabine resistant cells, Wang *et al.* found that Notch-2 and its ligand Jagged-1 were highly upregulated in gemcitabine-resistant pancreatic cancer cells [46]. Knockdown of Notch lead to a reduction in the invasive and EMT phenotype of the cells, indicating that activation of Notch signaling in gemcitabine-resistant cells may be linked to EMT.

MicroRNAs and gemcitabine resistance

MicroRNAs (miRNAs) are post-transcriptional regulators that bind to mRNA. Since their identification, research has revealed multiple roles for miRNAs in the negative and positive regulation of translation and transcription. Several researchers have demonstrated the role of miRNA-21 in the chemoresistance of gemcitabine in both pancreatic cancer cell lines and patients. Research in this field has established that miRNA-21 contributes to pancreatic cancer invasion, metastases and chemoresistance of gemcitabine [47]. Giovenetti *et al.* carried out a study in pancreatic cancer patients and found shortened overall survival in individuals with high miRNA-21 expression in both metastatic and adjuvant setting [48]. Similarly, inhibitory studies with antisense oligonucleotides to miRNA-21 increased apoptosis, arrested cell cycle and sensitized the cells to gemcitabine [49]. The roles of other miRNAs are under investigation and potentially provide us with new targets for overcoming resistance.

Contribution of stromal factors to drug resistance

Pancreatic cancer is characterized by dense desmoplastic reactions which can involve adjacent vital structures. Cancer cells are usually surrounded by a dense stroma consisting of myofibroblast-like cells, collagen and fibronectin. Several researchers have demonstrated the contribution of stromal factors to pancreatic cancer pathogenesis [50]. Similarly the role of tumor microenvironment in the innate resistance of pancreatic cancer has also been demonstrated [51,52].

The use of antiangiogenesis drugs has elicited survival benefits in many aggressive tumors; however, these inhibitors have failed to produce lasting clinical responses in pancreatic cancer patients [53]. A review by Bergers *et al.* describes the modes of resistance to antiangiogenesis drugs by adaptive/evasive resistance [54]. The biological

regulatory mechanisms of invasion and angiogenesis, as well as contributory factors in the tumor microenvironment, have revealed underlying mechanisms of resistance to antiangiogenic therapies. Pancreatic cancer is characterized by its hypoperfusion [55], and also proven consistently hypoxic possibly because of hypoperfusion. Failure of most of the chemotherapeutic combinations and even newer treatment including cetuximab and bevacizumab has led to the idea of hypoperfusion as factor leading to the poor delivery of anticancer agents [56]. This concept has been recently demonstrated by Olive *et al.*, who found that with the use of inhibitors of hedgehog signaling in genetically engineered nude mice with pancreatic cancer, fibrous stroma was depleted, which improved the microvascular density and delivery of gemcitabine into the cancer cells [57]. This is a very interesting study that could at least partially explain the general resistant behavior of pancreatic cancer to anticancer agents. Hedgehog signaling and its aberrant activation are well recognized in pancreatic cancer pathogenesis. Feldman *et al.* also demonstrated that inhibition of hedgehog signaling resulted in inhibition of metastatic spread in an orthotopic xenograft model [58]. However, practical and experimental limitations mean that we have a particularly poor understanding of the role of the pancreatic cancer microenvironment in gemcitabine, capecitabine and erlotinib chemoresistance.

Capecitabine

Capecitabine (Xeloda®, Genentech, Inc., CA, USA; Roche, Basel Switzerland) is an oral fluoropyrimidine carbamate that is used in colon cancer [59], gastric cancer [60], breast cancer [61] and pancreatic cancer [6]. Capecitabine is converted to 5-FU in three enzyme-controlled steps as illustrated in Figure 3. The first step is catalyzed by carboxylesterase, an enzyme located almost exclusively in the liver, the second step by cytidine deaminase, expressed in the liver and various types of tumors, and the last by thymidine phosphorylase (PD-ECGF/TP), which is thought to have higher expression in tumor than in normal tissues, thus ensuring an enhanced efficacy [62]. Capecitabine has potentially two pharmaceutical advantages: enhanced activation at the tumor site and decreased drug accumulation in healthy tissues, thereby decreasing systemic toxicity.

