Interferon-alpha Restitutes the Chemosensitivity in Pancreatic Cancer

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Abstract. Background: Multidrug resistance is a major obstacle in the treatment of pancreatic cancer. Immunochemotherapy including interferon-alpha increases response rates and survival. Materials and Methods: Pancreatic cancer was induced in an orthotopic mouse model. Animals received standard chemotherapy or combinative treatment with interferon-alpha. Expression and function of drug-resistance proteins were analyzed. Immunological phenotyping, cytotoxic activity assays and analysis of T-cell activation status were performed. Results: Addition of interferon-alpha to chemotherapy regimes significantly reduced chemotherapy-induced expression of multidrug resistance proteins and drug efflux activity of cancer cells. Tumor size and metastatic seeding decreased significantly upon combination therapy and survival was prolonged. A significantly higher proportion of activated and cytotoxic active CD8+ tumor infiltrating lymphocytes was detectable after induction of drug resistance. Conclusion: Restitution of chemosensitivity by the addition of interferon alpha to chemotherapy was demonstrated in experimental pancreatic cancer for the first time. Since drug-resistance proteins may function as tumor antigens, our data support immunochemotherapy as an encouraging new approach.

Pancreatic carcinoma is the fourth leading cause of cancer-related death in the Western world. The treatment of choice is surgical resection followed by adjuvant chemotherapy. Response rates to chemotherapy are below 20%, with a median life expectancy of about nineteen months (1, 2). The statistics for second-line therapy are even more dismal with response rates <10% and a median survival of four months (3). Compared to other gastrointestinal tumor entities, pancreatic cancer is one of the most resistant tumors, even to aggressive adjuvant and palliative chemotherapy. Multidrug resistance-associated proteins (MRPs) are overexpressed in pancreatic cancer; a correlation of protein expression with tumor grading and impaired survival has been reported (4-6). Since there is evidence of cancer immunosurveillance, new chemotherapeutic protocols include immunomodulatory agents such as interferon alpha (IFN-α) in their treatment regime. For pancreatic cancer impressive five-year survival rates of 55% after resection and adjuvant immunochemotherapy were reported (7, 8). Since similar protocols using 5-fluorouracil, cisplatin and external beam radiation showed only limited success, IFN-α seems to play a crucial role in these treatment regimes (9). In 2004, we initiated a randomized open, controlled, prospective, multi-center phase III trial comparing 5-fluorouracil, cisplatin, IFN-α and radiation therapy with 5-fluorouracil monotherapy in patients with resected pancreatic adenocarcinoma (CapRI) (10). In a translational research program, we analyze various mechanisms of IFN-α within this protocol. Antitumoral effects of IFN-α include an augmentation of the immune response with: a) activation of natural killer cells (NK cells); b) enhancement of the differentiation, maturation and function of dendritic cells (DC); c) induction of CD8+ memory cells, d) increase of macrophage activities and e) an induction of the immunoproteasome (11-14). Direct inhibitory effects on tumor cell growth as well as antiangiogenic properties and an enhancement of the immunogenicity of tumors via an increased MHC class I expression have been reported by our group (13, 15-18). Radiosensitizing effects of IFN-α have been demonstrated in vitro and in vivo (13, 19). Furthermore, chemosensitizing effects of IFN-α have been discussed (20-22). Here the influence of IFN-α on induction and inhibition of multidrug resistance in pancreatic cancer are evaluated in vitro and in vivo and the concomitant immunomodulatory effects analyzed.

Materials and Methods

Cell lines. PANC1, a human pancreatic cell line generated from ductal pancreatic carcinoma was used for in vitro experiments (23). Cells were treated with: group 1; 5-fluorouracil at 170 μg/ml plus IFN-α.
1x10^3 U/ml three days a week; group 2: cisplatin at 1.8 μg/ml plus IFN-α 1x10^3 U/ml three days a week; and group 3: gemcitabine at 380 μg/ml plus IFN-α 1x10^3 U/ml three days a week. Group 4 comprised the untreated control. Panc02, a murine pancreatic tumor cell line was used for in vivo experiments. All cells were cultivated in RPMI 1640 cell culture medium (Life Technologies Inc., Berlin, Germany) supplemented with 10% FCS (PAA, Cölbe, Germany), 100 U/ml penicillin, and 100 μg/ml streptomycin (Seromed, Julich, Germany) and incubated at 37°C under 5% CO2.

