Review

Pharmacogenetics of anticancer drug sensitivity in pancreatic cancer

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Abstract

Chemotherapy has produced unsatisfactory results in pancreas cancer and novel approaches, including treatment tailoring by pharmacogenetic analysis and new molecular-targeted drugs, are required. The scarcity of effective therapies may reflect the lack of knowledge about the influence of tumor-related molecular abnormalities on responsiveness to drugs. Advances in the understanding of pancreas cancer biology have been made over the past decade, including the discovery of critical mutations in oncogenes (i.e., *K-Ras*) as well as the loss of tumor suppressor genes, such as *TP53* and *p16^INK4*. Other studies showed the dysregulation of the expression of proteins involved in the control of cell cycle, proliferation, apoptosis, and invasiveness, such as Bcl-2, Akt, mdm2, and epidermal growth factor receptor. These characteristics might contribute to the aggressive behavior of pancreatic cancer and influence response to treatment. Indeed, the inactivation of p53 may explain the relative resistance to 5-fluorouracil, whereas Bcl-2 overexpression is associated with reduced sensitivity to gemcitabine. However, the future challenge of pancreas cancer chemotherapy relies on the identification of molecular markers that help in the selection of drugs best suited to the individual patient. Recent pharmacogenetic studies focused on genes encoding proteins directly involved in drug activity, showing the role of thymidylate synthase and human equilibrative nucleoside transporter-1 as prognostic factor in 5-fluorouracil- and gemcitabine-treated patients, respectively. Finally, inhibitors of signal transduction and angiogenesis are under extensive investigation, and several prospective trials have been devoted to this area. Pharmacogenetics is likely to play a central role in the personalization of treatment, to stratify patients based on their likelihood of response to both standard agents (i.e., gemcitabine/nucleoside transporters) and targeted treatments (i.e., epidermal growth factor receptor gene mutations and/or amplification and tyrosine kinase inhibitors), Thus, molecular analysis should be implemented in the optimal management of the patient affected by pancreatic adenocarcinoma. [Mol Cancer Ther 2006;5(6):1387–95]

Current Therapeutic Management

Pancreatic cancer is a highly malignant disease whose incidence has risen steadily and it is now the fourth cause of death from cancer in the western world (1); despite this fact, there has been little improvement in prognosis over the past 20 years (2). Due to the delay of clinical diagnosis, pancreatic cancer is often detected at an advanced stage and the prognosis is extremely poor, with survival of 4 to 6 months (2). However, this malignancy has a grim prognosis even following surgical resection (3), and the 5-year survival remains <4% (1, 2).

Unfortunately, systemic chemotherapy still relies on few drugs and has produced unsatisfactory results. Gemcitabine is now the standard drug for advanced pancreas cancer (4). Indeed, treatment with gemcitabine produces clinical benefit and symptom improvement in 20% to 30% of patients and the 2% 1-year survival rate in 5-fluorouracil (5-FU)–treated patients was raised to 18% by gemcitabine (5). Moreover, gemcitabine is expected to have a role in the adjuvant setting, although the ESPAC-3 trial, which aims to randomize 990 patients into observation, 5-FU/leucovorin and gemcitabine, is still under investigation (6). Results of a multicenter phase III trial of adjuvant chemotherapy with gemcitabine versus observation in 368 patients showed an increased disease-free survival in gemcitabine-treated patients (7). Many other chemotherapeutic agents have been examined, but only few of them, such as oxaliplatin and irinotecan, have been consistently shown to have beneficial effect, particularly as second-line chemotherapy as well as in combination with gemcitabine (8, 9). However, randomized studies to date have not shown a survival benefit for combination cytotoxic chemotherapy over gemcitabine alone (2).

Pattern of Molecular Abnormalities

The understanding of molecular events occurring during the development of pancreatic carcinoma may improve the

management of patients, enabling early diagnosis in high-risk individuals (10) and permitting the development of therapies targeting specific pathways. Infiltrating carcinomas may arise from *in situ* pancreatic duct lesions, and the progression to invasive cancer is associated with the accumulation of genetic alterations. For these reasons, molecular techniques that can identify critical mutations in oncogenes, such as *K-Ras*, or deletions of tumor suppressor genes, such as *TP53*, *p16^INK4*, or *Rb*, are being developed and may represent a sensitive approach to recognize incipient neoplasia and find targets for new drugs (11). Tissue microarrays showed that molecular abnormalities in pancreatic intraepithelial neoplasia can be stratified into ''early'' (e.g., overexpression of MUC5), ''intermediate'' (e.g., overexpression of cyclin D1), and ''late'' (e.g., overexpression of p53 or loss of Smad4/Dpc4; ref. 12). Multiple lines of evidence also suggest that *K-Ras* mutation and loss of *p16* are very early events during ductal cell carcinogenesis (13).