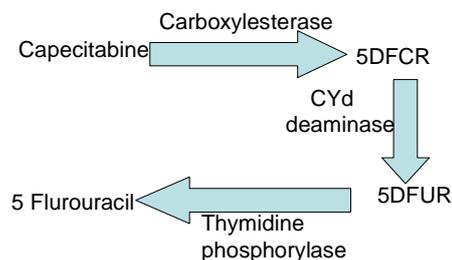


Figure 3. Metabolic conversion of capecitabine to 5-fluorouracil

5'DFCR: 5'-Deoxy-5-fluorocytidine; 5'DFUR: 5'Deoxy-furouridine

Metabolic conversion of Capecitabine to 5-fluorouracil

Capecitabine is orally administered as a prodrug converted to 5'-deoxy-5-fluorocytidine (5'DFUR), which is ultimately converted into 5-FU *in vivo* (as shown in Figure 3). Therefore, to understand the mechanism of action of capecitabine, it is important to understand the mechanism of 5-FU action. 5-FU exerts its anticancer effects through inhibition of thymidylate synthase (TS) and incorporation of its metabolites into RNA and DNA, as outlined in Figure 3.

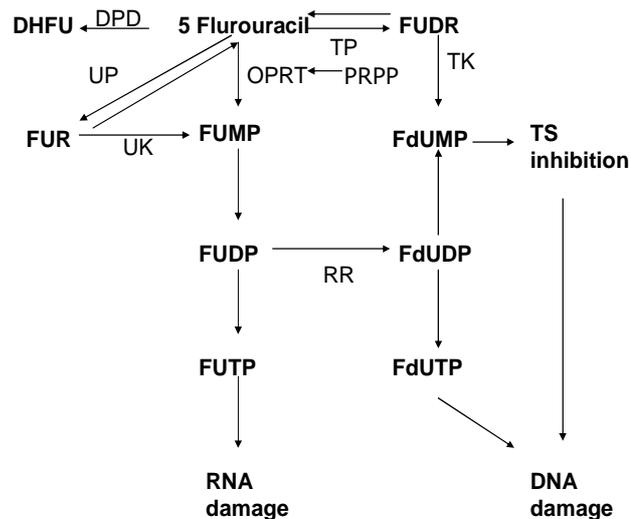


Figure 4. Cellular metabolic scheme for 5-fluorouracil.

FdUDP: Fluorodeoxyuridine diphosphate; FdUMP: Fluorodeoxyuridine monophosphate; FdUTP: Fluorodeoxyuridine triphosphate; FUDP: Fluorouridine diphosphate; FUTP: Fluorouridine triphosphate; FUMP: Fluorouridine monophosphate; OPRT: Orotate phosphoribosyltransferase; PRPP: phosphoribosyl pyrophosphate; FUR: Fluorouridine; UP: Uridine phosphorylase; UK: Uridine kinase; RR: Ribonucleotide reductase; DPD: Dihydropyrimidine dehydrogenase; TK: Thymidine kinase.

Cellular metabolic scheme for 5-FU

5-Fluorouracil is converted to three active metabolites: fluoroxyuridine monophosphate (FdUMP), fluoroxyuridine triphosphate (FdUTP) and fluorouridine triphosphate (FUTP), as shown in Figure 4. The mechanism of 5-FU activation is conversion to fluoroxyuridine monophosphate, either directly by orotate phosphoribosyltransferase (OPRT) with phosphoribosyl pyrophosphate as the cofactor, or indirectly via fluorouridine (FUR) through the sequential action of uridine phosphorylase (UP) and uridine kinase. Fluorouridine monophosphate is then phosphorylated to fluorouridine triphosphate, which can be either further phosphorylated to the active

metabolite FUTP, or converted to fluorodeoxyuridine diphosphate (FdUDP) by RNR. In turn, FdUDP can either be phosphorylated or dephosphorylated to generate the active metabolites FdUTP and FdUMP, respectively. An alternative activation pathway involves the thymidine phosphorylase-catalyzed conversion of 5-FU to fluorodeoxyuridine (FdUR), which is then phosphorylated by thymidine kinase (TK) to FdUMP. Dihydropyrimidine dehydrogenase (DPD)-mediated conversion of 5-FU to dihydrofluorouracil (DFHU) is the rate-limiting step of 5-FU catabolism in normal and tumor cells. Up to 80% of administered 5-FU is broken down by DPD in the liver [63].