Animal tumor model. Male 6 to 10-week-old C57BL/6 (h-2b) mice (Charles River Inc., Sulzfeld, Germany) weighing 100±20g were kept and treated in accordance to the principles laid down in the European Community’s Council Directives and approved by the local administration (reference number: 35-9185.81/G-147/05). Tumor inoculation in mice was performed with ultrasound-guidance under general anesthesia as described by Schneider et al. (24). Five μl Panc02 cells in a concentration of 5x10^6 cells/ml NaCl 0.9% were injected in the pancreas with a 25 μl gastight syringe. Treatment with chemotherapy was started seven days after tumor inoculation. A minimum of six mice in each group were treated as follows: group 1: 100 μl saline intraperitoneal five days a week (control group); group 2: 5-fluorouracil at 35 μg/g/day plus IFN-α 2x10^5 U three days a week; group 3: cisplatin at 4 μg/g plus IFN-α 2x10^5 U three days a week; and group 4: gemcitabine at 2.5 μg/g plus IFN-α 2x10^5 U three days a week. Tumor growth was assessed during treatment with ultrasound and animals were sacrificed when the tumor reached a volume of ≥0.6 cm^3 or when they became moribund. Exact tumor volume was measured after scarification with a vernier calliper using the formula:

\[ V = \text{length} \times \text{height} \times \text{width} / 6 \]

Peritoneal metastatic seeding as well as lung and liver metastases were documented.

Flow-cytometry. In in vivo experiments, pancreases of sacrificed mice were collected and single cells were passed through a 40 μm nylon mesh. Pancreatic cells were isolated and stained using monoclonal antibodies (mAbS) directed against cytokeratin 7 (CK7) (Chemicon International, Temecula, CA, USA), P-glycoprotein (clone JSB-1; Abcam, Cambridge, UK), MRPI (clone QCRL-2; Kamiya Biomedical Company, Seattle, WA, USA) and MRP3 (clone M3H-21; Abcam). For in vitro experiments, Panc1 cells were stained using mAbS against CK7 (clone RCK 105; Abcam), P-glycoprotein (clone JSB-1; Abcam), MRPI (clone QCRL2; Kamiya Biomedical Co., Seattle, WA, USA), MRP3 (clone H-16; Santa Cruz Biotechnology Inc.; Santa Cruz, CA, USA) and PanMRP (clone QCRL1; Calbiochem, San Diego, CA, USA), an antibody detecting the linear epitope of various human MRPs. For each flow cytometric measurement, gates of negative control were set to less than 2%. Data from 300,000-100,000 cells were collected and analyzed on an EPICS XL-MCL flow cytometer (Beckman Coulter, Krefeld, Germany). Negative controls consisted of the cells labeled with a corresponding isotype control.

Drug resistance quantification. The drug efflux activity of Panc1 cells was measured using a multidrug resistance quantification kit (Sigma Aldrich, Munich, Germany) according to the instructions of the company. Briefly, fluorescent Calcein-AM is used as substrate for targeted extrusion by MRPs. The degree of fluorescence observed in the test cells is inversely proportional to the protein activity. By selectively blocking the maximal protein activity, the MRP-specific activity and the baseline activity were obtained in triplicates for each sample. MAF_T, representing the total protein activity, MAF_{MRP}, representing the total MRP activity, and MAF_{MDR} representing the P-glycoprotein activity, were calculated.

