Growth inhibition and apoptosis induction in ovarian cancer cells

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Abstract. Standard therapy for the treatment of ovarian cancer is radical surgery followed by radiation and/or chemotherapy using cisplatin and paclitaxel. Unfortunately, some patients relapse after this first line chemotherapy and some patients become platinum-refractory. Therefore, we analyzed two different ovarian carcinoma cell lines for their sensitivity for γ-irradiation and treatment with cisplatin, irinotecan, paclitaxel and gemcitabine. We found that both cell lines were rather resistant against γ-irradiation and treatment with cisplatin and irinotecan whereas paclitaxel and gemcitabine resulted in a considerable reduction of the viability of the cancer cells. Both paclitaxel and gemcitabine treatment resulted in the induction of apoptosis. This sensitivity profile might be due to a particular subset of p53, which reacted with monoclonal antibodies DO-1 and PAb1801 but not with PAb1620 and PAb421. Gemcitabine and paclitaxel are highly efficient in the induction of apoptosis in ovarian cancer cells, which express a particular subset of the growth suppressor protein p53. Thus, a sensitivity profile for each ovarian carcinoma seems to be highly recommended before starting treatment.

Introduction

Ovarian cancer is one of the leading causes of cancer mortality. It is difficult to detect at an early stage and most of the patients suffer from massive metastatic disease at the time of diagnosis. Despite radical surgery and the application of several potent anti-neoplastic drugs like cisplatin the prognosis still remains poor. The survival rate of patients with advanced ovarian cancer is only 10-20% (1). Ovarian cancer is nowadays commonly treated with a combination of cisplatin or carboplatin and paclitaxel (2). The cytotoxic effects of cisplatin are primarily mediated by the formation of DNA interstrand and intrastrand cross-links (3). In addition, the cisplatin-induced accumulation of p53 following DNA strand breaks also leads to growth arrest or apoptosis (4). Paclitaxel acts by binding to γ-tubulin, which leads to irreversible microtubule stabilization, thus inhibiting the degradation of the mitotic spindle and subsequently, leads to G_2/M arrest and apoptosis (5) via p53 dependent and independent pathways (6). Unfortunately, most patients relapse after this first line chemotherapy (7). Some of them can be re-treated effectively with the same agents; but others become platinum-refractory. In such cases a number of other drugs are used such as anthracyclines, topoisomerase inhibitors, nucleoside analogues or vinca alkaloids (8). Irinotecan is one of these drugs that acts as a topoisomerase I inhibitor leading to DNA strand breaks (9). One interesting cytostatic drug, which is gaining attention is gemcitabine, a pyrimidine analogue with considerable success in the treatment of different types of cancer and with modest side effects. Gemcitabine inhibits ribonucleotide metabolism, hinders DNA processing, and increases accumulation of intrastrand adducts and interstrand cross-links, thereby leading to a G_1 block in the cell cycle. Gemcitabine is generally well-tolerated (10).

Despite its long history in the treatment of ovarian carcinoma and its proven curative role in patients with minimal residual disease, the proper role of radiotherapy in the management of this tumor is controversial and not clearly established. Similarly, the potential roles for radiotherapy in the consolidate treatment and as salvage therapy following chemotherapy failure remain controversial (11). There are examples where radiotherapy has proven to be successful (12) and where a combination of radiotherapy and hyperthermia led to the complete remission of an ovarian clear cell adenocarcinoma.

The main aim of all these therapies might be tumor regression through the induction of apoptosis. There are two principal ways of apoptosis, which finally lead to the fragmentation of the DNA and to the decay of the cell into apoptotic bodies, which are finally removed by phagocytes: the receptor-mediated extrinsic pathway and the mitochondrial intrinsic pathway. One of the late events in the apoptosis pathways is the cleavage of the poly-ADP-ribosyl-polymerase (PARP), a 116-kDa protein into fragments of 89 and 26 kDa (13). A failure in inducing apoptosis might well be due to a non-functional cellular regulator such as p53. p53 is activated and accumulates in the cell in response to DNA damage. By inhibiting the activity of the cyclin dependent kinases it forces

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the cell into growth arrest and supports DNA repair activity of the cell. Irreparable damage leads the cell into apoptosis by influencing the balance between pro- and anti-apoptotic proteins (14). In more than 50% of all tumors p53 is mutated and in many tumors a wild-type p53 is non-functional because of the interaction with an antagonist. As p53 is mainly active at the G_1/S check-point actual therapeutic strategies try to bypass this check-point, which is mostly defective in cancer cells and hit the G_2/M check-points of tumor cells by forcing cancer cells into mitotic catastrophe.