We will now discuss the potential mechanisms thought to be involved in innate and acquired resistance to capecitabine. Unfortunately, as little research has been conducted on capecitabine resistance mechanisms in pancreatic cancer, we must rely on indirect evidence gathered from knowledge of capecitabine resistance in other tumor types. Table 4 summarizes the main known resistance mechanisms to capecitabine and its downstream products.

Table 4. Factors involved in capecitabine/5-FU resistance

Mechanism	Marker of resistance	Ref
Membrane transport mechanisms	hENT1 & hENT2	[65]
	hCNT1	[64]
	Upregulation of MRP5	[68]
	Upregulation of MRP8	[69]
Enzymes important in DNA/RNA synthesis	TP	[74]
	DPD	[78]
	TS	[79]
Genes involved in cell cycle regulation, proliferation and apoptosis	Src kinase activation	[86]
	Overexpression NF-κB	[84]
	Over expression of antiapoptotic gene c-Flip	[84]

DPD: Dihydropyrimidine dehydrogenase; hCNT: Human concentrative nucleoside transporter; hENT1: Human equilibrative nucleoside transporter; MRP: Multidrug-resistance protein; TP: Thymidine phosphorylase; TS: Thymidylate synthase.

Cellular transport mechanisms

Currently, there is very little information available on the role of transporters in resistance to 5'-DFUR (the intermediate product of capecitabine) in pancreatic cancer. Mata *et al.* showed that 5'-DFUR is a substrate for the nucleoside transporter hCNT1, but not for

capecitabine or 5-FU, and its expression conferred sensitivity to the drug in a heterologously hCNT1-expressing CHO-K1 cell line [64]. In breast cancer cell lines, hENT1 is the main transporter of 5'-DFUR, and deficiency of this protein reduces the cytotoxicity of 5'-DFUR [65]. Inhibition of hENT1 has been demonstrated to block most of the transcriptional targets of 5'-DFUR action, including genes associated with apoptosis and cell cycle progression [66]. Therefore, there is evidence to suggest that hENT1 and hCNT1 are both transporters of 5'-DFUR and deficiency of these transporters reduces the cytotoxicity of 5'-DFUR. However, expression of these transporters in pancreatic cancer cells and its impact on the prognosis of pancreatic cancer has not yet been studied.

Role of efflux pumps in cancer resistance to capecitabine metabolites

Pancreatic cancer's intrinsic resistance to established chemotherapy drugs can be mediated by multidrug resistance proteins (MRPs) and the multi-drug resistant-1 gene (*MDR*; P-glycoprotein) [67]. However, the contribution of MDR to chemoresistance in pancreatic carcinoma is unclear. Hagmann *et al.* found MRP3, MRP4, and MRP5 were upregulated in 5-FU-resistant Capan-1 pancreatic cancer cells [68]. RNAi-mediated knockdown of MRP5 resulted in increased sensitivity to 5-FU in pancreatic carcinoma cells. Utilizing a PC6 small cell lung cancer cell line Oguri *et al.* developed resistance to 5-FU and found that reduced drug sensitivity was associated with over expression of the *MRP8* gene [69].

Sub cellular enzyme targets

In theory, either a deficiency of enzymes that are responsible for the activation of capecitabine intermediates or an increase in levels of enzymes responsible for inactivation of capecitabine could confer resistance. Three enzymes demonstrated to be involved in resistance to activated capecitabine products are TP, TS and DPD.

Thymidine phosphorylase is an enzyme present in cancer cells responsible for converting 5'-DFUR to its active metabolite, 5-FU, and is therefore a limiting factor in the anti-tumor effects of these drugs. Interestingly, this enzyme is identical to PDGF and also has angiogenic activity [70]. Increased pretreatment levels of TP in pancreatic [70] and colon [71] cancer is associated with unfavorable clinical outcome and poorer survival. In breast cancer, patients with TP-positive tumors showed a longer time to progression if they received taxanes before capecitabine than patients with TP-negative tumors who did not receive this treatment, providing evidence that TP expression in breast cancer could represent a biomarker of sensitivity to capecitabine treatment [72].

Meropol evaluated TP, TS, and DPD levels for their ability to predict response to capecitabine treatment in colon cancer [73]. Positive staining for TP by immunohistochemistry predicted for significantly higher response rate (65% v 27%) to the combination of capecitabine with irinotecan (CAPIRI), and survival that was nearly double for patients with TP-negative tumors.