**Cytotoxicity assay for pre-treated tumor cells.** Tumor-infiltrating lymphocytes (TILs) as well as splenic lymphocytes were isolated by passing tumor / spleen through a 70-μm cell strainer (BD Falcon; Becton Dickinson, Heidelberg, Germany), followed by lysis of erythrocytes (Ortho Diagnostic Systems, Neckargemünd, Germany) and magnetic bead conjugate mediated depletion of granulocytes (Serotec, Kidlington, UK). TILs and splenic lymphocytes of treated and untreated animals were separately incubated as effector cells, each either mixed with untreated non-resistant or treated resistant (MRP overexpressing, as determined by flow-cytometry) Panc02 cells stained with PKH-2 (Sigma, Deisenhofen, Germany) or PKH-26 (Sigma) over 4 hours at an effector to target ratio of 80:1. The percentage of stained cells was analyzed by flow-cytometry. Since the tumor cell lysis of untreated tumor cells at this given effector to target cell ratio is known, the approximately cell lysis of pre-treated tumor cells was calculated using the following formula:

\[ \text{Lysis}_{\text{pretreated}}(\%) \approx \frac{(b - e \times a \times (100\%-c)) \times 100}{d \times 100} \]

where \(a\) and \(b\) are the percentages of untreated and treated cells respectively in the control mix (without peripheral blood lymphocytes), \(c\) is the known lysis of untreated cells at a given E:T ratio (from 51Cr-release assay) (%), \(d\) and \(e\) are the percentages of untreated and treated cells respectively remaining after 4-h incubation with PBL (%); in which \(a + b \approx 100\%\) and \(d + e \approx 100\%\).

**Interferon-gamma ELISPOT.** The murine interferon-gamma (IFN-γ) ELISPOT Set was used according to the manufacturer’s instructions (BD Biosciences Pharmingen, San Diego, CA, USA). TILs as well as splenic lymphocytes of treated and untreated animals were seeded on 95 well plates at a density of 45,000 cells in triplicates and co-incubated with lysate of untreated non-resistant or treated resistant Panc02 cells over 20 hours. The resistance of Panc02 cells was determined by flow-cytometry defined as significant overexpression of MRPs compared to untreated Panc02 cells. Tumor lysate was prepared by rapidly freezing (−196°C) and thawing (37°C) three times and the content of protein was determined by BCA (Sigma Aldrich, Munich, Germany). Spot-forming cells were quantified using the KS ELISPOT System, release 4.1 (Carl Zeiss Light Microscopy, Göttingen, Germany).

**Interferon-γ staining.** TILs and splenic lymphocytes of treated and untreated animals were isolated and seeded at a density of 5x10^6 per well. Cells were stimulated with lysate of untreated non-resistant or treated resistant Panc02 cells overnight. The proportion of cytotoxic T-cells was quantified by flow-cytometry using a monoclonal antibody against CD8 (ImmunoTools, Friesoythe, Germany) and against IFN-γ (RD Systems, Minneapolis, MN, USA).
Generation of dendritic cells. Dendritic cells (DCs) were generated from spleen and bone marrow samples of untreated animals. Cells were isolated from spleen as described above. Bone marrow leukocytes were flushed from mouse femur and tibia and depleted of erythrocytes. Splenic and bone marrow DCs were depleted using the mouse CD11c MicroBeads isolation kit according to the manufacturer’s instructions (Miltenyi Biotec Inc., Bergisch Gladbach, Germany). The cells of the binding fraction were cultured in DC medium consisting of RPMI-1640, 10% autologous heat inactivated serum, 0.05M EDTA, 1% P/S, 25mM HEPES, murine granulocyte macrophage–colony stimulating factor (200 U/ml) and murine IL4 (1000 U/ml, Genetex; San Antonio, USA)). On day three, DCs were pulsed with lysate of untreated non-resistant or treated resistant Panc02 cells (100 μg/ml) and LPS (100 ng/ml). Medium was changed on day 4 and DCs matured by addition of TNF-α (Sigma-Aldrich; Munich, Germany), IL-1 (Genetex) and IL-6 (Sigma-Aldrich). In parallel, pancreatic tumors were induced in mice and treated 7 days after inoculation either with gemcitabine or a combination of gemcitabine with IFN-α. On day 9 after tumor inoculation, 5x10⁵ DCs were injected ultrasound-guided into the tumor. Ten days later, the pancreas of sacrificed mice were collected and prepared as described above for flow-cytometry. TILs were stained for T-cell subtypes (CD8, clone 53-6.7; CD4 clone RM4-5), regulatory T-cells (CD4+CD25+ clone PC61), naïve T-cells, central memory cells and effector memory cells (CD45RA, clone RA3-6B2; CD62L, clone MEL-14; CD8), co-stimulatory molecules on monocytes (CD40, clone 3/23; CD80, clone H1.2F3), naïve T-cells, central memory cells and effector memory cells (CD45RA, clone RA3-6B2; CD62L, clone MEL-14; CD8), co-stimulatory molecules on monocytes (CD40, clone 3/23; CD80, clone 16-10A1; CD86, clone GL1; CD11b, clone M1/70) and peripheral DC (CD33D1, clone 33D1; CD11c, clone HL3 and I-ab, clone AF6-120.1). All antibodies were purchased from BD Pharmingen.