To improve the treatment of ovarian cancer it would be beneficial to know the sensitivity profile of cancer cells towards the used chemotherapeutic drugs. We therefore established an *in vitro* system consisting of two different ovarian carcinoma cell lines: OV-MZ 32 is a serous adenocarcinoma cell line (15), and OvBH-1 cells are derived from an ovarian clear cell carcinoma (16). We treated these cell lines with the drugs, which are usually applied for the treatment of ovarian carcinoma such as cisplatin, paclitaxel, irinotecan and gemcitabine. Since there is also a report on a patient with ovarian cancer in whom complete remission was obtained by a combination of radiotherapy and hyperthermia (12) we moreover irradiated the ovarian carcinoma cell lines. As an important parameter for the success of the treatment we analyzed the induction of apoptosis in the different cell lines. Surprisingly, the conventional treatment with cisplatin and irinotecan and also γ-irradiation only showed a modest outcome. A very efficient induction of programmed cell death was demonstrated by the application of paclitaxel and especially, of gemcitabine. A combination of both drugs showed no gross synergistic effect. Thus, our results demonstrate the importance of checking each individual ovarian carcinoma for its resistance or sensitivity profile towards chemotherapeutic drugs in order to create an individual therapy. Moreover, we show that gemcitabine or paclitaxel treatment alone is sufficient for an efficient tumor therapy, at least *in vitro*.

Materials and methods

Cell lines. OV-MZ-32 is a serous human ovarian adeno-carcinoma cell line, which was kindly provided by Dr I. Runnebaum, Freiburg (15). OV-MZ-32 cells carry a 13-bp deletion from codon 314 to codon 318 of the p53 gene. OvBH-1 is a temperature-sensitive ovarian clear cell carcinoma cell line, characterized in detail elsewhere (16,17). Polish Patent Nr. P.327793 protects this cell line. Cells were maintained in DMEM medium with 10% fetal calf serum in a humidified incubator at 37˚C with 5% CO_2.

Treatment of the cells with different drugs and γ-irradiation. Cells were grown to 40% confluence 24 h before irradiation. Cells were γ-irradiated with doses of 0, 2, 4, 6, 8 and 16 Gy. Seventy-two hours after treatment surviving cells were counted in a haemocytometer after dye exclusion.

Gemcitabine hydrochloride (Gemzar®, Lilly S.A., France), irinotecan hydrochloride (Campto®, Aventis Pharma Ltd., Essex, UK), and cis-platinum (II)-diamine dichloride (Sigma, München, Germany) were dissolved in water in a concentration of 10 mM. Paclitaxel (Sigma) was dissolved as 5 mM stock solution in dimethyl sulfoxide (DMSO). Twenty-four hours after seeding the cells drugs were applied in concentrations of 1 nM, 10 nM, 100 nM, 1 μM and 10 μM. Seventy-two hours after treatment the viability of the cells was determined with an MTT assay or the cells were subjected to a Western blot or cytofluorimetric analysis.

MTT assay. Cell proliferation and viability was determined using a colorimetric MTT-based assay (MTT: 3-[4,5-dimethyl-thiazol-2-yl] 2,5-diphenyl tetrazolium bromide, Sigma-Aldrich, Deisenhofen, Germany). The assay is based on the reduction of the yellow tetrazolium salt MTT to purple formazan crystals by metabolically active cells. $3x10^3$ (OvBH-1) or $5x10^3$ (OV-MZ-32) cells/per well were grown in a 96-well plate in a final volume of 100 μl culture medium. The MTT labeling reagent (10 μl) (5 mg/ml PBS) was added to each well. The enzymatic reaction was allowed to take place for at least 4 h at 37˚C in a humidified atmosphere. The formation of purple crystals was monitored in a light microscope. The crystals were dissolved by adding 100 μl of a solubilization solution [10% w/v sodium dodecyl sulfate (SDS), 0.01 M HCl] overnight at 37˚C. The spectrophotometrical absorbance of the blue dye was determined in a 96-well plate in an ELISA reader at 595 nm.

Cytofluorimetry. For cytofluorimetric analysis cells were washed twice with cold phosphate-buffered saline (PBS) and harvested by trypsinizing. Cells were spun down (400 x g, 4˚C, 10 min) and resuspended in 200 μl PBS. Cells were fixed by adding 2 ml ice-cold 70% ethanol and left for at least 30 min at -20˚C. Cells were harvested by centrifugation and resuspended in 800 μl PBS. RNase (100 μl) (1 mg/ml) and 100 μl propidium iodide (400 μg/ml) were added and cells were incubated for 30 min at 37˚C and then sonicated for 30 sec at 4˚C. Cell cycle analysis of the suspension was performed using a Becton-Dickinson FACScan II cytofluorimeter.