Abnormalities of many other oncogenes and tumor suppressor genes have been identified during the last decade (Table 1). Several mutations have been found in specific types of tumors, suggesting that different causes and molecular mechanisms are involved. One example is the loss of heterozygosity at the *von Hippel-Lindau* gene locus in both wild-type and hereditary serous cystoadenomas (14) as well as the occurrence of activating *β-catenin* mutations in 90% of solid pseudopapillary tumors and the virtual absence of *K-Ras* mutation and *DPC4* and *TP53* abnormalities in acinar cell carcinomas, whereas all these mutations are frequently found in ductal adenocarcinomas (15). Indeed, specific gene mutations of ductal cells might explain why, among the heterogeneous population of epithelial cells forming the pancreas, a cell type accounting for <10% of the gland generates a vast majority of tumors.

The variety of genetic changes suggests that multiple etiologic factors contribute to carcinogenesis. For example, pancreatic cancer has, among all cancers, the highest

frequency of *K-Ras* mutations, which have been associated with smoking, alcohol consumption, and exposure to organic solvents (2, 16). Moreover, polymorphisms in DNA repair genes as well as in xenobiotic-metabolizing enzymes may increase pancreatic cancer risk and support a role for both carcinogen exposure and genetic susceptibility to the disease (17). About 10% to 15% of pancreatic cancer cases have a positive family history, including hereditary neoplastic syndromes associated with pancreatic cancer (i.e., Peutz-Jeghers and hereditary nonpolyposis colorectal cancer syndrome), hereditary pancreatitis, and familial pancreatic cancer (18), but several susceptibility genes are still unknown and linkage studies are under way (19). Screening protocols for high-risk subjects have been proposed using a combination of endoscopic ultrasound and tomography scanning (10), but some techniques, such as ERCP, are limited by a low sensitivity (3). Collection of the duodenal juice is an easier method and gene sequencing may be used to detect mutations of codon 12 of *K-Ras* gene, which characterize ∼90% of pancreatic adenocarcinoma, whereas it is not found in nonmalignant disease, such as chronic pancreatitis (20).

Potential Role of Pharmacogenetics in Treatment Optimization

The scarcity of available therapies for pancreas cancer might reflect the lack of knowledge on the way by which molecular abnormalities affect tumor responsiveness to anticancer agents. An overview of the best-characterized interactions between genes, gene products, and chemotherapeutic agents in pancreatic cancer is reported in Fig. 1. This issue deserves intensive investigation to select novel methodologic approaches for a new generation of chemotherapeutic strategies to make a significant effect in the treatment of pancreas cancer. Many studies showed the prognostic significance of expression of molecular determinants involved in proliferation and apoptosis as well as in invasiveness and angiogenesis, such as Akt (21), tissue factor (22), p53 and mdm2 (23), S100A6 calcyclin protein (24), epidermal growth factor receptor (EGFR; ref. 25), and Bax (ref. 26; Table 2). Therefore, the future challenge of chemotherapy of pancreas cancer relies on the identification of molecular and genetics markers that are predictive of response and may help in the selection of chemotherapeutic agent best suited to the individual patient. Indeed, pharmacogenetics studies may explain how the responses of patients to drugs are affected by their genetic profile, and due to the developments in this area, promising agents are gaining momentum, including inhibitors of intracellular signal transduction proteins (2, 27).

Molecular Determinants Related to Drug Sensitivity

Antimetabolites

Examples on how genetics might affect drug response are offered by gemcitabine, a deoxycytidine analogue with proven activity against a wide spectrum of tumors, including pancreatic cancer (2). Because of its hydrophilicity,

Table 1. Molecular alterations frequently detected in human pancreatic adenocarcinoma

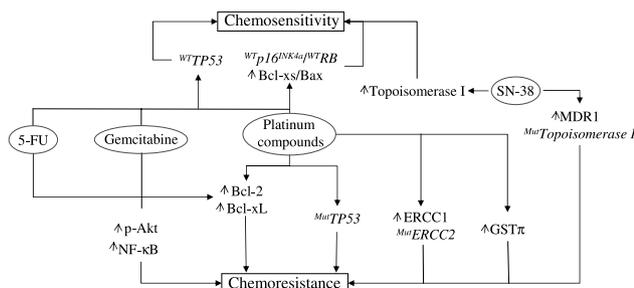|	Alteration frequency (%)
Oncogenes (amplification or mutation)	
K-ras	75–100
c-erb-3	60–70
c-erb-2 (HER-2/neu)	10–20
Tumor suppressor genes (mutations or deletions)	
TP53	50–75
p16^INK4	80–95
Rb	60–70
DPC4	50–55
Growth factors and their receptors (amplification or mutations)	
FGF	60–90
FGFR	50–70
EGFR	25–65
TGFβ	40–50