Studies have also demonstrated that intratumor levels of TP and DPD are indicators of tumor response to capecitabine, and that the ratio rather than the actual expression levels of TP or DPD alone is predictive of response to capecitabine treatment [74-76]. Schuller *et al.* showed that increased TP levels resulted in increased intratumoral 5-FU levels, thereby enhancing the effect of capecitabine in colorectal tumors [77]. Tsukamoto *et al.* presented data suggesting that high levels of DPD expression result in lower intratumoral 5FU levels through increased degradation *in vitro* [74].

Recent studies further support a correlation between the ratio of TP:DPD and capecitabine response. Ishikwa *et al.* showed that capecitabine can be effective in tumors expressing low TP, if DPD expression is low as well [78]. A recent study investigated TP and DPD levels in tumor tissue to assess their clinical significance as indicators for selecting colorectal cancer patients for 5'-DFUR-based adjuvant chemotherapy. Results showed that patients with high TP but low DPD expression had the best disease-free survival, whereas the low TP but high DPD group had the worst survival [75]. As capecitabine is ultimately converted in the cells to 5-FU by the action of TP, the alteration of enzymes and targets of 5-FU activation may also play a role in chemoresistance of capecitabine.

Another enzyme that has been very extensively studied in the resistance to 5-FU is TS. Peters *et al.* found that initial 5-FU treatment inhibited TS, but prolonged exposure induced TS levels [79]. TS is a key enzyme in the synthesis of 2 deoxy5monophosphate (dTMP) from 2 deoxyuridine monophosphate (dUMP), for which methyltetrahydrofolate is a methyl donor. TS is an important target of the active metabolite of 5-FU, and 5-FU treatment can induce TS expression. Okumara *et al.* suggested that TS mRNA expression level may be a predictor of chemosensitivity to 5-FU in colon cancer patients as TS mRNA expression was inversely related to 5-FU sensitivity [80]. Banerjee *et al.* found that high levels of TS occur as a result of increased copy number or increased translation/transcription, and is associated with intrinsic resistance to fluropyrimidines [81].

Other enzymes responsible for activation of 5-FU include uridinemonophosphate kinase (UMP5K) and orotate phosphorylase transferase (OPRT). Decreased levels of these enzymes have also been described as a resistance mechanism for 5-FU [82]. UMP5K is an enzyme that is responsible for conversion of 5-FU to 5FUTP and its incorporation into RNA, and it was demonstrated that resistance to bolus 5-FU was associated with lower expression of UMP5K. However, the activities of other 5-FU-metabolizing enzymes remain unchanged. More recently, Koopman *et al.* found that patients with high OPRT expression in stromal cells had a favorable prognosis for overall survival; however, high OPRT levels in tumor cells was an unfavorable prognostic parameter for progression-free survival and overall survival in the Capecitabine, Irinotecan and Oxaliplatin (CAIRO) study for advanced colorectal cancer [83].

Contribution of genes involved in cell cycle regulation, proliferation and apoptosis to resistance

As with gemcitabine, genes involved in apoptosis cell cycle regulation and proliferation play an important part in resistance of capecitabine.

Wang *et al.* profiled 5-FU-resistant and corresponding parental breast cancer cell lines by microarray analysis [84]. Enzymes involved in 5-FU activation were downregulated in resistant lines, including TK, UMPK and OPRT. Overexpression of genes involved in cell regulation, proliferation and apoptosis such as *TS*, *c-YES*, *NF- κ B*, *p65* and *c-Flip* were also detected in resistant cells. Cotransfection of *NF- κ B* and *p65* cDNA induced 5-FU resistance in MCF-7 cells corresponding with reduced expression of genes governing G₁-S and S-phase transition. This phenotype may protect FU-resistant cells from cell death induced by incorporation of 5-FU into DNA chains by allowing time to repair 5-FU induced damage.

Maxwell *et al.* analyzed the 5-FU-resistant H630-R10 and parental H630 colorectal cancer cell lines by microarrays and found basal expression levels of SSAT, annexin II, thymosin beta-10, and chaperonin-10 and MAT-8 expression dramatically increased in the 5-FU-resistant cell line compared with the parental line, suggesting that these genes may be useful biomarkers of resistance [85].