Western blot analysis. Proteins were analyzed by SDS poly-acrylamide gel electrophoresis according to the procedure of Laemmli (18). For Western blot analysis proteins were transferred to a PVDF membrane by tank blotting with 20 mM Tris/HCl, pH 8.7, 150 mM glycine as transfer buffer. Membranes were blocked in PBS with 0.1% Tween-20 and 5% dry milk for 1 h at room temperature. The membrane was incubated with the primary antibody in PBS Tween-20 with 1% dry milk for 1 h. The membrane was then washed with PBS Tween-20 three times before incubating with the peroxidase-coupled secondary antibody in a dilution of 1:10000 in PBS Tween-20 with 1% dry milk. Signals were developed and visualized by the Lumilight system of Roche Diagnostics (Mannheim, Germany).

We used monoclonal antibodies DO-1 (Santa Cruz), PAb1801 (Oncogene Science), PAb421 (19) and PAb1620 (20) for the detection of p53. The anti-PARP antibody (Ab-2) (Oncogene Science) was used in a dilution of 1:100 and incubated overnight.

Results

Viability of different ovarian carcinoma cells after treatment with γ-irradiation and chemotherapeutic drugs. We used an *in vitro* system to check the effect of cytostatica and radio-
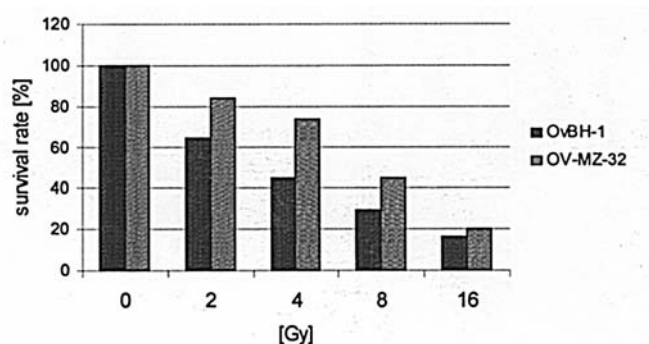
Figure 1. Survival of ovarian cancer cells after γ-irradiation. Cells were seeded 24 h before γ-irradiation. Cells were treated with doses of 0, 2, 4, 8 or 16 Gy. Seventy-two hours after the treatment living cells were counted in a haemocytometer. The number of non-irradiated cells corresponds to 100%.
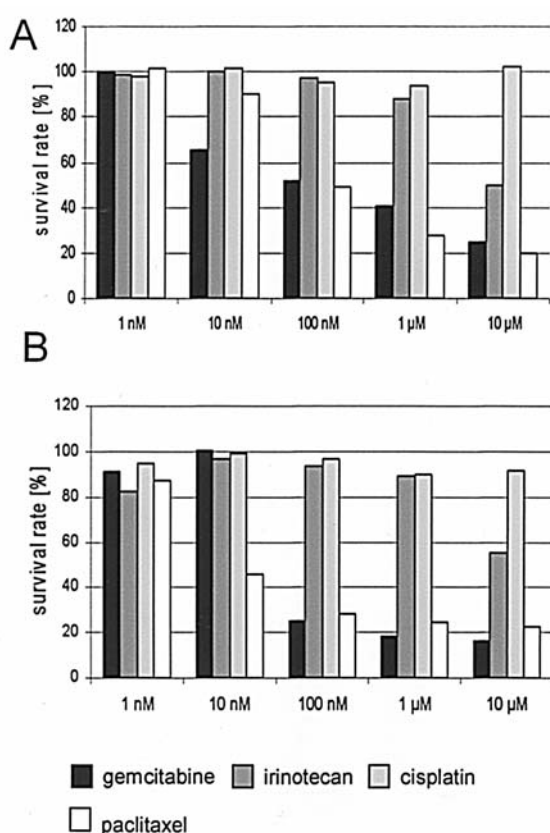