Figure 1. Interactions between genes involved in pancreas tumorigenesis and progression and anticancer agents. ↑, overexpression; ↓, downregulation; *Mut,* mutation; *WT,* wild-type.

gemcitabine does not cross the membrane by diffusion and it is transported into the cells by human equilibrative nucleoside transporters (hENT) and human concentrative nucleoside transporters (28). *In vitro* studies showed that gemcitabine enters pancreatic cancer cells mostly via the hENT1 transporter (29) and that treatment with nucleoside transport inhibitors, such as nitrobenzylmercaptopurine riboside or dipyridamole, reduced sensitivity to gemcitabine by 39- to 1,800-fold (28). An immunohistochemical study on neoplastic tissue from 21 patients with advanced pancreatic cancer showed that patients expressing detectable amounts of hENT1 had significantly longer median survival after gemcitabine than subjects with low or absent hENT1 (13 versus 4 months; $P = 0.01$; ref. 30). Similar results were obtained in a pharmacogenetic study on 83 pancreatic cancer patients; PCR analysis showed that overall survival (OS) was significantly longer in patients with high hENT1 expression with respect to patients with low hENT1 levels (median, 25.7; 95% confidence interval, 17.6-33.7 months versus median, 8.5; 95% confidence interval, 7.0-9.9 months) and the multivariate analysis confirmed the prognostic significance of hENT1 (31). Moreover, the modulation of hENT1 expression induced by thymidylate synthase (TS) inhibitors (32), as well as by the multitargeted antifolate pemetrexed (33), may represent a new way to explore effective modalities for pancreatic cancer treatment (Fig. 2).

Following cellular uptake, gemcitabine is phosphorylated to its active metabolites diphosphate and triphosphate (dFdCTP) that inhibit ribonucleotide reductase and DNA synthesis. Deoxycytidine kinase (dCK) is the rate-limiting enzyme in the biotransformation of nucleoside analogues. Studies on dCK have also shown that it has a complex regulation (feedback inhibition by the metabolic product dCTP and by ribonucleotides), and its saturation occurs at plasma gemcitabine concentrations of 15 to 20 μmol/L (27). The administration of gemcitabine by a fixed-dose rate infusion (~ 10 mg/m^2/min) has been shown to generate drug concentrations higher than those required to produce maximal intracellular levels of dFdCTP (4). A phase II trial comparing 49 patients treated with a dose of 2,200 mg/m^2 for 30 minutes and 43 patients treated with a dose of 1,500 mg/m^2 for 150 minutes, at the constant dosing rate of 10 mg/m^2/min, showed an improved overall response rate (11.6% versus 4.1%) and a higher 1-year survival rate (23.8% versus 7.3%) in the fixed-dose rate arm (34). However, none of the differences between treatment arms were statistically significant. Therefore, the results of this study can be viewed as hypothesis generating, and the fixed-dose rate strategy is being tested in a phase III trial (27).

Several studies have suggested that dCK is a limiting factor for the antitumor effect of gemcitabine because its deficiency is critically involved in acquired resistance to nucleoside analogues *in vitro*; indeed, the sensitivity to these drugs was restored by transfection with wild-type *dCK* (35). Moreover, a clear correlation between dCK activity and gemcitabine sensitivity in tumor xenografts has been shown (36). The crucial role of dCK was confirmed by the marked reduction of gemcitabine cytotoxicity against pancreatic cancer cells using the dCK substrate 2′-deoxycytidine, whereas transcriptome analysis suggested that the synergistic interaction with pemetrexed mainly relies on the increase of *dCK* mRNA expression (37). Indeed, as an inhibitor of *de novo* purine biosynthesis, pemetrexed may enhance the expression of enzymes involved in salvage nucleoside pathway, including dCK, as a compensatory mechanism. This result is in agreement with previous studies that indicated that several inhibitors of DNA biosynthesis, such as cladribine and fludarabine, had a