Xin *et al.* suggested that the activation of antiapoptotic genes after repeated drug exposure contributes to chemo-resistance of pancreatic cancer cells towards 5-FU, and that blockade of antiapoptotic genes might enhance chemosensitivity in pancreatic cancer [30]. Ischenko *et al.* developed a 5-FU-resistant pancreatic cancer cell line and tested the hypothesis that Src tyrosine kinase inhibition could augment the chemo-sensitivity of 5-FU-resistant human pancreatic cancer cells to 5-FU [86]. They found that combining 5-FU and Src kinase inhibitor restored the 5-FU-induced apoptosis in the 5-FU resistant cell line. Western blotting and RT-PCR analysis revealed that the expression of TS was higher in 5-FU-resistant cells; however, expression decreased significantly after pre-treatment with Src kinase inhibitor. Furthermore, the combination of 5-FU and Src inhibitor decreased the 5-FU-induced activation of EGF receptor (EGFR)-Akt pathway. Finally, 5-FU and Src inhibitor substantially decreased the *in vivo* tumor growth and inhibited distant metastases. Taken together, 5-FU chemoresistance can be reversed through indirect TS regulation by inhibiting Src tyrosine kinase, which may be linked to the inhibition of 5-FU-induced EGFR-Akt activation

Erlotinib

Erlotinib (Tarceva®, OSI Pharmaceuticals [NY, USA]/Genentech, Inc, Roche) is a small molecule tyrosine kinase inhibitor targeting the intracellular domain of EGFR and competes with ATP for binding to the kinase domain, thereby impeding downstream signaling.

Human epidermal growth factor receptor is a member of ErB receptor family of receptor tyrosine kinases; it is over expressed 30-65% of pancreatic ductal adenocarcinoma [87] and has been implicated in the carcinogenesis of the disease [88]. EGFR overexpression is associated with poor prognosis, poorly differentiated histology and a more advanced

stage of cancer [89]. Recent studies have also suggested that EGFR may be useful as a predictive marker for invasion and metastasis in pancreatic cancer [90].

Over expression of another member of the EGFR family, HER2, has been observed with variable incidence of 10-60% in this disease [91] and overexpression of HER2 is inversely related to survival [92].

It has been established that EGFR signaling pathways deregulation occur through various mechanisms including receptor or ligand overexpression, receptor mutation and receptor cross-talk [93]. In contrast to receptor homodimerization, heterodimerization of EGFR with HER2 provides a stronger growth stimulus, mediated through two pathways, P13K-Akt pathways and the Ras-Raf mitogen-activated pathway [94], which leads to a cascade of events resulting in cell survival and chemoresistance..

Predictability of response to erlotinib in pancreatic cancer

In contrast to lung cancer, *EGFR* mutation status and its response to erlotinib has been poorly studied. Although a study by Moore *et al.* showed modest benefit of erlotinib with gemcitabine versus gemcitabine alone, a 1.5 to 3.6% frequency of *EGFR* mutation in pancreatic cancer was reported, which is significantly lower than other cancer types [5]. A study by Tzeng *et al.* characterized *EGFR* mutation in pancreatic adenocarcinoma by using 9 pancreatic cancer cell lines and 31 specimens from pancreatic cancer patients, and observed that the EGFR tyrosine kinase domain is highly conserved in pancreatic cancer [95]. Clearly there needs to be a more significant and clinically focused investigation of the predictive value of the *EGFR* mutation and gene copy number on erlotinib response in pancreatic cancer to give a more definitive understanding of the role of these alterations in erlotinib sensitivity.

Frolov *et al.* found that heterodimerization of EGFR with ERB3 and downstream signaling from ERB3 is an important mechanism of carcinogenesis [96]. Miapaca-2 cells, which lack ERB3, displayed persistent activation and ongoing proliferation, despite the use of erlotinib. Erlotinib treatment inhibited Akt phosphorylation in ERB3-expressing cell lines. The same phenomenon was demonstrated by Buck *et al.* [97]. Their work on pancreatic and colon cancer cell line and demonstrated that coexpression of EGFR with ERB3 determine the sensitivity to erlotinib.

Unfortunately, there have been proportionately few investigations of the mechanisms underlying erlotinib resistance in pancreatic cancer, so there is an obvious need for further investigation. Therefore, we will review the recognized mechanism of resistance to erlotinib and related tyrosine kinase inhibitors in other cancers. Box 1 summarizes our knowledge of erlotinib resistance mechanisms based on a number of different cancer types.