Figure 2. Survival of ovarian carcinoma cells after cytostatica treatment. Twenty-four hours after seeding cells were exposed to the indicated concentrations of cisplatin, irinotecan, paclitaxel or gemcitabine. Seventy-two hours after the treatment the rate of living cells was determined with an MTT assay. An untreated control was set to 100% (data not shown) to which all other values were referred. (A), OvBH-1; (B), OV-MZ-32.
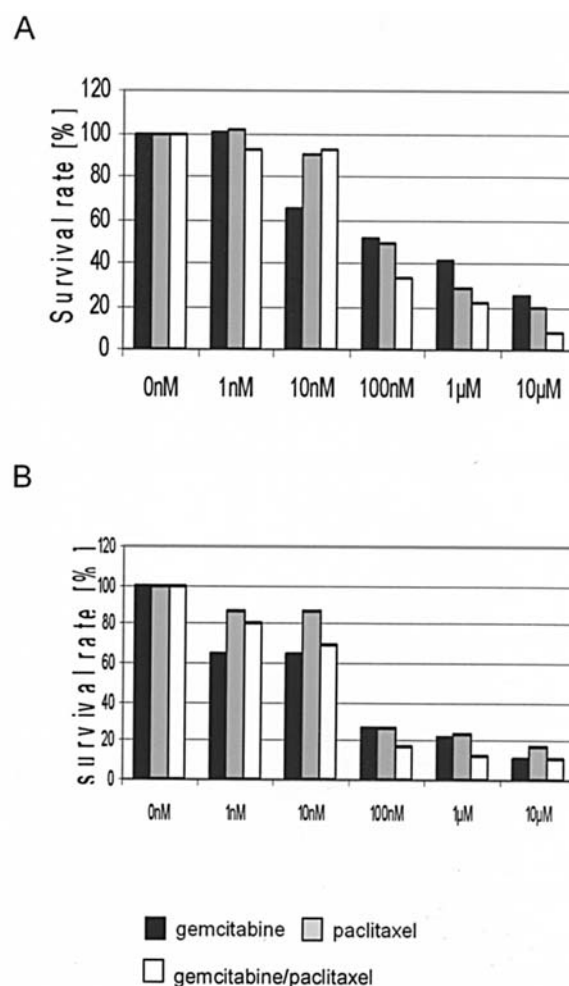

Figure 3. Survival of ovarian carcinoma cells after treatment with gemcitabine and paclitaxel. Twenty-four hours after seeding cells were exposed to the indicated concentrations of gemcitabine, paclitaxel or a combination of both. Seventy-two hours after treatment the rate of living cells was determined with an MTT assay. An untreated control (0 nM) was set to 100% to which all other values were referred. (A), OvBH-1; (B), OV-MZ-32.

therapy on ovarian cancer cells. Cells were γ-irradiated with doses up to 16 Gy and afterwards, living cells were counted in a haemocytometer. For the comparison of the effect of the radiotherapy the percentage of viable cells was plotted as a function of the radiation dose. The 50% inhibitory dose (ID_{50}) was determined for each cell line based on these curves. As shown in Fig. 1, in general, both cell lines were rather insensitive. However, OV-MZ-32 cells appeared to be more

resistant than OvBH-1 cells. Based on the data in Fig. 1 the ID_{50} doses ranged between around 4 Gy for OvBH-1 and around 8 Gy for OV-MZ-32 cells indicating that γ-irradiation is not the first choice for the treatment of ovarian carcinoma.

Next, we analyzed the sensitivity of the ovarian carcinoma cell lines for the treatment with paclitaxel and cisplatin. In addition, we also tested irinotecan and gemcitabine. Cells were treated with concentrations ranging from 1 nM up to 10 μM of cisplatin, irinotecan, paclitaxel or gemcitabine. After treatment for 72 h viability of the cells was controlled with a colorimetric assay. The percentages of the resulting vital cells compared to untreated control cells are presented in Fig. 2. For all cell lines treatment with cisplatin showed no effect on the growth of the cells. We made essentially the same observation when using irinotecan although at the highest concentration (10 μM) the viability of the cells was modestly reduced to a growth rate of 50-75% of the control cells.

