Profiling of signaling molecules in four different human prostate carcinoma cell lines before and after induction of apoptosis

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Received July 29, 2005; Accepted September 2, 2005

Abstract. We have treated four prostate tumor cell lines, DU-145, PC-3, LNCaP and 22RV1 with various concentrations of cisplatin in order to check for influence on viability and for onset of apoptosis induction. At a cisplatin concentration of 20 μM, 22RV1 and DU-145 cells showed ~22% and 18% and PC-3 and LNCaP cells showed ~4 and 10% dead cells, respectively. When checking for apoptosis induction, the differences among the cell lines became even more evident. DU-145 and 22RV1 cells showed apoptosis induction at 5- and 2-μM cisplatin, whereas in the case of LNCaP and PC-3 cells comparable apoptosis induction was observed at 100-μM cisplatin; hence, the difference between the two groups of cell lines with respect to apoptosis induction is 20- and 50-fold, respectively. We used 37 antibodies to screen the expression levels of key signaling molecules and their phosphorylation status where appropriate. DU-145 and PC-3 cells are androgen-receptor negative and harbor non-functional p53, whereas LNCaP and 22RV1 cells are androgen-receptor positive and harbor wild-type p53. The results of the profiling of DU-145 and PC-3 support the notion that an intact PTEN/AKT pathway (as found in DU-145 and 22RV1 cells) and the presence of active p38 are responsible for the high sensitivity to apoptosis induction and that neither the androgen receptor nor the p53 status is of primary importance for the differences observed with respect to apoptosis induction.

Introduction

Traditionally, tumors are classified according to the site of their appearance, e.g. prostate, breast, colon etc. The individual neoplasms are further classified with respect to stage and grade. The grading of a tumor, in particular, is highly dependent on the subjective evaluation of the pathologist, using mostly immunohistochemical methods where numerous markers are applied for measuring proliferation, differentiation and apoptosis in tissue slices. However, a systematic profiling concept, which would take into account signaling pathway networks, is not commonly used.

Since the major known signaling pathways, which control proliferation, cell cycle, apoptosis etc., apply to virtually all cells, it could be possible in the future to classify tumors not necessarily according to the site of occurrence but according to the alterations they exhibit in their signaling network. Hence, early neoplastic stages exhibiting identical alterations could be used as first indications of a new malignant development. One example is the phosphorylation status of protein kinase CHK2 at Thr68 by ATM kinase (1,2). In a large selection of bladder carcinomas at different degrees of differentiation and lung hyperplasias, the authors were able to show that CHK2 was already phosphorylated at Thr68 in pre-malignant neoplasias. This observation prompted us to set up a profiling approach in order to answer the question of whether there are basic alterations in various signaling molecules common to all tumors. Moreover, such investigations may be helpful under transfection experiments when looking for the specific effect of a chosen protein. Cell lines, although derived from the same type of tumor, may express proteins at different levels and/or modification status and, hence, the results obtained will differ accordingly. In order to reduce the many controversial data found in the literature, it would be helpful to know more about the basic signaling network of a specific cell line than only a few parameters such as androgen receptor or p53 status.

Tumors of the same origin respond differently to chemotherapy and radiation but, in most cases, they develop resistance to the applied treatment schemes. Hence, it is desirable to obtain information on their signaling status prior to therapeutic treatment. By using such an approach, it may be possible to explain why some tumors are more prone to therapeutic treatment than others.

Although cisplatin treatment of prostate cancer is only efficient in ~19% of patients (3), we have chosen this established drug for our investigations foremost to induce apoptosis and not because of any clinical relevance in prostate cancer treatment. However, new platinum analogs show promising results in treating prostate cancer (4).

Initially, we investigated established signaling pathways in four selected prostate carcinoma cell lines (Table I) in order to discover how they would differ with respect to the expression and modification status of key signaling molecules and in their response towards chemotherapeutic compounds.

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Key words: signaling molecules, prostate cancer, apoptosis, cisplatin

Table I. Overview of analyzed human prostate carcinoma cell lines.

Cell line	p53 status	AR status	Morphology	Origin
DU-145	Mutated	-	Epitheloid	Brain metastasis
PC-3	Stop codon 169	-	Epitheloid	Bone metastasis
LNCaP	Wild type	+	Fibroblastoid	Lymph node metastasis
22RV1	Wild type (?)	+	Epitheloid	Primary, xenograft CWR22R-2152

The table contains data about the four prostate carcinoma cell lines, LNCaP, DU-145, PC-3 and 22RV1, with respect to the p53 and androgen-receptor (AR) status as well as cell morphology and origin of the cell lines.

We were aware that the tumor cell lines may have undergone changes and, therefore, behave differently to the tumor material they are derived from. However, one may also argue that if a series of tumors were classified to be of the same type, they should either have retained or lost some of their original characteristics at the molecular level. If not, one would have to assume that the original primary tumors did not exhibit the same alterations at the molecular level, despite the application of the same classification criteria.

Prostate cancer is genetically and phenotypically heterogenous. This may be a consequence of mutations of different cell types (basal stem, transit amplifying or luminal cells) resulting in different malignant maturation pathways. One consistent characterization of these cancers, however, is their eventual progression to a hormonal refractory state. In order to check for the influence of a functional androgen receptor (AR) versus lack of AR, we have selected four established prostate cell lines, LNCaP/22RV1 (AR positive) and DU-145/PC-3 (AR negative, i.e. lacking the AR). The selected cell lines also vary with respect to their p53 status. The AR-positive cell lines harbor wild-type p53, whereas the AR-negative cell lines exhibit non-functional p53. It was possible to distinguish two groups of cell lines: i) those which respond to relatively low amounts of cisplatin and ii) those which show signs of apoptosis at higher drug concentrations. By applying the profiling strategy, we aimed to answer the question of why some tumor cell lines are more sensitive towards cisplatin treatment than others. Our results show that neither the AR nor the p53 status can explain the observed cisplatin sensitivity in the four prostate cell lines investigated. Moreover, we have found that the MAP kinase and PTEN/AKT signaling pathways are of central importance for a particular tumor cell line to undergo apoptosis readily or not.