Statistics. Mann-Whitney rank sum test was applied to reveal significant differences in the protein expression. The median tumor size and the metastatic status of the control group and all treated animals were compared. A value of ps≤0.05 was defined as the level of significance. All of the computations were performed with SigmaStat 1.0 software (Jandel Scientific, San Rafael, CA, USA).

Results

Multidrug resistance in vitro. A positive cellular surface expression was detectable for P-glycoprotein in 66.7±2%, for MRP1 in 37.4±4%, for MRP3 in 69.8±3% and for the common MRP epitope in 58.3±3% of tumor cells from the control group. Treatment with IFN-α monotherapy did not significantly influence the drug resistance-associated proteins. Cellular expression was positive in 68.2±6% for P-glycoprotein, 32.1±3% for MRP1, 35.3±3% for MRP3 and 43.4±4% for the MRP epitope of IFN-α treated cells.

Previously, we demonstrated that 5-fluorouracil, cisplatin or gemcitabine monotherapy increased the proportion of MRPs and their activity compared to untreated tumor cells (25). Here, treatment with gemcitabine plus IFN-α revealed a significant reduction of P-glycoprotein and MRP3 expression compared to the control (ps≤0.05). MRP1 and MRP epitope expression remained almost at the same levels as the control. Combined treatment with cisplatin and IFN-α showed a significant lower expression of P-glycoprotein, MRP3 and the MRP epitope in comparison to the control (ps≤0.05). Furthermore, there was a trend towards lower MRP1 expression. Combined treatment of 5-fluorouracil plus IFN-α had minor effects on cell surface expression of drug resistance proteins (Figure 1).

The quantication of MRP activity in Panc1 cells showed slightly lower efflux activity for the combined treatment with 5-fluorouracil plus IFN-α (MAF Total = 38 vs. MAF Total = 42), cisplatin plus IFN-α (MAF Total = 40 vs. MAF Total = 52) and gemcitabine plus IFN-α (MAF Total = 42 vs. MAF Total = 56) in comparison to monotherapy. Altogether, MRP activity after combination therapy was practically at the same level as in the untreated control (MAF Total = 42).