A considerable reduction in viability was achieved with gemcitabine and paclitaxel. OV-MZ-32 cells showed a
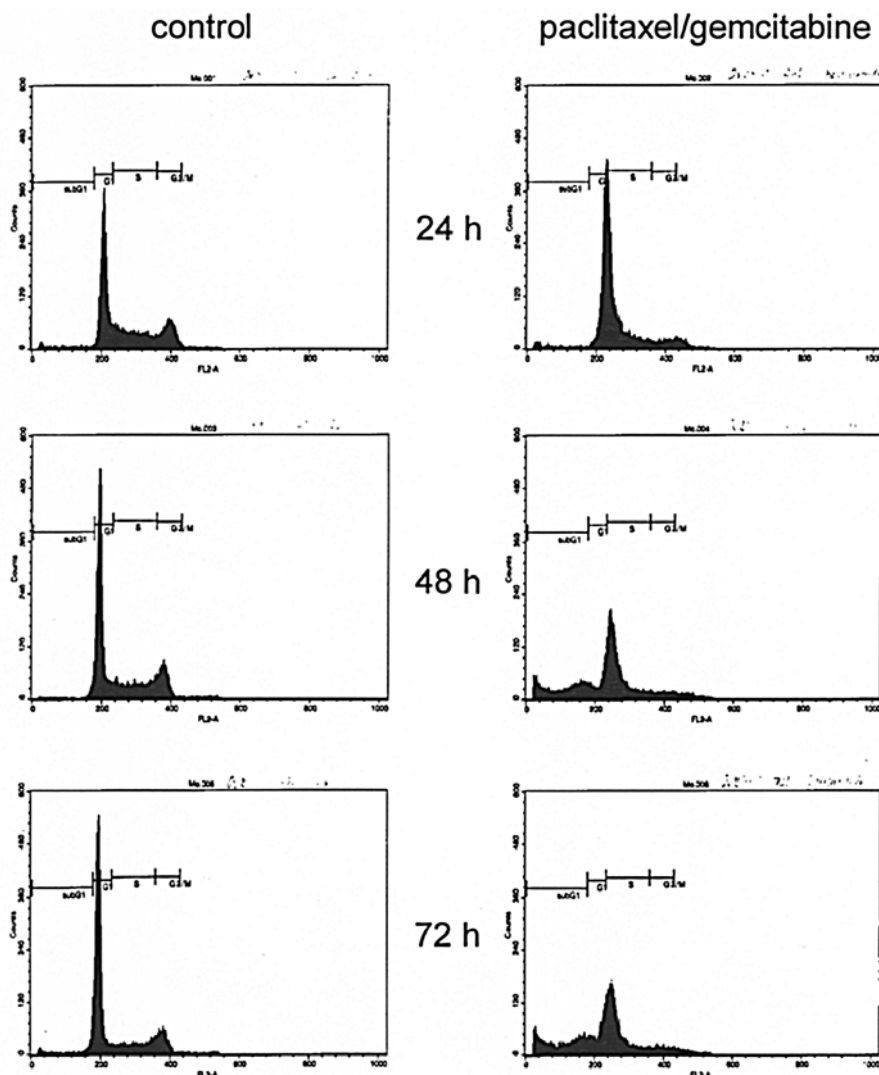
Figure 4. Cytofluorimetry analysis of OvBH-1 cells after treatment with paclitaxel/gemcitabine. OvBH-1 cells were treated with a combination of 100 nM gemcitabine and 100 nM paclitaxel for 24, 48 and 72 h. Cells were fixed with ethanol and stained with propidium iodide. Cell cycle analysis was performed using a FACScan II cytofluorimeter from Becton-Dickinson. Untreated cells served as control.

reduction of 75% in growth when using 100 nM gemcitabine. Paclitaxel was already effective at a concentration of 10 nM in around half of the cell population. Also, OvBH-1 cells showed a stronger sensitivity towards both drugs with an ID_{50} of 100 nM for gemcitabine or paclitaxel. In all cases both drugs were more efficient than cisplatin and irinotecan.

In order to analyze whether there is a synergistic effect the viability assays were repeated with a combination of both drugs. The results shown in Fig. 3 demonstrate that the combination of both drugs had some but not a significant effect on the reduction of the growth rate compared to gemcitabine or paclitaxel alone. Thus, we have demonstrated that the ovarian cancer cell lines were rather insensitive towards γ-irradiation and chemotherapy with cisplatin and irinotecan. However, viability of cells was considerably reduced by the use of paclitaxel and gemcitabine.

Apoptosis induction in ovarian cancer cells after treatment with gemcitabine and paclitaxel. Since we observed a considerable reduction in metabolically active cells after treatment with gemcitabine or paclitaxel we now asked whether treatment with these drugs provokes the induction of apoptosis. We performed two different assays for the detection of programmed cell death in treated cells: a cytofluorimetric analysis and a Western blot analysis to show the cleavage of poly-ADP-ribosyl-polymerase (PARP). For the cytofluorimetric analysis we treated cells for 72 h with 100 nM gemcitabine or 100 nM paclitaxel or a combination of 100 nM gemcitabine/100 nM paclitaxel. Cells were harvested and the DNA stained with propidium iodide. An example of a cell cycle profile is shown in Fig. 4 for OvBH-1, which had been treated with the combination of both drugs. In comparison to the control cells the sub-G_1-population significantly increased from about 4 to 26% after 48 h and 28% after 72 h. The sub-G_1-population represents cells with fragmented DNA. Thus, nearly one third of the cell population is committed to apoptosis. Moreover, we observed an increase in the S-phase cells from about 25-30% to 45-50%. Concomitantly, the amount of G_2- and especially G_1-cells decreased drastically. The data for the cell lines treated with both drugs separately or in combination are

Table I. Percentage values of treated and untreated cells in the different cell cycle phases.

	Sub-G_1	G_1	S	G_2/M
Ov-MZ-32, Pac 72 h	56.29	23.27	11.44	8.77
Ov-MZ-32, Gem 72 h	59.83	23.17	11.72	5.47
Ov-MZ-32, Pac/Gem 72 h	31.9	15.95	25.09	15.98
Ov-MZ-32, control	5.96	36.5	10.9	36.3
Ov-BH1, Pac 72 h	10.27	6.19	53.12	16.19
Ov-BH1, Gem 72 h	23.6	6.31	42.13	13.57
Ov-BH1, Pac/Gem 72 h	27.95	18.43	44.18	6.51
Ov-BH1, control	6.78	57.15	20.57	14.1

Pac, paclitaxel 100 nM; Gem, gemcitabine 100 nM.