Materials and methods

Materials. The human carcinoma cell lines, LNCaP, DU-145, PC-3 and 22RV1, were purchased from DSMZ. All materials for cell culturing are from Gibco BRL, Life Technologies. Okadaic acid and protease inhibitor cocktail (Complete) were purchased from Roche; cisplatin, casein and trypan blue dye were from Sigma; polyvinylidene difluoride (PVDF) membranes were from Bio-Rad Laboratories; chemiluminescence reagent, CDP-starTM, was from Tropix, Applied Biosciences; and [γ-^{32}P]-ATP was from Hartmann Analytic, Braunschweig. The following antibodies were used: ß-actin (A5441; 1:10,000), purchased from Sigma; AKT1 (610860; 1:500), GSK3ß (610201; 1:2,500), and PKCα (610107; 1:1,000) from BD Biosciences; AKT2 (sc-5270; 1:100), BCL-2 (sc-7382; 1:100), Cyclin D1 (sc-718; 1:200), Cyclin E (sc-198; 1:200), JNK2 (sc-7345; 1:100), MDM2 (sc-965; 1 μg/ml), MEK1 (sc-6250; 1:200), PKA Cα (sc-903; 1:500), PKC (sc-17769; 1:200), PKCδ (sc-937; 1:400), PKCζ (sc-17781; 1:100), PSA (sc-7316; 1:50), p38α (sc-7149; 1:100), and c-SRC (sc-18; 1:200) from Santa Cruz; phospho-Thr308-AKT (9275; 1:500), androgen receptor (3202; 1:1,000), Caspase-3 (9668; 1:1,000), Caspase-9 (9508; 1:1,000), phospho-Thr68-CHK2 (2661; 1:1,000), ERK1/2 (9102; 1:1,000), phospho-Thr202/Tyr204-ERK1/2 (4377; 1:1,000), phospho-Ser9-GSK3ß (9336; 1:1,000), MKK4 (9152; 1:1,000), phospho-ßIISer660-PKC (9371; 1:1,000), phospho-Thr180/Tyr182-p38α (9211; 1:500) from Cell Signaling; phospho-Ser473-AKT (06-801; 2 μg/ml), CHK2 (05-649; 1 μg/ml), PTEN (07-016; 1:500) from Upstate; p53 (OP09; 3 μg/ml), CK2α (218703; 2 μg/ml) and CK2ß (218712; 1 μg/ml) from Calbiochem; and PARP (6639GR; 1:1,400) from Pharmingen or the non-commercial primary antibody, CK2α', raised in rabbits against the peptide ^{334}SQPC ADNAVLSSGTAAR^{305} from human CK2α', (the order number and dilution used for detection on immunoblots are shown in brackets). We applied the nomenclature for the MAP kinases most commonly used but would like to add the approved HUGO gene symbols: p38/SAPK2a (MAPK14); JNK1 (MAPK8); JNK2 (MAPK9); ERK1 (MAPK3); and ERK2 (MAPK1). The following secondary antibodies were applied: alkaline phosphatase conjugated goat anti-mouse IgG (1:20,000), goat anti-rabbit IgG (1:40,000), rabbit anti-goat IgG (1:5,000) or rabbit anti-sheep IgG (1:20,000) (Jackson ImmunoResearch Laboratories).

Cell culture. LNCaP, DU-145, PC-3 and 22RV1 were maintained as monolayers in RMPI-1640 supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin. The cells were cultured in a humidified incubator at 37˚C and 5% CO_2. At ~80-90% confluence, the cells were subcultured using 0.05% trypsin/EDTA.

Preparation of cell extracts. The media was changed 1-2 days before harvesting the cells. The cells were harvested at ~80% confluence. The cell suspensions were centrifuged at 725 xg

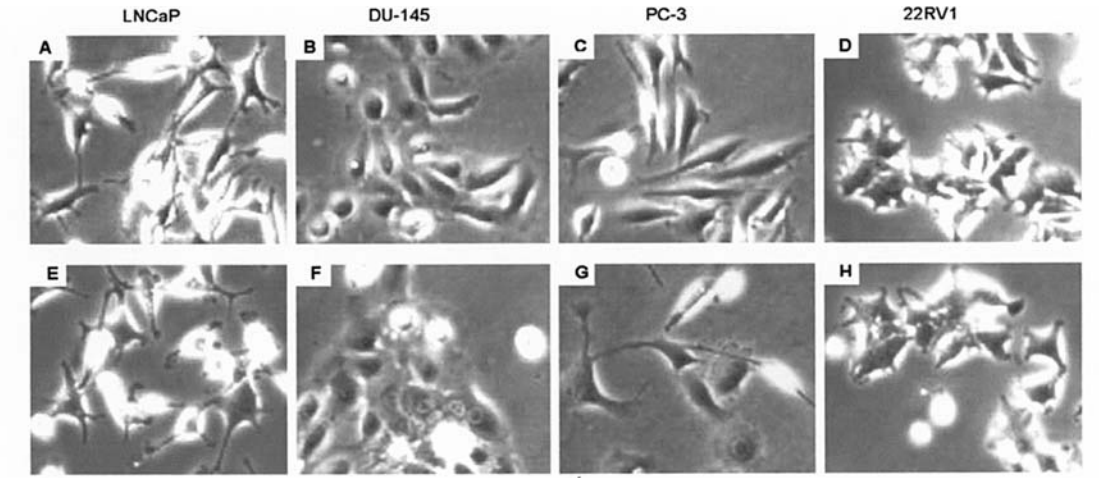

Figure 1. Effect of cisplatin on cell morphology. Phase contrast microscopy of untreated cells (A-D) and cisplatin-treated cells (E-H). For induction of apoptosis, LNCaP (A and E), DU-145 (B and F), PC-3 (C and G) and 22RV1 (D and H) cells were cultured for 24 h in the presence of 40-, 10-, 40- and 2-μM cisplatin, respectively. Original magnification, x100.