Box 1. Summary of the major known erlotinib resistance mechanisms

- Down regulation of PTEN [99]
- Amplification of MET [100]
- Mutation of EGFR or other genes as mechanism of resistance [101]
- Mutation in downstream EGFR targeting pathway genes [102]
- Alterations in the expression of related growth factor receptor pathways/proteins [106-107]

Overexpression/activation of signaling proteins

Phosphatase and tensin homolog is a lipid phosphatase and tumor suppressor protein that regulates P13K-Akt signaling pathway. The major substrate for PTEN is phosphatidylinositol 3, 4, 5-triphosphate, a second messenger of P13K. With the loss of PTEN function, phosphatidylinositol 3, 4, 5-triphosphate accumulates in the cell membrane and activates Akt increasing its cellular anti-apoptotic function [98]. Yamasaki *et al.* demonstrated that acquired resistance to erlotinib involves the activation of phosphorylated Akt (p-Akt) and downregulation of MMAC1/PTEN in the A-431 epidermoid cancer cell line [99]. Gefitinib is another EGFR inhibitor and Engelmann *et al.* found that amplification of *MET* causes gefitinib resistance in a gefitinib-resistant lung cancer cell line by driving *ERBB3* (HER3)-dependent activation of PI3K, a pathway thought to be specific to EGFR/ErbBB family receptors [100]. Hence a similar phenomenon may also occur with erlotinib.

Mutation of EGFR or other genes as a mechanism of erlotinib resistance

Pao *et al.* undertook a study of lung cancer patients whose disease progressed on erlotinib or gefitinib and found, in addition to a drug sensitive mutation in *EGFR*, a secondary mutation in exon 20, which leads to the substitution of methionine for threonine at position 790 (T790M) in the kinase domain [101]. This study showed that the secondary mutation (T790M) emerges in the resistant subclones in the presence of a drug. Interestingly this secondary mutation was not seen in untreated tumor samples. The authors showed that this T790M mutation directly confers resistance to other *EGFR* mutants usually sensitive to either erlotinib or gefitinib. This study also showed that 24% of the patients who were refractory to erlotinib treatment had a *k-ras* mutation and none of the erlotinib-sensitive tumors had such a mutation. *K-ras* mutation has been associated with primary resistance to erlotinib treatment in non-small-cell lung cancer. In colon cancer treatment, a very clear link has been established between the efficacy of EGFR-targeted treatments and *k-ras* mutation status [102]. Karapetis *et al.* undertook a study in colon cancer patients and their tumor samples, and assessed whether *k-ras* mutation status is associated with response to cetuximab (EGFR-targeted agent). They found that patients with mutated *k-ras* did not benefit from cetuximab treatment, whereas patients

with tumors with wild-type *k-ras* did [102]. This association has, as yet, not been established in pancreatic cancer.

Role of receptor activation as a mechanism of resistance to erlotinib

The IGF-1 receptor (IGF-1R) activates many of the same down-stream pathways as EGFR and overexpression can lead to carcinogenesis, increased proliferation, angiogenesis and metastasis [103]. PI3K-Akt signaling is a critical component of the downstream mediation of EGFR and also plays a functional role in IGF-1R signaling [103]. Chakravarti *et al.* identified two glioblastoma cell lines that both overexpressed EGFR, but exhibited very different responses to EGFR inhibitors [104]. The resistant cell line significantly overexpressed IGF-1R and showed further increases in IGF-1R expression in response to EGFR inhibition by AG1478, an EGFR tyrosine kinase inhibitor. PI3K-Akt signaling persisted in the resistant cell line in response to AG1478 treatment, and these cells also maintained their invasive and antiapoptotic characteristics. These findings point towards an ability to switch from EGFR signaling to IGF-1R signaling in this cell model in the presence of EGFR inhibition. Therefore, inhibiting both pathways simultaneously may provide a mechanism to circumvent this ability to switch oncogenic drivers, reduce resistance potential, and thereby reduce the growth and invasiveness of cancerous cells [105].

Morgillo *et al.* also pointed towards another interesting phenomenon of erlotinib resistance [106]. Their data suggested that heterodimerization of EGFR/IGF-1R and its downstream signaling, including mTOR, stimulated *de novo* protein synthesis of EGFR and survivin with the treatment of erlotinib. They also demonstrated *in vitro* and *in vivo* that knockdown of survivin, inhibition of IGF-1R activation and suppression of mTOR-mediated protein synthesis abolished erlotinib resistance.