Figure 6. Identification of p53 in ovarian cancer cells with different p53 antibodies. Protein (50 μg) from a cell extract from OvBH-1 and OV-MZ-32 cells were loaded on a 10% SDS polyacrylamide gel and analyzed by Western blot with the p53 specific monoclonal antibodies DO-1, PAb421, PAb1620 and PAb1801. Protein bands were visualized with a POD-conjugated secondary anti-mouse antibody and the Lumilight system. (Lane 1), OvBH-1; (lane 2), OV-MZ-32.

Figure 7. Expression of p53 in ovarian carcinoma cells after γ-irradiation. Cells were γ-irradiated with 4 Gy and harvested after 1 h (lane 2) or after 3 h (lane 3), respectively. Non-irradiated cells served as control (lane 1). Cell extract (30 μg) was loaded on a 10% SDS polyacrylamide gel and analyzed by Western blot with the p53-specific antibody DO-1. Signals were visualized with a POD-conjugated secondary anti-mouse antibody and the Lumilight system. A GAPDH-specific antibody was used as loading control. (A), OvBH-1; (B), OV-MZ-32.
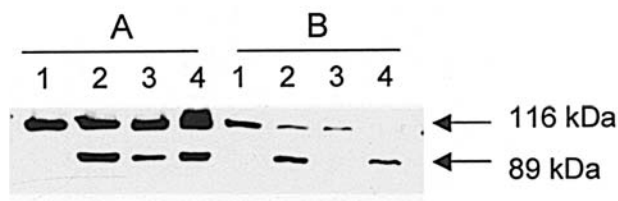

Figure 5. PARP cleavage after treatment of ovarian cancer cells with gemcitabine and paclitaxel. Cells were treated with 100 nM gemcitabine (lane 2), 100 nM paclitaxel (lane 3) or a combination of both (lane 4) for 72 h. Untreated cells (lane 1) served as control. Cell extract (150 μg) was loaded on a 10% SDS polyacrylamide gel and analyzed by Western blot with the PARP-specific antibody Ab-2. Signals were visualized with a POD-conjugated secondary anti-mouse antibody and the Lumilight system. (A), OV-MZ-32; (B), OvBH-1.

summarized in Table I. Although there are some variations in the efficiency for both drugs for the studied cell lines the combination of paclitaxel and gemcitabine showed no clearly visible synergistic effect. So far our data provided strong evidence that the chemotherapy of ovarian cancer with paclitaxel and gemcitabine applied separately and in combination induced apoptosis.

To verify the data concerning the induction of apoptosis we performed a Western blot analysis in which we checked the cleavage of PARP. PARP is a 116-kDa protein, which is processed by caspase 3 into fragments of about 89 and 26 kDa. In the Western blot analysis we used an antibody, which recognizes the full-length protein and the 89-kDa fragment and thus allows a follow-up of the apoptosis induction in response to the treatment. The results are shown in Fig. 5. In every
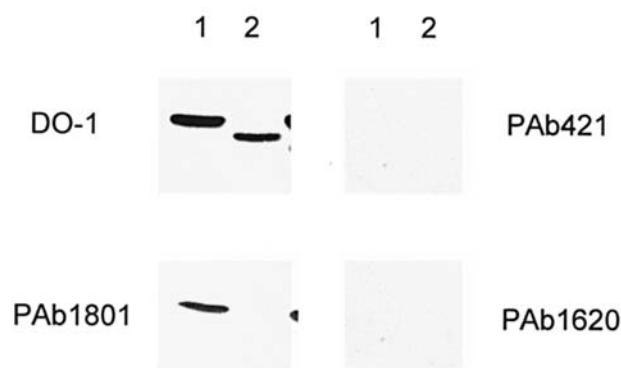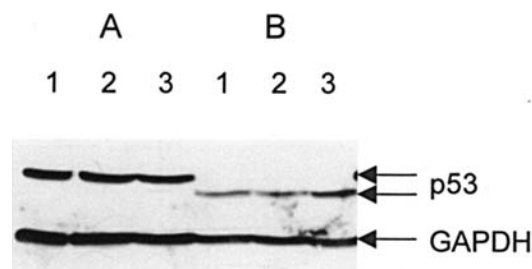
case we detected the full-length protein. Upon treatment with 100 nM gemcitabine we constantly observed the processing of the full-length protein to the 89-kDa fragment. We also reproducibly detected the cleavage of PARP in response to the treatment with paclitaxel, but in contrast to the treatment with gemcitabine it turned out to be much weaker. The combination of both drugs also led to the cleavage of PARP, but the effect was obviously not stronger than with gemcitabine alone. Thus, these data support the observation that by the treatment with cytostatic drugs cells are committed to apoptosis; for the induction of apoptosis in the studied cell lines treatment with paclitaxel or with gemcitabine alone seems to be sufficient and efficient.