for 5 min at 4˚C. Lysis of the cells was performed by incubation on ice for 20 min in ice-cold lysis buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1% Triton-X-100, 10% glycerol, 1 mM DTT, 30 mM Na_4PP_i, 10 mM NaF, 1 mM activated Na_3VO_4, 1 x protease inhibitor cocktail with EDTA). The cell lysates used for detection of phospho-AKT and AKT activity were made using lysis buffer supplemented with 100 nM okadaic acid; lysis was performed by adding the lysis buffer directly to the cells in the cell culture dishes. The lysates were centrifuged for 20 min at 17,860 xg and 4˚C. The supernatant, i.e. the cell lysate, was used for further experiments.

Western blotting. Cell lysates (100 μg) were electrophoresed on 12.5% sodium-dodecyl-sulphate (SDS)-polyacrylamide gels and transferred to PVDF membranes by wet electroblotting at 350 mA for 1 h. The membranes were blocked for 1 h at room temperature or overnight at 4˚C in 0.2% (w/v) casein, 0.1% Tween-20 in PBS. Membranes were incubated for 1 h or overnight with the primary antibodies, and for 1 h with the appropriate secondary antibodies. Antibody-antigen complexes were detected using the chemiluminescence reagent, CDP-star™ . Detection of ß-actin was performed as a loading control.

AKT activity test. AKT kinase activity was measured in a total volume of 40 μl containing 10 μg of cell lysate, 10 mM Tris-HCl pH 7.5, 12.5 mM ß-glycerol phosphate, 2.5 mM EGTA, 0.5 mM Na_3VO_4, 0.5 mM DTT, 18.75 μM $MgCl_2$, 125 μM ATP, and 2.5 μCi [γ-^{32}P]-ATP (3,000 Ci/mmol). The AKT peptide, RPRAATF, was used at a final concentration of 100 μM as kinase substrate . The samples were mixed on ice and made in triplicate. Protein phosphorylation was performed by incubation for 10 min at 30˚C. The samples were cooled on ice for 3 min. Of each sample, 20 μl was spotted onto P81 paper. The paper was washed three times for 5 min in 0.75% phosphoric acid and once in acetone. Incorporation of radioactive-labelled phosphate in proteins was measured by counting the samples in 3 ml scintillation cocktail in a liquid scintillation counter.

Induction of apoptosis. The cells were seeded at 40-50% confluence 24 h prior to the induction of apoptosis. Apoptosis was induced by the addition of different amounts of cisplatin in fresh media. As a control, some cells were given fresh media. The cells were harvested 24 h later as described above. For analysis of the level of phosphorylated AKT in the cisplatin-treated cells, the cells were treated with 100 nM okadaic acid for 1 h prior to harvesting. The cells were then lysed using lysis buffer without okadaic acid. Of cell lysates from cisplatin-treated DU-145 and PC-3, 100 μg was applied to SDS-PAGE and Western blot analysis to determine the expression level of the chosen proteins. For detection of the 116-kDa full-length poly-adenosine ribose polymerase (PARP) and the 85-kDa cleavage product of PARP, 50 μg of cell lysates were applied.

Trypan blue dye exclusion assay. At ~80% confluence, the cells were trypsinized and harvested by centrifugation. The cells were resuspended in a small volume of medium and, after counting in a Neubauer hemocytometer, they were diluted in PBS to 3x10^6 cells/ml. Of the suspensions, 10 μl was mixed with 10 μl of 0.4% trypan blue solution and incubated at 37˚C for 4 min. Using a Neubauer hemocytometer, at least 500 cells were counted. The proportion of blue cells vs. total cells revealed the percentage of dead cells.

Results and discussion

Description of cell lines. Table I shows a short description of the four cell lines used under the present investigations. We focused on published information concerning morphology, androgen sensitivity, p53 status, and the tissue from where the cell lines were derived.

DU-145 and PC-3 cells are AR negative (no mRNA, no protein) (5). Additionally, they have a non-functional p53 in common and are both derived from metastases, either from brain or bone respectively. In contrast to androgen-positive cells, AR-negative cell lines produce bFGF and readily form tumors in athymic mice (6).

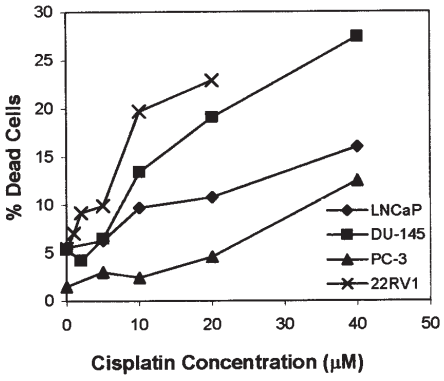

Figure 2. Trypan blue staining of cisplatin-treated cells. Trypan blue dye exclusion test was performed for LNCaP, DU-145, PC-3 and 22RV1 to evaluate the effect of 24 h of cisplatin treatment on cell viability. The percentage of dead cells is calculated as the number of blue cells in the total number of cells.

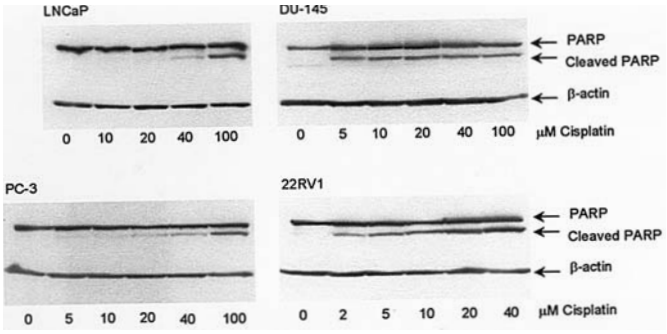

Figure 3. Effect of cisplatin treatment on PARP-cleavage. LNCaP, DU-145, PC-3 and 22RV1 were treated with varying concentrations of cisplatin for 24 h as indicated. After harvesting, 50 μg of cell lysates were used for Western blot analysis for detection of full-length PARP (116 kDa) and its cleavage product (85 kDa). ß-actin was used as loading control.