Table 2. Molecular determinants of clinical outcome in pancreatic cancer

| Determinant	Correlation with clinical outcome	Rationale
Akt	$P < 0.01$ between p-Akt low expression and longer OS	Akt has antiapoptotic activity and favors angiogenesis and cancer invasiveness.
Tissue factor	$P < 0.0001$ between tissue factor low expression level and longer OS	Tissue factor expression correlates with invasiveness and angiogenesis.
mdm2	$P < 0.05$ between mdm2 expression and shorter OS	The *mdm2* oncogene product affects normal p53 function.
S100A6	$P = 0.003$ between high nuclear S100A6 expression and shorter OS	S100A6 may be involved in promoting cancer growth, invasiveness, and metastasis.
EGFR	$P = 0.02$ between EGFR cytosolic overexpression and shorter OS	EGFR is involved in mitogenic signaling pathways.
Bax	$P < 0.05$ between Bax detection and longer OS	*Bax* gene is a promoter of apoptosis.
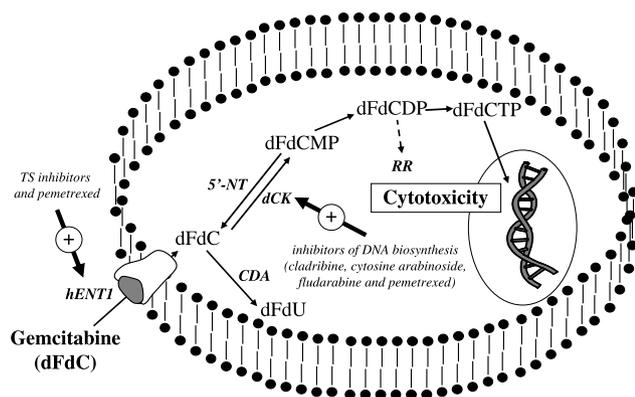
Figure 2. Gemcitabine metabolism and mechanism of action.

stimulatory effect on dCK (38). These findings may have implications for rational design of drug regimens incorporating gemcitabine and other agents modulating the activity of the key enzyme dCK (Fig. 2).

The amount of phosphorylated metabolites of gemcitabine may be reduced by cellular 5′-nucleotidase (5′-NT); moreover, gemcitabine itself is inactivated by cytidine deaminase (CDA; ref. 4), and high expression of these catabolic enzymes has been found in tumor cells resistant to nucleoside analogues (39). A recent study has also shown a clear correlation between higher CDA expression in peripheral blood mononuclear cells and shorter survival in gemcitabine-treated patients with advanced pancreatic carcinoma (40).

In addition to be incorporated into DNA, gemcitabine exerts its cytotoxicity by inhibiting ribonucleotide reductase (RR). Data support the hypothesis that the M1 subunit of RR is the most important cellular target, although resistance to gemcitabine is observed in both RRM1- and RRM2-overexpressing cells (41, 42). A clinical study reported that non–small cell lung cancer patients with lower expression of RRM1 significantly benefited from gemcitabine/cisplatin neoadjuvant chemotherapy (43). Finally, RRM1 promoter allelotypes 37CC-524TT were found to influence OS and disease-free survival in 286 non–small cell lung cancer patients (44). Thus, a mechanism for gemcitabine resistance other than decreased activity of dCK and enhanced activity of 5′-NT or CDA could be a mutation or overexpression of RR, thereby providing the rational basis for the selection of cancer patients to be treated with the drug.

Pancreatic cancer cell lines express variable amounts of Bcl-2, and its overexpression is associated with a significant increase in the gemcitabine-induced apoptosis (45). Similarly, the enhanced expression of Bax significantly increased the sensitivity of pancreatic ASPC-1 cells to gemcitabine and 5-FU (46).

On the other hand, two antiapoptotic signal transduction pathways that have been linked to chemoresistance of pancreatic cancer cells are phosphatidylinositol 3-kinase/Akt and nuclear factor-κB (NF-κB). Recent investigations showed that the reduction of phosphorylated protein kinase B/Akt correlated with the enhancement of gemcitabine-induced apoptosis and antitumor activity, suggesting that the phosphatidylinositol 3-kinase/Akt pathway plays a significant role in mediating drug resistance in several pancreatic cancer cells (47). No such correlation was observed in a study in BxPc-3, Capan-1, and PancTu-1 cells and the inhibition of phosphatidylinositol 3-kinase/Akt by LY294002 did not affect gemcitabine-induced apoptosis (48). These controversial findings may be attributed to the high basal NF-κB activity. Indeed, NF-κB inhibition enhanced the activity of gemcitabine, suggesting that modulation of NF-κB pathway may have therapeutic potential when combined with gemcitabine (48). Furthermore, an immunohistochemical analysis in 65 patients who underwent resection revealed that p-Akt levels were prognostic for OS; thus, Akt inhibition might be a possible pharmacologic target for pancreatic cancer (21).