Multidrug resistance in vivo. Effects of combined therapy with 5-fluorouracil, cisplatin or gemcitabine plus IFN-α were investigated in an orthotopic pancreatic carcinoma mouse model. Previously, we demonstrated a significant induction of multidrug resistance after monotherapy with 5-fluorouracil, cisplatin and gemcitabine in vivo (25). Here, animals treated with gemcitabine plus IFN-α had a significantly lower proportion of MRP1-positive cells compared to the control (ps≤0.05). P-glycoprotein as well as MRP3 expression showed a trend towards down-regulation in comparison to the control. Furthermore, treatment with cisplatin plus IFN-α revealed a significant decrease of P-glycoprotein, MRP1 and MRP3 (ps≤0.05). Treatment with 5-fluorouracil plus IFN-α had minor effects on (Figure 2).
Tumor kinetics and growth. Ascites, peritoneal seeding and liver metastases were found in the majority of control group animals. Lung metastases were only detectable in the control group. Combined treatment of chemotherapy plus IFN-α had positive effects. Treatment with cisplatin plus IFN-α significantly reduced liver metastases and peritoneal carcinomatosis. Mice treated with a combination of gemcitabine plus IFN-α had significantly less liver metastases (Table I). At the time of scarification, the tumor size was 0.53±0.07 cm$^3$ in animals of the control group. Treatment with 5-fluorouracil plus IFN-α, cisplatin plus IFN-α or gemcitabine plus IFN-α reduced the tumor size significantly compared to the control (p≤0.05) (Figure 3). Animals treated with 5-fluorouracil or gemcitabine in combination with IFN-α showed no side-effects. Animals treated with cisplatin plus IFN-α presented more often with diarrhea after administration. The median survival of untreated control group animals was 18±1 days. Combination of IFN-α and 5-fluorouracil therapy led to a median survival of 21±1 days. In combination of IFN-α and cisplatin or gemcitabine, survival was 19±1 days and 18±1 days, respectively.

Immunological response. Several parameters were evaluated for immunological response in order to investigate the potential role of MRPs as antigens. Spleen derived leukocytes and TILs were analyzed for antigen-specificity. Cells were prepared from the indicated tissue and stimulated with lysate of gemcitabine-treated resistant (MRP overexpressing) tumor cells or lysate of untreated non-resistant cells. Numbers of CD8$^+$ cells TILs were the same in the control group (61±13%) and in animals treated with immunochemotherapy (63±13%). Response, defined as IFN-γ secretion, was low in spleen-derived lymphocytes after stimulation with non-resistant tumor cell lysate or resistant tumor cell lysate. In TILs, stimulation with non-resistant tumor cell lysate had minor effects on IFN-γ secretion independently of the animals’ treatment. When stimulated with resistant tumor lysate, TILs from animals having undergone chemotherapy showed a significant increase of IFN-γ secretion. This effect was detectable after gemcitabine and 5-fluorouracil treatment compared to the control (p≤0.05). In contrast, the numbers of activated IFN-γ-secreting TILs tended to be lower after the animal had therapy with gemcitabine or 5-fluorouracil compared to the control (p≤0.05). As seen in IFN-γ staining these effects were less pronounced in animals treated with chemotherapy plus IFN-α.

Furthermore, we analyzed whether pre-treatment with chemotherapy enhanced the susceptibility of tumor cells to immunological effector cells. Therefore, splenic lymphocytes from healthy mice were incubated either with untreated non-resistant or chemotherapy-treated resistant (MRP overexpressing) PANC02 cells. The cytotoxic activity increased after contact with chemotherapy-treated tumor cells. In a next step, the susceptibility of tumor cells against splenic lymphocytes of tumor-bearing mice (antigen-experienced lymphocytes) was investigated. A nearly threefold increase in cytosis of chemotherapy-treated resistant tumor cells compared to untreated non-resistant...
cells was detected \( (p \leq 0.05) \). Finally, it was analyzed whether the untreated non-resistant or pre-treated resistant tumor cells were more susceptible to lymphocytes from tumor-bearing gemcitabine-treated animals. Again, a nearly threefold higher cytolysis of pre-treated resistant tumor cells compared to untreated non-resistant cells was detectable (Figure 5).