The LNCaP cell line is the most frequently used *in vitro* model for androgen-dependent tumors, partly based on the fact that LNCaP cells do not grow in castrated animals. However, the LNCaP cell line was established from a hormone-refractory patient and contains a mutation in the AR gene. This mutation creates a promiscuous AR that can bind to other steroids, which makes it questionable that this cell line is truly androgen-dependent (5).

The other AR-positive cell line, 22RV1, exhibits a 114-kDa protein (instead of the 110-kDa which is usually found for AR) due to a duplication of exon 3. An additional 80-kDa protein was also described (7-9). 22RV1 is androgen-responsive, yet androgens are not required for growth, although the cell line shows growth response in their presence. Both cell lines express wild-type p53.

Cell viability during induction of apoptosis. We first aimed to determine the optimum cisplatin concentration needed to induce apoptosis without severely affecting the viability of the cells. Fig. 1 shows the effect of cisplatin on the cellular morphology of the four analyzed prostate carcinoma cell lines. LNCaP cells exhibit fibroblastoid morphology (Fig. 1A), whereas DU-145, PC-3 and 22RV1 cells are epitheloid cells (Fig. 1B, C and D). Fig. 1 shows that cisplatin treatment causes some of the cells to detach from the culture dishes, resulting in an increase in the number of cells floating around in the medium (Fig. 1A-H). Almost the same numbers of cells were found in the medium when 40-μM cisplatin was applied for LNCaP and PC-3, and 10- or 2-μM was applied for DU-145 and 22RV1 respectively. The treatments resulted in a change in the cellular shape (DU-145 and PC-3; Fig. 1F and G). The morphological alterations are clearly seen for some of the PC-3 cells (Fig. 1C and G), which change from an elongated to a more rounded form. This is the first sign of the detachment accompanied by induction of apoptosis.

Trypan blue dye exclusion tests have been performed to measure the number of viable and dead cells in the cell cultures. Fig. 2 shows viability expressed as percentage of dead cells after 24 h of treatment with increasing amounts of cisplatin. PC-3 cells were the least affected, whereas 22RV1 cells were the most affected. Considering the influence of 20-μM cisplatin, an ~5-fold difference exists between these two cell lines with respect to cisplatin tolerance. We divided the four cell lines into two groups, i.e. PC-3 and LNCaP cells as the less sensitive and DU-145 and 22RV1 cells as the more sensitive with respect to cell viability.

Determination of the minimum required cisplatin concentration to induce apoptosis. Concurrently, we investigated the effect of cisplatin on apoptosis induction. We applied the PARP cleavage test to find the minimum cisplatin dosage leading to PARP cleavage (Fig. 3). LNCaP and PC-3 cells, which have been shown to be least sensitive to cisplatin with respect to cell viability, also require much higher dosages of cisplatin to undergo apoptosis. In the case of LNCaP, the first sign of a cleavage product can be seen at 40 μM, whereas it is well visible at a cisplatin concentration of 100 μM. The same occurs with PC-3 cells. DU-145 and 22RV1 cells already exhibit a clear PARP cleavage signal at 5-μM and 2-μM cisplatin, respectively. No significant PARP cleavage was detectable at cisplatin concentrations below 5 μM for DU-145 (results not shown). Hence, there is a 20- and 50-fold difference in sensitivity towards apoptosis induction between DU-145 and 22RV1 cells in comparison to LNCaP and PC-3 cells respectively. These results are in line with the observed effects on cell viability after cisplatin treatment (Fig. 2).

Profiling of LNCaP, PC-3, DU-145 and 22RV1 cell lines. We have used all four prostate carcinoma cell lines and 37 different antibodies to screen for signaling molecules and their phosphorylation status where appropriate. The chosen set of signaling molecules is representative for known key pathways. We are aware that this is a biased selection and many more molecules should have been profiled; however, this profiling of signaling molecules in order to look for general principles governing the response of various tumors towards known chemotherapeutic agents is a new and tentative approach. These investigations should shed light on the naive and yet important question of why some tumors are more sensitive towards chemotherapy than others.

Fig. 4 shows immunoblots using the chosen antibodies. In all experiments, we used ß-actin as a loading marker. Some molecules, such as the AR and p53, were used as confirmatory

