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Review

Molecular Background of Chemoresistance in Pancreatic Cancer

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Abstract. *The survival data of patients with ductal pancreatic adenocarcinoma are rather poor, partly because the disease is frequently diagnosed at an advanced stage, partly because it is characterized by a chemoresistant phenotype. Even first-line chemotherapeutic drugs result in a modest objective response. This drug resistance is attributed to many different, unrelated mechanisms, including abnormal membrane receptor transport, ineffective metabolic drug conversion or enhanced metabolite inactivation, increased DNA repair and alterations in the apoptotic pathways. The role of NF-kappaB, cyclin D1 and stromal factors is also emphasized by many groups. The involvement of the ABC-transporters is not a universal feature, their alterations are important only in the resistance against specific cytostatics. Although several well-known molecular mechanisms have been elucidated, our understanding of drug insensitivity is still fragmentary, especially because recent microarray studies revealed that hundreds of genes are up- or down-regulated in resistant tumor cells, but their exact significance is still unclear. The reversal of the drug resistance is an area of intensive investigation, but to date, the compounds investigated are effective mainly in experimental systems and prospective studies are needed to validate their clinical applicability.*

Pancreatic cancer is one of the deadliest malignancies. This tumor is usually presented at advanced stage, when the surgical therapy is limited. Indeed, only 15-20% of patients are operable, for the majority of cases the only promise is that of cytostatic treatment. At present, gemcitabine is the first-line chemotherapeutic agent for pancreatic carcinomas, but the objective tumor response rate is low (about 15-20%), a complete response can be achieved in only 2-4% of cases, a partial response in about 15-18%, while the 1-year survival rate does not exceed 30%, albeit that disease-related symptoms are significantly improved. Several other cytostatics have been compared with this drug, but no superior effects were observed when administered as single agents (1). Similarly, combination treatments with various chemotherapeutics improved the outcomes in objective response rates, but had a little impact on the prolongation of life (2). It is evident that the vast majority of pancreatic carcinoma cells are resistant to cytostatic drugs.

Drug resistance of malignant tumors is a highly complex phenomenon. To be effective, the chemotherapeutic agents must reach the tumor cells, but in solid tumors the presence of vascular channels is highly variable. Moreover, in some tumors the blood flow shows fluctuation, sometimes intermittent cessation of the blood supply is observable in different areas, reducing the net tumor cell exposure to the antineoplastic agents, simulating resistance (3). True drug resistance can be due to changes in the membrane receptors, altered drug transport through the cell membrane, ineffective metabolic conversion to active compounds, or in turn, cytoplasmic inactivation of the cytostatic molecule, increased DNA repair mechanism, dysregulation of the apoptosis pathway, to name but a few. These mechanisms may be acting in combination making the understanding of resistance more complicated. Moreover, the alterations are not always constant, the tumor cells may continuously adapt to changing biological surroundings in order to ensure their survival: apart from intrinsic resistance, the originally sensitive tumor clones can acquire resistance during treatment. Nevertheless, in this review the most important mechanisms by which pancreatic cancer cells can evade the antitumoral effect of chemotherapeutic agents are outlined.

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Vasculature of the Pancreatic Cancer

Many pancreatic cancer cases characteristically display a desmoplastic stroma, rich in pancreatic stellate cells,

cancer-associated fibroblasts, myofibroblastic cells, extracellular matrix, collagen I, III, V, and newly formed vascular channels too. The first question is whether this stroma contains blood vessels in an amount necessary for the cytostatics to reach the tumor cells at a high concentration, or if the dense, collagenous stroma prevents this. Theoretically, a small number of vessels would result in lower levels of drug reaching the tumor. Although the pancreatic carcinoma is not a highly-vascularized tumor, several studies demonstrated that the intratumoral microvessel density was a good tool for predicting survival (4). The median survival time of patients with hypervascularized tumors was significantly shorter than that of the hypovascularized group (5, 6); a high microvessel density was correlated with shorter postoperative survival (7). The presence of more microvessels is associated with a higher occurrence of liver metastases and is significantly correlated with higher proliferative activity and poorer histological differentiation (8). The poorly differentiated carcinomas displayed a more pronounced vascularity, but this was not associated with the tumor size (9). These findings are in accordance with the well-known fact that the aggressiveness of pancreatic cancer is inversely associated with the degree of differentiation. However, the distribution of the blood vessels is heterogeneous within the tumor: using Doppler ultrasound it was demonstrated that 65% of cases displayed definitive vascular signals, but in 35% of the patients, almost no vascular signals were seen (10). Again, in the former group, liver metastases were more frequently seen and the 'hot spots' in areas of neoplastic parenchyma were found to be associated with shorter survival (10, 11).