Similar genetic and molecular considerations might be relevant to the chemotherapeutic activity of 5-FU, because several studies investigated the association between clinical response and intratumoral gene expression of the enzyme of 5-FU catabolism [dihydropyrimidine dehydrogenase (DPD)] and of the target enzyme TS. An immunohistochemical evaluation of DPD expression in 68 pancreatic tumors revealed that in the DPD low-expression group the adjuvant 5-FU chemotherapy subgroup showed a significantly higher survival rate than the untreated subgroup. Moreover, among the treated patients, OS was significantly better in the DPD-negative subgroup than in the DPD-positive subgroup (49). Similarly, a recent study showed that high TS immunoreactivity, detected in 43% and 47% of the primary and metastatic lesions, respectively, had a significant influence on the outcome of patients with resectable pancreatic cancer, and the rate of survival of the high TS group was significantly higher than that of the negative or low reactivity groups. However, high TS immunoreactivity did not have a significant influence on survival of the patients with unresectable tumors nor was an independent prognostic factor (50). In a following study on 132 patients, using a monoclonal antibody showing a close correlation with *TS* mRNA expression measured by reverse transcription-PCR, median survival among subjects with low intratumoral TS expression (63%) was longer than that among patients with high TS expression (37%), and in multivariate analysis, high TS expression turned out to be an independent predictor of mortality ($P = 0.029$). However, among patients with high TS-expressing tumors, 5-FU-based adjuvant therapy significantly reduced the risk of death (relative risk, 0.37; $P = 0.0006$), whereas 5-FU-based adjuvant therapy did not influence survival among patients with low TS-expressing pancreatic cancer (51).

TS inhibition-mediated DNA damage can result indirectly in an increase of wild-type p53 expression and, subsequently, of the cell cycle kinase inhibitor p21. This effect results in cell cycle arrest, during which apoptosis or

DNA repair can occur. In a high percentage of cancers, this protective mechanism against DNA damage is disrupted through mutation in *TP53*, and because *TP53* is the second most frequently inactivated tumor suppressor gene in pancreatic cancer (2, 3), it may explain its relative resistance to 5-FU. Indeed, there is evidence of an association of *TP53* mutations or positive p53 immunostaining in pancreatic cancer with a more advanced clinical stage and shorter postoperative survival, although other analyses reported contradictory results (3).

Finally, several studies have focused on thymidine phosphorylase (TP). Owing to the poor oral bioavailability of 5-FU due to DPD activity in the gut and liver, administration of 5-FU and a DPD inhibitor (i.e., ethynyluracil), or a 5-FU prodrug, such as capecitabine, has proven to be an effective strategy. Capecitabine is a promising tumor-specific agent used in clinical trials against pancreatic cancer (27). Indeed, it releases 5-FU in cancer cells expressing high levels of TP, and a recent immunohistochemical analysis in pancreatic cancer and adjacent nonmalignant pancreatic tissue showed that the mean of thymidine phosphorylase–positive epithelial and endothelial cells was significantly higher in pancreatic cancer tissue (52).

Topoisomerase Inhibitors

Topoisomerase inhibitors have also shown activity in gastrointestinal cancers (8); in particular, irinotecan has an active metabolite, SN-38, which inhibits topoisomerase I activity by stabilizing the topoisomerase I-DNA cleavable complex. Conversion of irinotecan to SN-38 occurs by carboxyl esterase enzyme, and the modulation of carboxyl esterase expression may affect the chemosensitivity to irinotecan (53). Nevertheless, the knowledge of expression levels of genes encoding drug targets would be instrumental in planning a successful chemotherapeutic regimen. In a study with both Northern and Western blotting analyses and RNA/PCR quantitation method, acquired resistant pancreatic tumor cells have shown decreased levels of *topoisomerase I* mRNA compared with their parental cell lines. Moreover, specific topoisomerase I activity of the native resistant cell lines was fairly lower than that of sensitive cells, suggesting that immunoreactive topoisomerase I protein contains low levels of active form enzyme (54). Recently, gene sequencing revealed the presence of several mutations, potentially associated with drug resistance, and mainly involving exons 12, 13, 15, and 20 (55). Therefore, although similar data are still lacking for pancreatic cancer, it is conceivable that mutational analysis of topoisomerase I might be considered to exclude patients from receiving irinotecan if their genotype suggests drug resistance.