The role of p53 in the apoptosis induction in ovarian cancer cells. Trying to analyze the p53 protein in both cell lines we found that p53 could only be detected with the monoclonal antibodies DO-1 and PAb1801 but not with PAb421 and PAb1620 (Fig. 6). Next, we analyzed the response of the p53 protein upon γ-irradiation. Cells were γ-irradiated with 4 Gy and extracted either after 1 h or after 3 h. The cell extracts were analyzed on an SDS polyacrylamide gel followed by a Western blot.
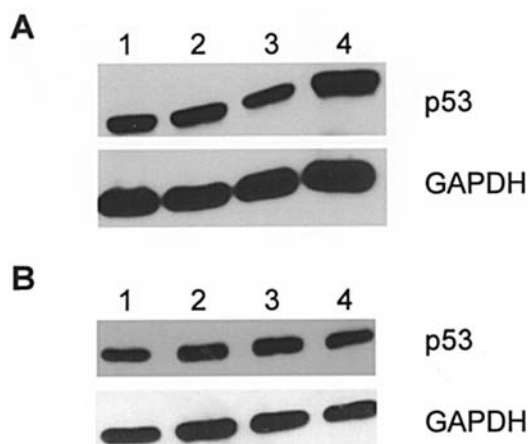
Figure 8. Expression of p53 in ovarian carcinoma cells after treatment with gemcitabine and paclitaxel. Cells were treated with 100 nM gemcitabine (lane 2), 100 nM paclitaxel (lane 3) or a combination of both (lane 4) for 72 h. Untreated cells (lane 1) served as control. Cell extract (50 μg) was loaded on a 10% SDS polyacrylamide gel and analyzed by Western blot with the p53-specific antibody DO-1. Signals were visualized with a POD-conjugated secondary anti-mouse antibody and the Lumilight system. A GAPDH-specific antibody was used as loading control. (A), OV-MZ-32; (B), OvBH-1.

p53 was detected with monoclonal antibody DO-1. As shown in Fig. 7, p53 was expressed in comparable amounts in both cell lines and independently of the time-points after radiation. Thus, these data indicated that there is no stabilization of p53. Moreover, there was no induction of p21^{WAF1}, one of the downstream targets of p53 (data not shown).

Subsequently, we analyzed the reaction of p53 after treatment with gemcitabine and paclitaxel. Seventy-two hours after drug application cells were harvested and protein extracts were subjected to a Western blot analysis with the p53 specific monoclonal antibody DO-1. As shown in Fig. 8, p53 could be detected in both cell lines, either treated or untreated. Upon treatment with the different drugs we did not observe accumulation of the p53 protein. Moreover, also in this case the p53 protein does not seem to be active as transcription factor since at least one of the major transactivated targets p21^{WAF1} could not be identified. Thus, the apoptosis induction by paclitaxel or gemcitabine does not rely on the presence of a functionally active p53. Thus, we conclude that the chemotherapy of the ovarian cancer cells leads to apoptosis without the induction of p53.

Discussion

Clear cell ovarian carcinoma has a poor prognosis mainly because its resistance to cytostatica and in particular against platinum compounds. Recently, a patient was reported with clear cell carcinoma of the ovary in whom complete remission was obtained by a combination of radiotherapy and hyperthermia (12). Moreover, also an established clear cell adenocarcinoma cell line turned out to be rather resistant to chemotherapy with cisplatin, irinotecan, paclitaxel and gemcitabine whereas it was very sensitive to γ-irradiation. A 50% growth inhibition was achieved with a dose of 1.8 Gy (21) indicating a high radiation sensitivity of this type of ovarian cancer. In contrast our ovarian clear cell carcinoma cell line was rather resistant to γ-irradiation with an ID$_{50}$ between 4 and 8 Gy. Thus, it seems to us that a general statement about a radiation therapy is impossible and each patient with ovarian clear cell carcinoma possesses an individual sensitivity.

It is known that cisplatin induced apoptosis in human ovarian cancer cells (22). However, many other tumors are intrinsically resistant against cisplatin. Some tumors, which were initially sensitive to cisplatin, develop resistance during treatment. It was the most surprising result of our study that the ovarian carcinoma cell lines analyzed appeared to be insensitive for cisplatin treatment. This might be explained by the observation that cisplatin resistant tumor cells show an elevated level of the p53 protein. Sequence analysis revealed that this p53 could either be mutant or wild-type (22,23). Moreover, these tumor cells also exhibited a less reduced inhibition of DNA synthesis after γ-irradiation.