Nowadays, antiangiogenic therapy is one of the 'hot spots' in the scenario of molecular-targeted cancer therapies aiming to reduce the blood flow within the tumors. Theoretically, in parallel with a reduced number of vessels, the concentration of the cytostatic drugs should be lower, but the experimental and clinical data do not support this view. In an experimental system using orthotopically implanted pancreatic cancer xenografts a significant decrease of the microvessel density was observed when an antiangiogenic compound (TNP-470) was combined with gemcitabine, but it did not reduce the efficacy of the cytostatic drug, and in fact a significant improvement in the survival rate was seen (12). In recent clinical trials where bevacizumab (Avastin) plus gemcitabine were administered to patients with an advanced stage of pancreatic carcinoma, more than 40% of stable disease and about 20% of partial response were noted (13, 14), again indicating that the antiangiogenic therapy did not change the antineoplastic action of the chemotherapy. Thus, the chemoresistance of pancreatic cancer is not related to the blood supply of the organ.

Resistance to Gemcitabine

This cytidine analog (2',2"-difluoro 2'-deoxycytidine; Gemzar®), serving as a standard drug in the treatment of advanced pancreatic cancer, is taken up by the tumor cells by human equilibrative nucleoside transporters 1 and 2 (hENT1, hENT2), and concentrative nucleoside transporters 1 and 3 (hCNT1, hCNT3). They consist of 11 (hENT1, hENT2) or 13 (hCNT1, hCNT3) transmembrane helices with an intracytoplasmic N-terminus and a glycosylated extracellular C-terminus (15). Their activity is essential for the sensitivity to gemcitabine, while the transporter-deficient cells are highly resistant to the drug (16); but the data concerning the clinical relevance are rather limited. *In vitro* experiments revealed that human pancreatic cell lines expressed a high level of hENT1, but the hCNT1 levels varied depending on the cell density (17). The importance of hENT1 in the outcome of patients was also underlined by human studies: gemcitabine-treated patients had a significantly longer survival when hENT1 was detectable immunohistochemically than those with a negative transporter status (13 *versus* 4 months, $p<0.01$). At the same time, hCNT3 was present at a moderate to high intensity in all the pancreatic cancer tissues (18). Recent studies also underlined the prognostic significance of hENT1 in survival figures (19). Although these data are promising in potentially selecting the gemcitabine-resistant or -sensitive patients, these premature data need further investigations through prospective, randomized trials.

Other mechanisms of gemcitabine resistance also need to be taken into account because the drug has multiple intracellular targets. Following influx through the cell membrane, it is phosphorylated by deoxycytidine kinase (dCK) and converted into active metabolites which interfere with DNA-synthesis. A strong correlation was observed with gemcitabine sensitivity and dCK activity (20), but this observation cannot be regarded as a universal finding because *in vitro* data showed that high therapeutic resistance was found to be associated with deficient dCK expression, however, only in the later stages. The earlier resistant cells exhibited dCK activity, while they had a reduced nucleoside transporter activity, indicating that both enzyme systems are important for the acquisition of resistance (21). Moreover, immunohistochemically, this enzyme protein is expressed in more than 90% of pancreatic cancers analyzed and available data demonstrate that genetic alterations of dCK are not a common mechanism of resistance to gemcitabine (22). Early xenograft studies also showed that, among the sensitive tumors both high and low activities were measured indicating that even the low activities are enough to activate gemcitabine (23). Thus, the alteration of this enzyme activity is one, but not the most important, factor in the development of the resistant phenotype of pancreatic

carcinoma. Similarly, deoxycytidine deaminase, a key enzyme involved in the inactivation of gemcitabine, does not play a role in the development of resistance (24).