For further investigation of the potential role of MRPs as antigens, DCs were generated from healthy animals. Stimulation was carried out either with lysate of untreated non-resistant control tumor cells or chemotherapy-treated resistant (MRP overexpressing) tumor cells. DCs of these two different groups were transferred separately into tumor-bearing animals. These animals received either therapy with gemcitabine or gemcitabine plus IFN-α. The following groups of animals were analyzed: 1: DCs pulsed with lysate of drug-resistant (MRP overexpressing) cells, animals receiving therapy with gemcitabine 2: DCs pulsed with lysate of drug-resistant (MRP overexpressing) cells, animals receiving therapy with gemcitabine plus IFN-α and 3: DCs pulsed with lysate of non-resistant control cells, animals receiving therapy with gemcitabine plus IFN-α. There were no differences in total numbers of TILS or monocytes in any group. In tumors of animals receiving – immunochemotherapy and implanted DCs pulsed with lysate of drug resistant cells (group 2) central memory as well as effector memory cells increased in comparison to animals receiving immunochemotherapy and implanted DCs pulsed with lysate of non-resistant cells (group 3). In the same animals, more CD8+ cells, a trend towards decrease of naïve T-cells) as well as CD11c/I-Ab+ dendritic cells almost at the same level were detectable (Figure 6).
Pancreatic cancer is one of the most intrinsically drug-resistant type of tumors (26). New strategies to overcome the high resistance against multiple cytotoxic agents are urgently required. The overexpression of P-glycoprotein, MRP1 and MRP3 has been identified in human pancreatic carcinoma. A correlation of protein expression with tumor grading and impaired survival has been reported (4, 5). Previously, we demonstrated an induction of multidrug resistance by cytostatic therapy in pancreatic cancer (25). This study shows that immunotherapy with IFN-α restores the chemosensitivity in pancreatic cancer in vitro and in vivo.

Immunotherapeutic strategies demonstrated impressive 5-year survival rates for pancreatic cancer (7, 8). The immunomodulatory agent IFN-α seems to play a crucial role within these regimes. Previous in vitro studies demonstrated that IFN-α has direct inhibitory properties on tumor cells, acts as a radiosensitizer and circumvents tumor cell re-growth after cisplatin therapy (13). Here, we were able to detect significantly lower expression of P-glycoprotein, MRP1 and MRP3 after combined treatment with chemotherapy and IFN-α, indicating a modulation of drug resistance. Additionally, the drug efflux activity of pancreatic cancer cells decreased after immunotherapy. To date, an influence of IFN-α on multidrug resistance has been described for human melanoma, renal cancer, osteosarcoma and hepatoma cell lines (22, 27-29). A restoration of cytotoxic effects of anticancer drugs was reported after combination of chemotherapy with IFN-α for multidrug resistant cells derived from human colon carcinoma and breast carcinoma (30, 31). Manara et al. reported on the increased chemosensitivity of drug resistant osteosarcoma cells due to reduced expression of P-glycoprotein after exposure to IFN-α (29). A potential mechanism for the chemosensitivity-restoring effect of IFN-α might be the higher sensitivity of P-glycoprotein overexpressing cells to type 1 IFNs correlating with higher expression of the activator of transcription STAT-2 and STAT-3, two intracellular mediators of the IFN-α signaling pathway (29). Furthermore, IFN-α was found to induce growth arrest through regulation of proliferative genes (32). This antiproliferative activity is partly mediated via induction of IL-2, IFN-γ and TNF-α (33). P-glycoprotein has been shown to be involved in the transport of IL-2, IFN-γ and IL-4 (34). Since IFN-α induces cytokines and cytokine secretion was found to inhibit P-glycoprotein-mediated doxorubicin extrusion from leukemia cells, this might be a further mechanism involved in restoration of chemosensitivity of tumor cells by IFN-α therapy (35).

The present in vivo experiments showed a significant decrease of MRP and P-glycoprotein expression after immunochemotherapy. Furthermore, treatment with 5-fluorouracil, cisplatin or gemcitabine plus IFN-α displayed a significantly reduced tumor size. Peritoneal carcinomatosis, hepatic seeding and pulmonary metastases were significantly reduced compared to the control. 5-fluorouracil and gemcitabine are standard chemotherapeutics in pancreatic cancer but response rates to monotherapy are below 20% (1); MRP-associated cellular efflux significantly contributes to the resistance against both (36-38). Chemosensitizing effects of the addition of IFN-α to 5-fluorouracil are described (13). Nevertheless, the clear mechanism behind this has not yet been elucidated. Cisplatin is widely used in cancer therapy of primary digestive tumors but the effectiveness of single agent therapy was so far clearly limited due to the high intrinsic resistance of the targeted cells. Beside an increased extrusion from cells via MRPs, enhancement of DNA repair and stimulation of metabolic detoxification are described as mechanisms for cisplatin resistance (39, 40). Similar to our results, a significant decrease of P-glycoprotein and suppressed MRP levels were detected by Takeuchi et al. after the exposure of HepG2 cells to cisplatin and IFN-α (22). A significant decrease of MRP mRNA expression after IFN-α therapy was also described by Miracco et al. (24).