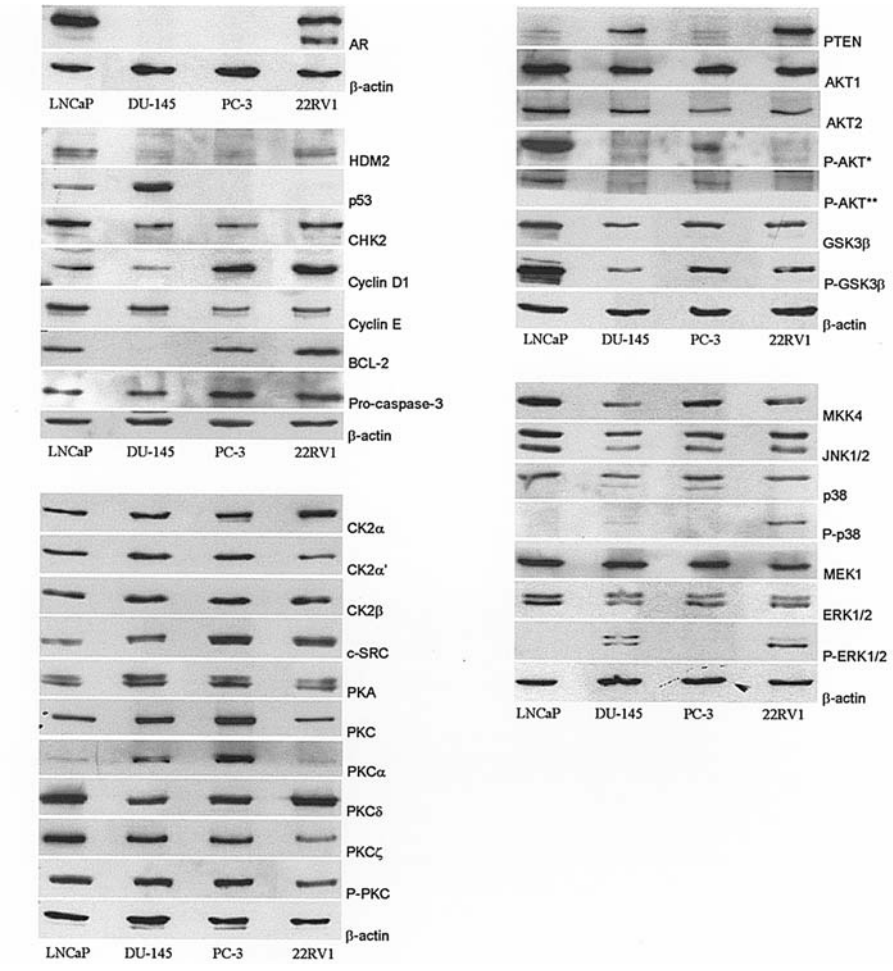

Figure 4. Protein analysis of human prostate cancer cell lines. Primary antibodies directed against the proteins under investigation were applied for Western blot analysis. Cell lysates (100 μg) from LNCaP, DU-145, PC-3 and 22RV1 were used. The phosphorylation status of the following proteins was analyzed: P-p53, phospho-Ser392-p53; P-PKC, phospho-ßIISer660-PKC; P-AKT*, phospho-Thr308-AKT; P-AKT**, phospho-Ser473-AKT; P-GSK3ß, phospho-Ser9-GSK3ß; P-p38, phospho-Thr180/Tyr182-p38; P-ERK1/2, phospho-Thr202/Tyr204-ERK1/2.

molecules because several groups have independently established their status for the investigated cell lines.

Our analyses (Fig. 4, Table II) confirm earlier results (8) describing a slightly stronger AR expression in LNCaP compared to 22RV1 cells. DU-145 and PC-3 cells both lack an AR. The additional signal (lower band) detected by the antibody directed against the AR in 22RV1 cells is the cleavage product of the AR which has been previously described for this cell line (7-9). LNCaP and 22RV1 cells are both known to express wild-type p53; therefore, owing to the short half-life of p53, one would not expect to detect a strong signal on the immunoblots. In fact, the signal from p53 in the 22RV1 cells is not visible in Fig.4. When p53 is mutated, it is stabilized and correspondingly exhibits a much stronger signal on the immunoblot than that seen for the DU-145 cell line. In the case of PC-3 cells, no p53 signal is seen due to a stop codon at position 169 (10).

Recent studies indicate that p53 abnormalities occur in ~30% of primary prostate cancer (11,12). Specific p53 mutations contribute to cancer progression and p53 mutations in the primary tumor may be predictive of metastases to distinct sites (13). Others have reported a correlation between the AR and p53 status and sensitivity towards cisplatin. Mujoo *et al* (4) investigated the efficacy of cisplatin treatment on various prostate cancer cell lines and found that cisplatin was substantially more effective against the prostate cancer cell line, LNCaP (wild-type p53), than towards DU-145 and PC-3 cells (inactive p53). Our results (Fig. 2) indicate that p53 status may not be the major responsible factor for the difference in cisplatin sensitivity. The signal intensity of the PARP cleavage product in LNCaP cells obtained with 100-μM cisplatin, is comparable to the PARP cleavage signal at 20-μM cisplatin in 22RV1 cells. This is an ~5-fold difference, although both cell lines are AR positive and exhibit wild-type p53. Additionally, DU-145 and PC-3 cells harbor inactive p53, yet they differ by a factor of five in their sensitivity towards cisplatin, as measured by the trypan blue dye exclusion test (Fig. 2). Using the PARP test as a direct measure of apoptosis an ~20-fold higher cisplatin concentration is needed in the case of PC-3 cells in order to obtain a PARP cleavage product of the same intensity as that in DU-145. Both cell lines are AR negative and harbor inactive p53. Cemazar *et al* (14), who investigated the effects of 'electrogenetherapy' combined with cisplatin on survival of human tumor cell lines with different p53 status, found that cisplatin sensitivity of the cell lines (among them DU-145 and PC-3 cells) was independent of the p53 status. This is in agreement with our observations. Since LNCaP and 22RV1 are AR-positive cells (DU-145 and

Table II. Evaluation of the relative intensities of the investigated proteins in human prostate cancer cell lines.

	LNCaP	DU-145	PC-3	22RV1
AR	++	-	-	+
HDM2	++	+	+	++
p53	++	+++	-	+
CHK2	+++	+	+	++
Cyclin D1	+	+	+++	+++
Cyclin E	+++	++	+	++
BCL-2	++	+	++	+++
Pro-caspase-3	+	+	++	++
CK2α	+	+	+	+
CK2α'	+	+	+	+
CK2ß	+	+	+	+
c-SRC	+	++	+++	+++
PKA	+	+	+	+
PKC	+	++	+++	+
PKCα	+	++	+++	+
PKCδ	+++	+	++	+++
PKCζ	+++	++	++	+
P-PKC	++	++	++	+
PTEN	-	+	-	++
AKT1	++	+	+	+
AKT2	++	+	+	+
P-AKT*	++	-	+	-
P-AKT**	++	-	+	-
GSK3ß	+++	+	++	++
P-GSK3ß	+++	+	++	++
MKK4	+++	+	++	+
JNK1	+++	+	++	++
JNK2	+++	+	++	++
p38	+	+	+	+
P-p38	-	-	-	+
MEK1	+	+	+	+
ERK1	++	+	+	+
ERK2	++	+	+	+
P-ERK1	-	++	-	+
P-ERK2	-	+	-	++

Signal intensities from Western blot analysis (Fig. 4) are evaluated. Signals for phosphorylated proteins reflect the phosphorylation status of the following residues: P-p53, phospho-Ser392-p53; P-PKC, phospho-ßIISer660-PKC; P-AKT*, phospho-Thr308-AKT; P-AKT**, phospho-Ser473-AKT; P-GSK3ß, phospho-Ser9-GSK3ß; P-p38, phospho-Thr180/Tyr182-p38; P-ERK1/2, phospho-Thr202/Tyr204-ERK1/2.