Irinotecan is a cornerstone drug in the management of metastatic colorectal cancer (24). It is a camptothecin derivative that inhibits topoisomerase I. It is believed that such an inhibitor stabilizes a DNA/topoisomerase I complex and interacts with the replication machinery to cause cell death. In the present study irinotecan inhibited cell growth of ovarian cancer cells only at high concentrations. However, there was no indication for cell death.

Paclitaxel is an antimicrotubule agent blocking cell division by interfering with the function of the mitotic spindle (25). Ovarian carcinomas with mutant p53 were described to be more responsive to a paclitaxel-based chemotherapy than p53 wild-type expressing tumors (26). In contrast, Debernardis *et al* could not find a correlation between the sensitivity of human ovarian cancer cells to paclitaxel and the p53 status and therefore suggested that paclitaxel may have the ability to activate apoptosis in the absence of a functional wild-type p53 (27). This later finding is in accordance with other observations in glioma cells and in renal cell carcinoma (28,29). Our results indicate that paclitaxel is very efficient in the treatment of ovarian carcinoma cells inducing apoptosis. Paclitaxel is also a highly effective chemotherapeutic agent against adenocarcinomas and squamous cell carcinomas of the esophagus where it was also shown to induce apoptosis (30).

Cytotoxicity of gemcitabine is related to the cellular accumulation of gemcitabine triphosphate inducing a G_0/G_1- and S-phase arrest in tumor cells (31). Gemcitabine (2',2'-difluorodeoxycytidine) has improved the survival of patients with many different tumors (32). Mechanistic studies have demonstrated that there are at least two major pathways through which gemcitabine acts: i) direct inhibition of DNA synthesis by formation of the triphosphate form, and ii) inhibition of ribonucleotide reductase, which results in the depletion of deoxynucleoside triphosphates necessary for DNA synthesis (33). In the case of pancreatic cancer gemcitabine-based combination chemotherapy has been shown to give better results than gemcitabine alone (34). In the case of breast and non-small cell lung cancers it was shown that paclitaxel increased the accumulation of dFdCTP, incorporation of gemcitabine into RNA and apoptosis (35,36). In the case of ovarian cancer cells we did not observe a significant increase in the response using a combination of both drugs. As shown herein, both paclitaxel and gemcitabine applied separately

are very efficient in its activity in inducing growth arrest and apoptosis. A combination of paclitaxel and gemcitabine failed to show any clearly visible synergistic effect.

The molecular pathway by which gemcitabine caused apoptosis has not been fully defined. In the case of non-small lung cancer (NSCLC) it was found that gemcitabine induced apoptotic cell death via the Bcl-2 dependent caspase-9 activation pathway. Moreover, upon gemcitabine treatment the extracellular signal-regulated kinase (ERK) pathway was induced. Since these studies were performed in p53$^{-/-}$ cells it was clear that the induction of these pathways is p53 independent (37).

Alterations in the p53 gene in ovarian carcinoma cell lines are rarely described (22,23,38). We found that both cell lines expressed high levels of p53 indicating a stabilization and inactivation of the protein. In the case of OvBH-1 cells this stabilization is not due to a mutation in the p53 gene because sequence analysis revealed only a neutral genetic polymorphism (17,39). OV-MZ-32 cells express a p53 mutant with a deletion between amino acids 314 and 318 (15). This deletion affects the cdc2 phosphorylation site at position 315, which is known to regulate the sequence specific DNA binding of p53 and conformational changes in the p53 protein (40). It was a very interesting observation that p53 from both cell lines reacted only with the p53 specific monoclonal antibodies DO-1 and PAb1801, but not with PAb421 and PAb1620. Antibody DO-1 reacts with the epitope [20]SDLWKL[25] in the N-terminus of p53 in an unphosphorylated form (41,42). A failure to interact with monoclonal antibody PAb421 indicates that p53 is phosphorylated at Ser 378 or acetylated at Ser 373 (43,44). Monoclonal antibody PAb1620 recognizes p53 in the wild-type conformation (45,46). PAb1801 reacts with the sequence between amino acids 46 and 55 (47) which seems not to be affected by specific modifications or functions of p53. Thus, our data indicate the presence of a particular subset of p53 in these ovarian carcinoma cells. This is further supported by the observation that neither p21^{WAF1} nor mdm-2 is trans-activated upon treatment with the different drugs indicating the presence of an at least partially inactive p53. Furthermore, these data demonstrate that gemcitabine and paclitaxel induce a p53 independent pathway of apoptosis in the ovarian cancer cells.

In conclusion, our data favor the view that a general therapy strategy for ovarian cancer seems to be inadequate. Information on the p53 status seems to be important before starting therapy. As shown here a sensitivity profile for γ-irradiation or for different drugs seems to be individually differentiated.

Acknowledgements

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