The complexity of Gemzar resistance is further evidenced by some other studies. Microarray analysis showed a specific expression of selenoprotein P (which reduces intracellular reactive oxygen species levels) in gemcitabine-resistant but not sensitive pancreatic cancer cell lines, and administration of this protein led to inhibition of the cytotoxic effect of the chemotherapeutic drug (25). In colonic cancer cell lines, the acquired resistance was attributed to a striking increase in the expression of the ribonucleotide reductase subunit M1 (RRM1) (24) and this gene expression was also detectable in most human pancreatic carcinomas (19). NF-κB also seems to play an important role: the resistant cell lines (*e.g.* BxPc-3, Capan-1 or PancTu-1) exhibited a high basal activity in contrast to the sensitive ones, and the inhibition of NF-κB strongly reduced the resistance to gemcitabine (26). Indeed, it is constitutively active (overexpressed) in human pancreatic cancers (27).

Another important mechanism of chemoresistance is the alteration in the apoptotic pathway evidenced by many studies, indicating that all apoptotic regulatory molecules are up-regulated in this carcinoma. While in the normal pancreatic tissue the immunohistochemical expression of the antiapoptotic bcl-2 protein is about 5%, in the malignant samples its presence is much higher, different papers reporting 20%, 36%, or 72% (28-30). Almost all pancreatic cancers (92%), also overexpressed the bcl-XL (28). At the same time, however, the proapoptotic bax molecule was also found at elevated levels (49% to 72% of cases) (28, 29). The bax gene seems to be directly involved in resistance because *in vitro* data provided evidence that on transfection of human pancreatic carcinoma cells with the bax gene the sensitivity to gemcitabine was significantly increased (31). The clinical significance of these data, however, is intriguing, because there is no direct impact on the outcome of the disease: an inverse correlation was found between bcl-2 overexpression and the tumor stage, while patients with strong bcl-2 immunopositivity exhibited a longer survival than those with negative bcl-2 status (30). Similar results were found in another study: the postoperative survival of bcl-2-positive cancer patients was 14.3 months, while the negative patients lived 7.3 months (29), suggesting again that although the apoptotic/antiapoptotic genes/proteins are profoundly altered in pancreatic carcinomas, their presence or absence alone cannot be regarded as a decisive factor in the prognosis.

Apart from the bcl-family, other molecules involved in the apoptotic pathway are also of importance. It was reported that the antiapoptotic p8 protein is specifically overexpressed in resistant cell lines, and forced overexpression in gemcitabine-sensitive cells increased their resistance to drug-induced apoptosis (32). Interleukin-1beta and nitric oxide have also been claimed as major determinants of chemoresistance *via* inactivation of caspases (33), and recently the X-linked inhibitor of apoptosis (XIAP) was found to be an important factor of chemoresistance. When XIAP was inhibited, the apoptosis index after gemcitabine treatment increased significantly (34). Microarray studies have also revealed that expression of the hypoxia-induced proapoptotic gene, BNIP3, decreased in pancreatic cancer as compared to the normal pancreas: it was undetectable in 59%, and down-regulated in more than 90% of the samples. It was found to be expressed at lower levels in resistant cell lines and the loss of BNIP3 resulted in an increased resistance to gemcitabine (35, 36). Hypoxia within the tumor was also reported to induce resistance to gemcitabine mainly through the phosphatidylinositol 3'-kinase (PI3K)/Akt pathway, the effect of which can be reversed by epidermal growth factor receptor tyrosine kinase inhibitor (37). Survivin, a potent caspase blocking factor, is frequently overexpressed in malignant pancreatic ductal tumors, and it is also implicated in the resistance to different apoptotic stimuli, including chemotherapy (38). The role of apoptosis in the pancreatic cancer is extensively reviewed by Westphal and Kalthoff (39).

The data mentioned above clearly show that the loss of sensitivity in pancreatic cancer cannot be attributed to a single molecular alteration. Indeed, gemcitabine resistance seems to be the result of an orchestra of genetic changes. Microarray analyses have identified several dozens of genes showing differential expression in this tumor, some of them are relatively overexpressed in sensitive cell lines, while some others are overexpressed in resistant ones (36, 40). The order of their action, their mutual relationships or the possibilities of their manipulations, are, however, still obscure and need to be further elucidated.

The Role of the ABC Transporters

The ATP Binding Cassette (ABC) transporters represent a special family of membrane proteins enabling the transport, metabolism, cellular effectivity and toxicity of pharmacological agents. These are subdivided into seven subfamilies (ABCA to ABCG) altogether consisting of 48 members. The major genes involved in the chemoresistance of malignant tumors are those of multidrug resistance 1 (MDR1, ABCB1), the multidrug resistance proteins (MRP1-5, ABCC1-5) and mitoxantrone resistance (BCRP, MXR, ABCG2). The lung resistance protein (LRP) is not an ABC transporter, but was also reported to correlate with drug resistance in some tumors.