Alkylating Agents

Over the past few years, there has been an increase in the use of gemcitabine-platinum combinations in the treatment of pancreatic cancer (9). The choice of such combination was based on lack of overlapping dose-limiting toxicities and results of experiments against pancreatic cancer cells (56). In particular, the synergism between gemcitabine and cisplatin seemed to be mainly dependent on an increase in platinum-adduct formation possibly related to changes caused by gemcitabine incorporation in DNA (57). Indeed, platinum compounds inhibit cell proliferation by damaging DNA through the formation of intrastrand and interstrand cross-links. Therefore, drug resistance occurs mostly because of detoxification or efficient repair of DNA by the nucleotide excision repair system. Among the members of the nucleotide excision repair superfamily, the *excision repair cross-complementing group 1* (*ERCC1*) gene product is responsible for the endonuclease activity. A clinical trial showed that low *ERCC1* gene expression is associated with improved response and survival of patients with colorectal cancer treated with oxaliplatin (58). On the contrary, 66% of pancreatic adenocarcinoma specimens presented a striking up-regulation in the expression of the gene *rad51*, which enhances survival of cells after induction of DNA double-strand breaks by mediating DNA repair via homologous recombination (59). These data agree with microarray experiments showing *rad51* overexpression in cell lines and tumor tissues and suggest that perturbations of DNA repair may contribute to the malignant phenotype of pancreatic cancer (60). Several polymorphisms have been reported in nucleotide excision repair genes, and data suggested that genetic variation may be associated with cancer risk as well as with clinical outcome after chemotherapy (43). In particular, the *X-ray repair cross-complementing group 1* [399]Gln allele is a potentially important determinant of susceptibility to smoking-induced pancreatic cancer (17). However, there are not yet data on correlation of polymorphisms and clinical outcome in pancreatic cancer patients treated with platinum compounds and the efficacy of these drugs may be affected by additional mechanisms. For example, cisplatin chemosensitivity may be modified by glutathione S-transferase π (GSTπ), which catalyzes the conjugation of electrophilic molecules with glutathione and is overexpressed in pancreatic cancer (61), whereas cisplatin itself may significantly limit the resistance to 5-FU by down-regulation of DPD, TS, GSTπ, and multidrug resistance–associated protein (MRP) family (62). Indeed, additional factors that protect cancer cells from toxicity are represented by the ATP-binding cassette superfamily, which includes P-glycoprotein and members of the MRP family (63). Positive P-glycoprotein immunostaining was observed in 73.2% of pancreatic ductal adenocarcinomas, where it was inversely related with aggressiveness of tumors, depending on a lower histologic grade, although the chemotherapeutic response was impaired (64). Moreover, an *in vitro* study showed that P-glycoprotein and MRP1 overexpression caused a cellular stress resulting in increased gemcitabine metabolism and sensitivity, whereas reversal of collateral gemcitabine sensitivity by verapamil also suggests a direct relation between presence of membrane efflux pumps and gemcitabine sensitivity (65). Finally, a recent study showed that the expression of *MRP3* mRNA was up-regulated in pancreatic samples and was correlated with tumor grading, whereas the *MRP5* mRNA level was significantly higher in carcinoma compared with normal pancreatic tissue. These

data suggest that *MRP3* and *MRP5* are involved in drug resistance and analysis of their expression may contribute to predict the benefit of chemotherapy in pancreatic cancer patients (63).

Novel Cancer Therapeutics Based on Genetic Abnormalities

Based on molecular mechanisms responsible for carcinogenesis, several newer compounds, including biological agents targeting signal transduction pathways, have been synthesized and tested against pancreatic cancer (27).

An important aspect of multistep tumorigenesis is the mutational activation of *Ras* gene. Oncogenic forms of *Ras* are locked in their active state and transduce signals essential for transformation, invasion, and metastasis via downstream pathways involving the RAF/mitogen-activated protein kinase cascade, phosphatidylinositol 3-kinase, etc. (3). *K-Ras* mutations occur in ∼90% of pancreatic adenocarcinomas (2), and tumor cells overexpressing *Ras* display reduced sensitivity to cisplatin and ionizing radiation. However, activated *Ras* seems to enhance the tumor cell sensitivity to topoisomerase II inhibitors (66). Ras proteins require post-translational modifications with a farnesyl moiety; therefore, the function of Ras may be targeted by farnesyl transferase inhibitors (67). However, a phase III study in 688 patients showed that median OS for the gemcitabine plus tipifarnib arm was 193 days compared with 182 days for gemcitabine and the placebo arm, with no difference in progression-free survival and in the 1-year survival rate (68).