PC-3 do not express AR), the observed difference in cisplatin sensitivity cannot be assigned to the androgen-receptor status either.

HDM2 can be detected in all four cell lines; however, it is mostly expressed in LNCaP and 22RV1 cells, the two cell lines which harbor wild-type p53 (Fig. 4, Table II). In the case of DU-145 and PC-3 cells, both of which do not harbor active p53, the HDM2 signal is barely visible, supporting the notion of a down-regulation, if p53 is absent in a wild-type form. Zhang *et al* (15) showed that knock-down of HDM2 by antisense oligonucleotide (ASO) treatment in LNCaP cells, leads to an expected concomitant raise of the p53 level, whereas it had no effect on p53 expression or stability in DU-145 cells. From our data, we conclude that, in cells with non-functional p53, the presence of HDM2 is not required, at least not at a high level. By contrast, cells with active p53, e.g. LNCaP and 22RV1, express HDM2.

We detected CHK2 in all four cell lines and found a higher expression in p53 wild-type cells (Fig. 4, Table II). Phospho-Thr68 of CHK2, the target site of ATM kinase, was detected only as a faint signal (data not shown), supporting the notion that CHK2 is present in all four cell lines, predominantly in the inactive form. Whether the presence of higher amounts of inactive CHK2 is of any importance in undamaged cells is unknown. However, it is possible that, when damage occurs, the presence of more ATM target molecules may enforce the signaling to downstream molecules of CHK2 such as p53.

Two cyclins were profiled, Cyclin D1 and Cyclin E. Whereas the latter did not differ significantly between the four cell lines, Cyclin D1 was remarkably well expressed in PC-3 and 22RV1 cells (Fig. 4, Table II). This result is interesting with respect to recent findings that the cyclins are not strictly essential for cell-cycle progression; however, it is possible that their overexpression in some cells may have consequences for growth control (16). The importance of overexpression of Cyclin D1 cannot be assessed using our data. Since knockouts of all cyclins showed that they are not strictly essential for cell-cycle progression, one could argue that cells harboring a lower number of these molecules will not exhibit a behavior towards apoptosis-inducing agents which is different to those with higher expression levels.

BCL-2 is overexpressed in 50-70% of solid organ malignancies, including prostate cancer, enhances tumorigenic and metastatic capability, and decreases the efficacy of ionizing radiation and chemotherapies (17). Fig. 4 shows that BCL-2 is equally expressed in LNCaP and PC-3 and exhibits a higher level in 22RV1 cells. After extended exposure, BCL-2 was faintly detectable in DU-145 cells (results not shown). When the responses of LNCaP, PC-3 and DU-145 to apoptosis induction (Fig. 3) are compared, our results fit the described relationship between high expression of BCL-2 and reduced responsiveness towards chemotherapeutic drugs. However, since 22RV1 cells, which also show a marked expression of BCL-2 (Fig.4, Table II), are readily entering apoptosis upon cisplatin treatment, factors other than the BCL-2 status may be responsible for the sensitivity towards cisplatin. One explanation may be the origin of the cells. Whereas LNCaP, PC-3 and DU-145 cells are derived from metastases, (Table I) 22RV1 cells are derived from a human prostate carcinoma xenograft from mice. Fahy *et al* (18), who investigated cell lines from pancreas, prostate, lung and breast, showed that BCL-2 levels varied, both between and within tumor types. With respect to the analyzed prostate cell lines, their data showed that BCL-2 levels are higher in LNCaP and lower in PC-3 cells; however, these results were not confirmed by our investigations.

Pro-caspase-3 was used as an additional marker for apoptosis induction where the reduction of the pro-caspase and the formation of the cleavage product were applied as indicators of apoptosis. All four cell lines showed the presence of pro-caspase-3 (Fig. 4, Table II) but no cleavage product (results not shown).

We further checked the expression levels of the subunits of protein kinase CK2. No significant differences were detected among the four cell lines (Fig. 4, Table II).

The expression level of c-SRC was highest in PC-3 and 22RV1 cells, medium in DU-145 and lowest in LNCaP cells (Fig. 4, Table II). There was no correlation between the expression levels and the different apoptotic responses.

PKA was shown to activate the AR (19). We did not detect any differences in PKA levels within the four cell lines (Fig. 4, Table II) including another subtype of PKA (shown as a second band on the immunoblot). This is not surprising, since we detected the catalytic subunit. Under physiological conditions, PKA is present as an inactive tetramer, i.e. the cAMP-dependent holoenzyme, which upon appropriate modulators, such as forskolin, leads to a PKA-catalyzed phosphorylation of AR (19). However, such a scenario was not detected by the applied techniques; thus, the activity of PKA may be different in the four cell lines, although the expression level was the same.