Choi *et al.* worked on non-small-cell lung cancer cell lines (H1650, PC-9, HCC-827) containing *EGFR* mutations that were primarily resistant to tyrosine kinase inhibitors [107]. Combined treatment of gefitinib with an IGF-1R inhibitor induced growth inhibition, apoptosis and downregulation of phosphorylation of Akt, EGFR and IGF-1R. Their data pointed towards combined use of IGF-1R and EGFR inhibitors in patients with non-small-cell lung cancers that are refractory to treatment with tyrosine kinase inhibitors.

Taken together, although there is very limited information on the underlying mechanisms of erlotinib resistance in pancreatic cancer, extrapolation from observations of erlotinib resistance in other tumor backgrounds suggests that it may be feasible to identify markers of such resistance, such as *k-ras*, and/or prevent or overcome resistance by targeting more than one point in key growth-driving pathways in the tumor cells.

Expert commentary and five year view

Pancreatic cancer is a disease with extremely poor prognosis. Although the addition of erlotinib to gemcitabine has improved median survival, this benefit is quite modest owing

to the resistance characteristics of this malignancy. Resistance to pancreatic cancer treatment represents a significant clinical and scientific challenge. We have so far identified some putative laboratory markers of resistance that could be helpful in properly selecting patients for treatment with, for example, gemcitabine and capecitabine, but the therapeutic potential/implications of those markers has not been explored in sufficient depth to incorporate them into current treatment. There is a clear and distinct need to identify and characterize robust markers, especially those which might be expressed in serum (owing to the inherent ethical and clinical challenges associated with tumor sampling in this particular malignancy). Our selection process for such markers should focus specifically on early detection, characterization of the intrinsic sensitivity/resistance to standard chemotherapies and early response markers owing to the frequency of resistance and the rapid progression of the disease. Looking away from the tumor itself, there is an appreciable amount of evidence suggesting that the tumor microenvironment plays an important role in this malignancy, and clearly a significant research effort must also be directed at this phenomenon to identify potential novel treatment approaches that may synergize with more directly tumor-targeting therapies. Advancing these specific aspects of pancreatic cancer research will allow us to make much better use of the existing arsenal of anticancer drugs and also offers the hope of developing completely new, and possibly less toxic, targeted therapeutics for pancreatic cancer treatment, as has been the case with some other malignancies.

Research into better treatment strategies for pancreatic cancer will continue, driven by the dearth of knowledge of that particular malignancy, coupled with the slow but inexorable global increase in the incidence of the disease. Five years is a proportionately short time in clinical cancer research, so our strategies at this time are likely to be informed by extrapolation from our overlapped knowledge with other malignancies and the underlying contributions of key alterations in signaling pathways driving the disease and confounding treatment response. Extrapolation of existing research suggests that we may be in a position to conduct larger-scale clinical evaluations of putative markers and especially marker profiles to confirm the presence and magnitude of the disease. Some of these putative markers may also provide early, semi-quantitative information on the response of the malignancy to treatment. The oncology pharmaceutical industry is awash with new drugs targeting new aberrations and pathway alterations, as well as second-generation agents with improvements in over existing targeted drugs, and it appears highly likely that at least some of these agents will be evaluated in regimens in pancreatic cancer, hopefully providing increases in rates and extent of patient remission. If we can identify agents that give durable response in patients, this may also drive an increased acceptance of routine molecular and pathological characterization of the patient's tumor prior to treatment.

While pancreatic cancer is a particularly challenging malignancy to treat, and its recent history has attracted a share of therapeutic "false starts," extrapolation of our current knowledge does give cause for optimism and suggests that significant advances in our understanding of the disease and the improvements in treatment options that derive from that will be forthcoming, allowing better stratification of patients for treatment and improved response.

Key issues

- Median survival of locally advanced pancreatic cancer is 9 months and metastatic pancreatic cancer is approximately 6 months.
- To date we can only detect some putative laboratory markers of resistance but the therapeutic potential/implications of those markers have not been explored sufficiently to incorporate them into treatment.
- We are still at an early stage in the understanding of this disease as we are looking for key aberrations that bring about the ubiquitous resistance phenotypes.
- We have no clear indicators of treatment response, particularly for erlotinib, despite the emergence of indicators for the treatment efficacy of this agent in other malignancies.
- Better understanding the molecular pathways, role of key genes and role of tissue microenvironment in pancreatic cancer resistance will help us to develop new targeted therapies with improved efficacy.

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