The potential role of MRPs as antigens was also evaluated. Chemotherapy leads to an up-regulation of antigens such as P-glycoprotein, which in turn marks the tumor cell ready for destruction by cytotoxic T-cells. There are reports that a P-
glycoprotein-specific CD8+–mediated immune response can be induced (41). The existence of naturally occurring P-glycoprotein specific cytotoxic T-cells has been described previously by Azuma et al. in the context of a murine leukemia model (42). Niethammer et al. activated in their study CD8+ T-cells against P-glycoprotein and demonstrated a prolonged life span in the treated animals (43). We isolated CD8+ cells from tumors of chemotherapy-treated animals and incubated them with lysate of drug resistant tumor cells. In these CD8+ cells, we were able to confirm a clearly higher IFN-γ secretion compared to CD8+ cells from chemonaïve animals by two different methods. Interestingly, these effects were not detectable in CD8+ cells from animals treated with chemotherapy plus IFN-α. It can be hypothesized that this is based on the reduced cell surface expression of MRPs after immunochemotherapy and a consecutive change in the antigen presentation to the immune system. It is well known that cancer cells have an increased susceptibility to immunotherapy compared with somatic tissue (44). An induction of the immunoproteosome, with enhanced vulnerability of pancreatic tumor cells against T-cells was described (14). We demonstrated that chemotherapy enhances the susceptibility of tumor cells against immunological effector cells from healthy animals. This effect was even higher against effector cells from antigen-experienced tumor-bearing but untreated animals. Lymphocytes from tumor-bearing treated animals showed a threefold increased cytolytic activity against treated tumor cells compared to untreated. Nevertheless, the overall effect was smaller than with lymphocytes from untreated tumor-bearing animals which might be caused by a lower antigen expression potentially induced by chemotherapy. Additionally, the destruction of vulnerable cell populations by immunochemotherapy leads to negative selection of subpopulations, which may be distinctively different from the majority of cells first encountered.

In a translational research program accompanying the phase III CapRI trial comparing radio-chemo-immunotherapy with standard chemotherapy for pancreatic cancer, we demonstrated an immediate activation of antigen presenting cells and NK cells, followed by antigen-specific T-cell activation in patients receiving low dose IFN-α (45). Here, DCs were stimulated with lysate of drug resistant (MRP overexpressing) tumor cells and transferred into murine pancreatic tumors. Consistent with previous observations in vitro and in our patients, a decrease of NK cells and naïve T-cells but an increase of CD8+ and effector memory cells was detectable in animals which underwent immunochemotherapy (14, 45). To investigate if this immunostimulatory reaction was related to drug resistance proteins DCs stimulated with lysate of non-resistant tumor cells were also transferred into animals treated with immunochemotherapy. Again, in animals with antigen-experienced DCs, the proportion of naïve T-cells and CD8+ cells decreased but increased effector memory as well as central memory cells were detected compared to animals with transferred antigen-naïve DCs.

**Conclusion**

This study shows a modulation of chemotherapy-induced multidrug resistance by IFN-α in experimental pancreatic cancer. It was also demonstrated that drug resistance proteins play a role as tumor antigens in pancreatic tumors. Since combination therapy with IFN-α significantly reduced tumor growth and restored chemosensitivity, our data support treatment schedules starting with chemotherapy and proceeding with immunochemotherapy as an encouraging new approach in pancreatic cancer.

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**References**