The MDR1 gene encodes a membrane protein named P-glycoprotein (P-gp), which acts as an ATP-dependent efflux-pump leading to decreased intratumoral drug

accumulation, and confers simultaneous resistance against several antineoplastic agents. Its role in pancreatic cancer, however, remains controversial. Japanese authors found a significantly elevated MDR1-gene expression in carcinoma patients over the normal pancreata and it was shown that in more than 70% of tumor samples, P-gp was overexpressed. Unfortunately, however, there was no correlation between the clinical aggressiveness of the tumor and the presence/absence of P-gp (41). Immunohisto-chemically, positive P-gp staining was observed in over 79% of pancreatic carcinomas, but it failed to predict their biological behavior (42). Similarly, no significant survival differences were observed between the MDR1-positive and MDR1-negative surgically treated patients (43). These data, however, do not contradict the importance of this MDR1-encoded membrane protein because the connection between its expression and the sensitivity to the chemotherapeutic drugs in these reports was not investigated. PANC-1, BxPC-3, AsPC-1, Capan-1 pancreatic cancer cell lines showed no evidence of P-gp in the rhodamine 123 assays (44), but other groups reported MDR1 gene expressions in 2 of 3 cell lines using RT-PCR and immunohistochemistry (45). In earlier studies, analyzing more than 400 types of malignant tumors, the MDR1 mRNA levels were usually found to be elevated in untreated, intrinsically drug-resistant tumors including those derived from the pancreas (46). In our human pancreatic carcinoma xenografts, 60% of tumors cells displayed an immunohistochemically detectable overexpression of P-gp (47).

The P-glycoprotein plays an important role in the transport of many cytostatic drugs including anthracyclines, vinca-alkaloids, taxanes, etoposide, and actinomycin D (48). For example, it is primarily responsible for docetaxel (Taxotere®) resistance. In Taxotere-insensitive pancreatic cancer cell lines, RT-PCR demonstrated strong expression of P-gp and the refractory feature could be reversed by verapamil, a known multidrug-resistance modulator (49). The drug was able to inhibit the *in vitro* invasiveness of the sensitive cells, but not the resistant ones (50). Resistance against another drug, paclitaxel, is also commonly seen, displaying a wide variety of mechanisms but the overexpression of the MDR1-gene is regularly observed (51). Irinotecan (Campto®), a topoisomerase I inhibitor, has extremely complex pharmacokinetics, but the elimination of this drug is primarily dependent on P-glycoprotein (52).

Multidrug resistance proteins (MRP) also confer insensitivity on pancreatic cancer (44). Analysis of human pancreatic cancer specimens and normal pancreata for mRNA expression of MRP family members has shown that the expression of MRP3 was up-regulated in the carcinomatous tissues and was correlated with the grade. The MRP5 mRNA level was also significantly increased over that of the normal pancreas, while the BCRP and MRP1 mRNA levels were not different in the normal and carcinomatous pancreatic tissues (53). The importance of MRP4 was also demonstrated: *in vitro* this protein rendered significant resistance to cyclophosphamide, camptothecin, irinotecan, rubitecan, but not to paclitaxel, etoposide or 5-fluorouracil (54). Interesting results were published concerning doxorubicin resistance *in vitro*: in a P-gp-negative human pancreatic cancer cell line, this resistance was attributed to overexpression of the vesicular marker lung resistance-related protein (LRP), but larger doses of this drug led to the activation of multidrug-resistance-associated protein indicating dynamic changes in the tumor cells (55). These and other data on the inducibility of resistance clearly show that the drug sensitive/resistant phenotype cannot be regarded as a constant finding in pancreatic cancer.