We looked for the expression of PKC using an antibody which is directed against a domain common to all PKC isoenzymes and, therefore, detects all PKC species (a so-called pan antibody). In addition, we used antibodies directed against individual isoenzymes of PKC, such as PKCα, a cPKC exhibiting a full functional regulatory domain requiring DAG and Ca^{2+} binding for full catalytic activity; PKCδ, an nPKC which has lost the capability to respond to alterations in Ca^{2+} levels; and PKCζ, an aPKC which is DAG and Ca^{2+} independent. High PKC expression is detected in DU-145 and PC-3 cells (Fig. 4, Table II), which may be due mainly to PKCα because only this particular PKC isoenzyme is significantly expressed in these two cell lines. LNCaP and 22RV1 are virtually devoid of a PKCα signal. The other two representatives of the PKC family, PKCδ and PKCζ, are expressed in all four cell lines. PKCδ is detected at high levels in LNCaP and 22RV1, whereas the signal of PKCζ is strongest in LNCaP, medium in DU-145 and PC-3 and lowest in 22RV1 (Fig. 4, Table II). The antibody detecting phospho-Ser660 of PKC is directed against this residue in PKCß; however, it recognizes only the corresponding serines from cPKCs and nPKCs and not aPKCs because the latter contain a glutamic acid residue instead (20). Phosphorylated PKC is detected less in 22RV1 than in the other cell lines (Fig. 4, Table II). Three phosphorylation events (two for aPKCs) are responsible for activation of PKCs (20). It has previously been shown that activation of PKCα and PKCδ in LNCaP is involved in induction of apoptosis upon treatment of cells with phorbol esters or diacylglycerol-lactones. This indicates that PKCα and PKCδ play pro-apoptotic roles in prostate cells (21-23). In disagreement with this, the ribozyme-mediated reduction in the PKCα level sensitizes the androgen-independent DU-145 and PC-3 cells to cisplatin-induced apoptosis (24). However, these functions of PKC isoenzymes cannot be confirmed by our results as there is no correlation between the expression level of these kinases and cisplatin-induced apoptosis.

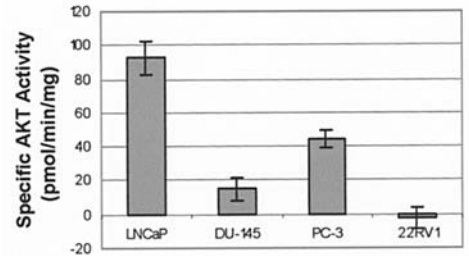

Figure 5. AKT activity test of human prostate cancer cell lines. AKT kinase activity towards AKT peptide, RPRAATF, was tested using 5 μg of cell lysates from LNCaP, DU-145, PC-3 and 22RV1. The activities are averages of three replicates. Standard deviations are shown. For 22RV1 cells, both very low positive and small negative activity values were obtained indicating that there is no significant basal AKT activity in this cell line.

The four analyzed prostate carcinoma cell lines express AKT1 and AKT2; however, higher expression levels of these proteins are observed in LNCaP cells (Fig. 4, Table II). LNCaP and PC-3 cells have been reported to be PTEN negative due to mutations in the PTEN gene (25) resulting in constitutive activation of AKT. These results are confirmed by our own investigations (Fig. 4, Table II) where constitutive phosphorylation of AKT at Thr308 and Ser473 was seen in PC-3 and LNCaP cells. The latter cell line exhibited a stronger AKT-phosphorylation in comparison to PC-3 cells (Fig. 4, Table II). LNCaP and PC-3 cells were the least responsive to cisplatin treatment (Fig. 3). Numerous reports show a correlation between AKT activation and the AR. Activated AKT co-immunoprecipates with the AR, suggesting that the endogenous AR is associated with activated AKT *in vivo* (26). However, other analyses dispute these results. Using biochemical and functional approaches, Sharma *et al* (27) did not detect physical protein-protein interaction between AKT and the AR or *in vitro* phosphorylation of the AR by AKT. Irrespective of the AR status, only those cells which harbor a functional PTEN, i.e. DU-145 and 22RV1 cells, enter apoptosis readily. Therefore, we believe that the androgen status may not play a central role in determining a cell's fate with respect to drug-induced apoptosis. A downstream target of AKT is GSK3ß, which is expressed at equal levels in all four cell lines. Of interest is the phosphorylation of GSK3ß at Ser9, which is catalyzed by AKT. As expected, the phosphorylation of Ser9 is highest in cells with a non-functional PTEN (LNCaP and PC-3) and least in DU-145 and 22RV1 cells (Fig. 4, Table II). AKT activity using a specific peptide substrate harboring the AKT consensus sequence was determined in all four cell lines in order to confirm the results obtained by immunodetection of AKT (Fig. 5). The highest AKT activities were measured in LNCaP and PC-3 cells. A minor activity close to the detection limit of the method was found in DU-145 cells and virtually no activity was found in 22RV1 cells where the detection level was so low that it led to negative measurements in some of the triplicates. Given that immunodetection is only a semi-quantitative method, the data obtained from the two different methods are in good agreement.

AKT phosphorylation of GSK3ß has been shown to influence the WNT signaling pathway downstream of GSK3ß, especially ß-catenin. Although ß-catenin action has been