Other targets for solid tumors are involved in mitogenic signaling pathways. The excessive activation of such signaling cascades is determined by tyrosine kinase growth factor receptors and their ligands, which are frequently overexpressed in pancreatic cancers. These include the EGFR and related receptors (i.e., HER-2), multiple ligands that bind to EGFR and fibroblast growth factor receptors and ligands (69). A recent study showed that patients with cytoplasmic EGFR overexpression had shorter OS than those without EGFR overexpression ($P = 0.02$; ref. 25). Pancreatic cancers also frequently overexpress transforming growth factor-β that usually inhibit cell growth but underexpress the type I transforming growth factor-β receptor and harbor mutations in the *smad4* gene. These alterations prevent transforming growth factor-β from inhibiting cancer growth (4). However, the signaling pathways can be blocked both by antibodies binding to the external domain and by small-molecule tyrosine kinase inhibitors that bind to the ATP-binding site of the receptor. For example, EGFR blockade with the cetuximab antibody plus gemcitabine resulted in an additive effect on pancreatic carcinoma growing orthotopically in mice (70), and a phase II study in patients positive for EGFR staining showed promising results (52% patients reported stable disease and the OS was 203 days with a 18% progression-free survival rate and 33% alive at 1 year); therefore, phase III trial with this

combination is being planned (71). Similar results in efficacy (OS of 7 months and 1-year survival rate of 19%) and mild toxicity were obtained with the combination of gemcitabine and the monoclonal antibody trastuzumab in a phase II study conducted in 32 patients with HER-2-positive metastatic pancreatic adenocarcinoma (72). Small-molecule tyrosine kinase inhibitors gefitinib and erlotinib have also been combined with gemcitabine and a recent report in 569 patients with advanced pancreatic cancer showed an OS of 6.4 months in the erlotinib/gemcitabine group compared with 5.9 months in the gemcitabine group (73). In this study, patients responded equally well to treatment with erlotinib regardless of whether their tumors had abnormally levels of EGFR. However, the clinical development of these drugs is far from simple and we need to better understand biological criteria for patient selection to best use the different available agents. In particular, the discovery of EGFR mutations in non–small cell lung cancer patients responding to therapy and the identification of markers that might predict response could help optimize the use of these agents in the future (74).

Another potential drug target is the serine/threonine kinase Aurora-2, which associates with the centrosome, and shows both gene amplification and up-regulation in pancreatic cancer cells and tissues (60, 75). In particular, an immunohistochemical analysis showed an overexpression of Aurora-2 in 26 of 28 pancreatic cancers compared with 18 normal pancreas samples (75). These findings are quite reminiscent of the situation in the early exploration of HER-2/*neu* in breast cancer, indicating the potential of Aurora-2 as a therapeutic target in pancreatic cancer.

Because marked cyclooxygenase-2 expression was observed in pancreatic cancer tissue (76), other agents being investigated in combination with gemcitabine included the cyclooxygenase-2 inhibitor celecoxib. Indeed, experimental data suggest that cyclooxygenase-2 may play an important role in pancreatic tumorigenesis and metastasis and may be a promising target for treatment (77). In preclinical studies, celecoxib enhanced the antitumor efficacy of chemoradiation. However, the preclinical and the clinical studies have revealed more toxicity with this combination than with gemcitabine and radiotherapy alone. These observations require further study but are cause for concern when combining gemcitabine, radiotherapy, and celecoxib (78).

Additional molecular markers of pancreas cancer include dysregulation of p53 and Bcl-2/Bax (3). Understanding how these factors interact with drugs or affect their action represents the basis for a translational research for innovative therapy.

Immunohistochemical staging using both p53 and Bcl-2 significantly predicted survival duration by multivariate analysis in patients who received multimodality therapy for resectable adenocarcinoma of the pancreas; patients whose tumors stained positively for p53 and/or overexpressed Bcl-2 had a significantly longer survival than those whose tumors stained negative for both proteins (79). Another study showed that in pancreatic cancers there was an inverse

relationship between the expression of Bcl-2 and p53; malignant behavior of pancreatic cancer may be associated with the phenotype Bcl-2⁻-p53⁺ (80). In particular, Bcl-2⁺ patients with invasive adenocarcinoma showed higher survival than Bcl-2⁻ patients for both p53⁺ and p53⁻ patients, suggesting that Bcl-2 expression has a stronger effect on the survival than p53 expression. On the other hand, Bcl-2 expression had no influence on survival of the adjuvant chemotherapy group and the surgery alone group, whereas the adjuvant chemotherapy group enjoyed a significantly better survival than the surgery group for p53⁺ patients. Therefore, Bcl-2 expression was a prognostic factor, whereas p53 expression may be beneficial in the prediction of the effects of adjuvant chemotherapy on patients with invasive pancreatic cancer (81). For this reason, the administration of taxane-based chemotherapy might be better suited in this setting because these drugs promote p53-independent apoptosis by phosphorylation of the antiapoptotic proteins Bcl-2/Bcl-xL (82).