Effects of the Stroma and Tumor-Stroma Interactions

Malignant tumors are composed of parenchyma and stroma, which mutually influence each other (56). It was demonstrated that co-culturing stromal fibroblasts and pancreatic cancer cells decreased their sensitivity to etoposide by 50% and almost the same results were obtained when conditioned medium of fibroblasts was added to the carcinoma cell lines (57). The fibroblasts released NO and also stimulated IL-1β secretion, which was found to maintain the chemoresistant phenotype of the tumor cells by autocrine secretion (58). The extracellular matrix (ECM) also seems to be important: *in vitro* results have shown that adhering the carcinoma cells to any of the ECM proteins resulted in the decreased cytotoxicity of antineoplastic drugs, except for gemcitabine (59). As for gemcitabine, other ECM components are taken into account, for example the carcinoembryonic antigen-related cell adhesion molecule (CEACAM) 6, while it is stably overexpressed in gemcitabine-resistant Capan2 cells, the silencing of its gene markedly increased the sensitivity to the drug. The overexpression of CEACAM 6 seemed to prevent the cytochrome c-induced caspase-3 activation, and hence apoptosis (60). Integrin-linked kinase (ILK) that facilitates the signal transduction between the ECM and subcellular compartments was found to be overexpressed in Gemzar-resistant tumors, while knockdown of ILK induced chemosensitivity and increased apoptosis (61). These data represent new aspects of the resistance of pancreatic cancer and may serve as new targets to achieve better therapeutic results.

Other Mechanisms of Drug Resistance

Several other factors and mechanisms may contribute to the resistant phenotype of pancreatic carcinomas. It has long been

known that the overexpression of cyclin D1 is associated with poor prognosis (62, 63). Indeed, increased levels of cyclin D1 have been detected at both the mRNA and protein levels in 82% and 68% of the tumor tissues examined (62, 63), and, *in vitro*, markedly increased resistance to chemotherapy (cisplatin) was observed (64). Antisense ablation of cyclin D1 in Panc-1 cell line suppressed growth and enhanced their responsiveness to several chemotherapeutic agents through decreased levels of thymidine synthase, MDR1 and MRP (65). The validity of this relationship was further supported with an elastase-myc pancreatic cancer cell line overexpressing cyclin D1: in addition to enhanced cell proliferation and cell cycle progression, it was associated with up-regulation of NF-κB activity, a higher survival rate, significantly decreased cisplatin sensitivity, increased resistance to apoptosis and maintenance of bcl-2, bcl-XL protein levels (66). Although these results are convincing, chronic pancreatitis has also been reported to express 2-fold cyclin D1 mRNA levels and an increased immunohistochemical staining indicating a hyperproliferative state (67). In the chemoresistant pancreatic cancer cell lines exhibiting high NF-κB activity, the level of the E3-ubiquitin ligase receptor subunit betaTRCP1 was also significantly elevated and blockade of betaTRCP1 expression reduced this resistance (68). Neuropilin-1, a novel co-receptor for VEGF, is not found in normal pancreata, but it is expressed in carcinomatous tissues. Its overexpression renders an enhanced resistance and increased survival of cancer cells to gemcitabine or 5-fluorouracil, and increased the expression of the antiapoptotic regulator, MCL-1. In turn, down-regulation of neuropilin-1 resulted in a markedly increased chemosensitivity to gemcitabine (more than 50% cell death) (69).