Bcl-2 is expressed in 23%, Bax in 53%, Bcl-xL in 90%, and Mcl-l in 90% of the invasive ductal adenocarcinomas (83). An imbalance between antiapoptotic proteins (such as Bcl-2, Bcl-xL, and Mcl-1) and proapoptotic proteins (such as Bax and Bcl-xs) is involved in the distinctive biological features of adenocarcinomas of the pancreas. Indeed, the enhanced expression of Bcl-xL is related to a shorter patient survival, whereas the up-regulation of Bax is associated with longer survival and these findings suggest that the modulation of apoptotic pathways might be one of the reasons why pancreatic cancer shows only limited sensitivity to anticancer treatment (26).

The tumor necrosis factor–related apoptosis-inducing ligand receptor is another central mediator of programmed cell death and a recent study showed that geldanamycin and bortezomib synergistically enhance tumor necrosis factor-α-triggered and tumor necrosis factor–related apoptosis-inducing ligand–triggered apoptosis by increasing the activation of caspase cascades by both blocking NF-κB activation and disrupting the Akt/protein kinase B signaling pathway as well as cell cycle progression (84). This study suggests that geldanamycin, bortezomib, and tumor necrosis factor–related apoptosis-inducing ligand combination may be a novel therapeutic strategy. Indeed, a phase I study of bortezomib-gemcitabine combination showed antitumor activity in previously treated pancreatic cancer patients and further studies are warranted (27).

Conclusions

Despite the considerable advancement in the comprehension of the molecular and genetic pathways leading to solid tumors, such a progress has not yet been translated into a better management of patients with pancreatic cancer.

However, the study of the influence of genotype on drug efficacy (pharmacogenetics) and the novel approach to targeted drug discovery are gaining momentum in understanding the unpredictable activity of cancer chemotherapy and help in the selection of drugs best suited for pancreatic cancer patients.

Indeed, genetic variability may alter drug uptake (i.e., hENT1 for gemcitabine), catabolism (i.e., DPD for 5-FU), and anabolism (i.e., thymidine phosphorylase for capecitabine). Moreover, increased expression of transporter systems (i.e., the ATP-binding cassette superfamily) can be associated with reduction of the cytoplasmic levels of drugs that may be unable to exert a cytotoxic effect. Additional systems could protect tumor cells from drug-induced cytotoxicity, including the DNA repair machinery (i.e., nucleotide excision repair), antiapoptotic systems (i.e., Bcl-2), and several signal transduction pathways (i.e., phosphatidylinositol 3-kinase/Akt). Finally, alterations of drug targets may be associated with a decrease in the effectiveness of chemotherapy (i.e., mutations affecting topoisomerase I for irinotecan and EGFR for tyrosine kinase inhibitors and increased expression of RRM1 for gemcitabine). Therefore, genetic analysis has the potential to predict treatment efficacy, and future directions for systemic therapy of pancreatic cancer should include the individuation of possible molecular markers of chemosensitivity and the exploration of techniques to rapidly interrogate cancer cells recovered from patients to individualize therapy with pharmacogenetic analysis.

Moreover, although an extensive validation of available technology is still needed, and there are difficulties in enrolling large number of patients to obtain a suitable amount of pancreatic tissue samples, the progress in molecular biology has enabled to array the gene transcripts in solid matrices to scale the process of expression analysis to several thousand genes per sample (60).

For example, a recent meta-analysis of microarray data generated a set of 568 genes that were significantly dysregulated in pancreatic cancer and could represent good candidates for novel diagnostic and therapeutic approaches (85).

Finally, progress into a better management of patients with cancer will be obtained only with large well-designed prospective clinical trials in which a direct comparison is done between patient treatment based on conventional (empiric) criteria and treatment selection suggested by analysis of the genetic background of tumors. Other intriguing issues will be the identification of the optimal drug sequence in combination regimens and the pharmacologic research of novel cancer therapeutics based on genetic abnormalities, including biological agents targeting signal transduction pathways (i.e., EGFR inhibitors). This perspective implies that the classic approach of standard chemotherapy for the treatment of pancreas cancer irrespective of its molecular characteristics may be abandoned in favor of individually tailored cancer chemotherapeutics based on genetic pattern of disease.

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