Potential Reversal of Chemoresistance

Many experimental data reinforce the notion that chemoresistance of malignant tumors can be reversed by using various chemicals, but the first and second generation compounds possessed serious side-effects, or their effect was unpredictable (70, 71); the newest drugs seem to be safer and more effective. The MDR1-reverants display a wide range of structural and pharmacological properties, but the reversal brought about by the P-glycoprotein seems to depend on the lipophilicity, the molecular weight and the presence of at least one basic tertiary nitrogen atom (72). For pancreatic cancer, the MDR-phenotype could be reversed by small interfering RNAs (siRNAs) (73), by depleting cellular glutathione and the inhibition of glyoxalase I (74), or by enhancement of the c-Jun NH_2-terminal kinase (JNK) (75). Recently, various synthetic organosilicon compounds proved to strongly inhibit the efflux pump. These chemicals have been effective *in vitro* on MDR1-transfected mouse lymphoma and colonic cancer cell lines increasing the intracellular drug accumulation, as measured by rhodamine 123 assay. The compounds have been shown to act directly on the membrane P-glycoprotein without affecting the MDR1 gene expression, and in combination with cytostatic drugs they resulted in an enhancement of the cytotoxic effects (76). One of these organosilicons SILA-409 (1,3-dimethyl-1,3-bis(4-fluorophenyl)-1,3-bis(3-morpholino-propyl)-disiloxane-dihydro chloride) was also tested in human pancreatic cancer xenografts and encouraging results were obtained: at a dose of 10 mg/kg it resulted in a growth delay in the tumors, induced an increase in apoptotic activity and a significant reduction in the number P-gp-positive cancer cells was observed (47). Although this dose was much higher than that used *in vitro*, no obvious toxic effects were seen. Based on these experiments, combination treatments have been introduced using gemcitabine, vincristine or irinotecan plus SILA-409. Ten-ten immunosuppressed mice bearing subcutaneously growing PZX-40 human pancreatic adenocarcinomas were treated for 4 weeks and the tumor volumes were measured weekly. The PZX-40 carcinomas express P-gp in about 60% of the tumor cells. Although gemcitabine is not a substrate for P-gp, but this drug is the first-line cytostatic in pancreatic cancer, so the possible beneficial effect of co-administration was also investigated. In the combination group no beneficial effect was seen (tumor growth delay was observed in 50% of the animals, stable size was seen in 10% and the tumor volume was reduced in 40%). There was no shift in the proportion of the P-gp-positive tumor cells, although the staining intensities were lower. When vincristine was administered alone, volume reduction was seen in tumors of half of the animals, while coadministration of SILA-409 plus vincristine resulted growth delay in 30%, volume stabilization in 10% and volume reduction in 60%. The organosilicon treatment significantly decreased the P-gp positivity in the tumor cell membranes, and, in addition, the staining was also less intensive. Interesting results were found in experiment where SILA-409 and irinotecan (Campto) were simultaneously administered: tumor volume reduction was found in 70% of the animals, but Campto alone completely abolished P-gp expression, so the additional reversal effect of the organosilicon could not be evaluated. These experiments have shown that the synthetic morpholino-organosilicon compound can reduce the P-glycoprotein expression in a large proportion of tumor cells in human pancreatic cancer xenografts and this effect is also associated with some enhanced antineoplastic actions.

Conclusion

The major obstacle to the successful treatment of pancreatic cancer is its chemoresistant nature; even gemcitabine, the first-line cytostatic drug results in only a modest prolongation of life and rare objective tumor responses. But

the background to this feature is highly complex, one cannot generalize. Although various mechanisms related to chemoresistance have been revealed, this type of tumor remains a great challenge in oncology. Experimental data provide ample evidence that the refractory phenotype results from very different molecular defects and cannot be attributed to a single cellular aberration. The molecular alterations outlined above all taken as a whole seem to be really important, but are not individually. It has long been recognized that this tumor is characterized by frequent cytogenetic changes involving different chromosomes (77). Microarray data clearly show several hundreds of dysregulated genes (both up-regulated and down-regulated ones) and the genes that are associated with drug resistance pathways are frequently over-expressed in pancreatic carcinomas (78). Insensitivity prevails at different subcellular levels. Comparative genomic hybridization studies have also revealed genomic gains and/or losses in chromosomal regions encoding putative genes including various members of the ABC transporters, family members of cytochrome P450 monooxygenases, DNA repair enzymes, and factors involved in cell cycle regulation and apoptosis (79). Moreover, drug resistance to various chemotherapeutic agents involves different mechanisms. Theoretically, a combination of cytostatic drugs would prevent the development of resistance because of alternative points of attacks, however, the combined treatment modalities do not provide such a survival benefit for the patients as expected.

Among the possible mechanisms, vascular factors do not seem to play an important role, however, an orchestra of many other alterations are evidenced: nucleoside transporters, drug-metabolizing enzyme activities, NF-κB, apoptotic/antiapoptotic proteins, ABC-transporters, stromal elements and the ECM, cyclin D1, neuropilin-1 all contribute in the determination of chemoresistance. Many promising compounds have been synthesized to reverse drug insensitivity including MDR1-revertants, but to date no breakthrough has been achieved. Recent deeper insights into the genetic abnormalities have revealed several hundreds of dysregulated genes, but this important information at present does not allow us to provide practical guidance as to, for example, how to fight the resistance, what genes are of utmost significance and what the order of gene switching is. However, it seems likely that consecutive progressive steps are needed for the development of chemoresistance in pancreatic cancer, thus, to overcome drug insensitivity, multidirectional, gene-oriented approaches would be necessary in the therapy.

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