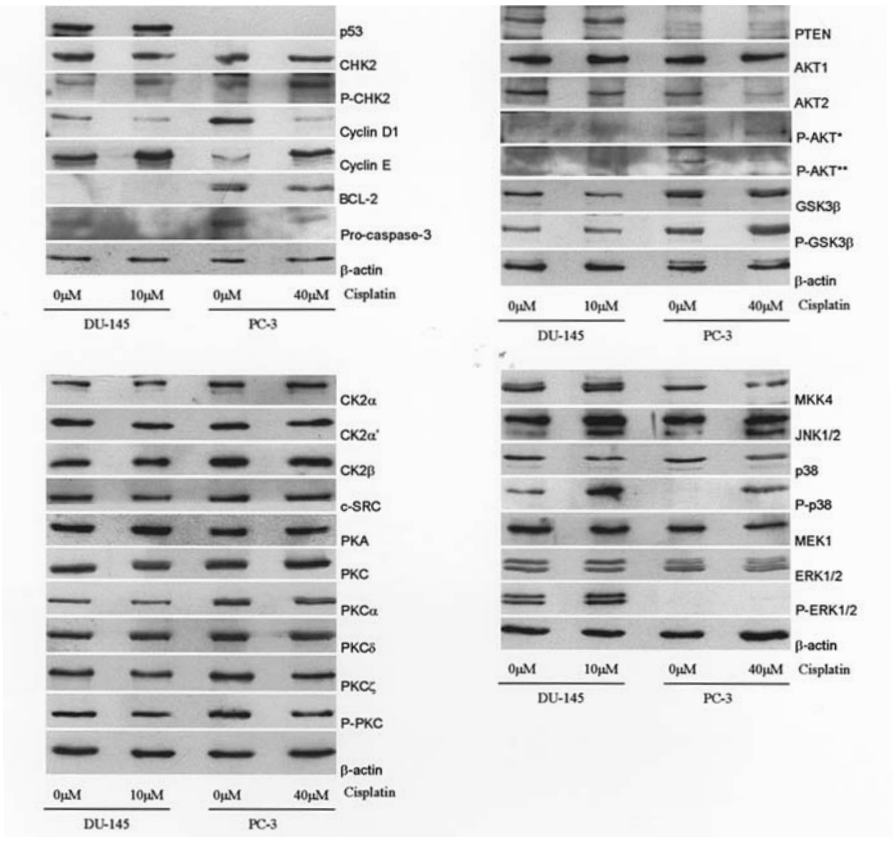

Figure 6. Protein analysis of cisplatin-treated human prostate cancer cell lines. DU-145 and PC-3 cells were treated for 24 h with or without cisplatin. Cell lysates (100 μg) from the treated cells were applied for Western blot analysis of the proteins under investigation. Detection of the phosphorylation status of the following residues was performed: P-CHK2, phospho-Thr68-CHK2; P-PKC, phospho-ßIISer660-PKC; P-AKT*, phospho-Thr308-AKT; P-AKT**, phospho-Ser473-AKT; P-GSK3ß, phospho-Ser9-GSK3ß; P-p38, phospho-Thr180/Tyr182-p38; P-ERK1/2, phospho-Thr202/Tyr204-ERK1/2.

implicated in tumorigenesis via its interaction with the Tcf/LEF transcription factors (28), such an effect was not detected in prostate cancer cells, despite the expression of TcF/LEF (29). Sharma *et al* (27) tried to connect WNT signaling with the PI3K/AKT pathway: PTEN phosphorylates and inactivates GSK3ß via PI3K/AKT; GSK3ß-dependent inactivation of cytoplasmic ß-catenin is attenuated; ß-catenin shuttles to the nucleus and augments ligand-stimulated transcription by the AR. Despite the convincing evidence linking ß-catenin to androgen signaling we have to consider our results obtained with PC-3 cells. As PC-3 cells are AR negative but express non-functional PTEN, leading to inactivation of GSK3ß (Fig. 4), an increased level of ß-catenin must target factors other than AR and Tcf/LEF; possibly NFκB, since the AKT pathway has also been shown to activate NFκB and ß-catenin (30).

We conclude that the PTEN/AKT status of a prostate cell line would be a suitable marker for predicting whether cells will enter apoptosis readily or not. Therefore, impairment of this pathway (e.g. by mutation of PTEN) could be responsible for the relative resistance towards cisplatin. By contrast, a functional PTEN/AKT would allow entry into apoptosis at considerably lower dosages of the drug.

Three major mammalian MAP kinase sub-families have been described: the extracellular-regulated kinase (ERK), the c-Jun N-terminal kinases (JNK) and the p38 MAP kinases. Each MAP kinase is activated through a specific phosphorylation cascade. The ERK pathway plays a major role in regulating cell growth and differentiation, being highly induced in response to growth factors, cytokines and phorbol esters (31-33). In contrast, JNK and p38 are only faintly activated by growth factors but are highly activated by a variety of stress signals (34-38).

High levels of MAP kinases were detected in recurrent tumors. Activation of MAP kinase pathways correlates with cancer progression in a variety of settings (39), e.g. activation of the ERK pathway increases the cell death threshold (40). Conversely, activation of the JNK and p38 cascades are generally, although not universally, associated with enhanced activation of the apoptotic program (41). Because of the central role of the MAP kinase signaling pathways, we have looked at the expression levels and the phosphorylation status of individual MAP kinases.

MKK4 and JNK1/2 showed different expression levels in the four cell lines. MKK4 showed the highest expression levels in LNCaP cells and PC-3 cells, whereas JNK1/2 was most highly expressed in LNCaP, less strong in PC-3 and 22RV1 cells and only marginal in DU-145 (Fig. 4, Table II).

With respect to p38 kinase, there was no significant difference between the expression levels of the four cell lines. Phosphorylation of p38 (Thr180/Tyr182) is detected in 22RV1 cells and to a minor extent in DU-145 cells. Thus, our experiments favor the existence of a correlation between the level of phosphorylated p38 (Fig. 4) and sensitivity towards

Table III. Evaluating relative signal intensities of proteins from cisplatin-treated human DU-145 and PC-3 prostate cancer cell lines.

	DU-145		PC-3	
	0 μM	10 μM	0 μM	40 μM
p53	+	+	-	-
CHK2	+	+	+	+
P-CHK2	+	+	+	++
Cyclin D1	++	+	+++	+
Cyclin E	++	+++	+	++
BCL-2	+	+	++	++
Pro-caspase-3	+	+	++	+
CK2α	+	+	+	+
CK2α'	+	+	+	+
CK2ß	+	+	+	+
c-SRC	+	+	+	+
PKA	++	++	+	+
PKC	+	+	+	+
PKCα	+	+	++	++
PKCδ	+	+	+	+
PKCζ	+	+	+	+
P-PKC	++	+	++	+
PTEN	+	+	-	-
AKT1	+	+	+	+
AKT2	+++	++	++	+
P-AKT*	-	-	+	+
P-AKT**	-	-	++	+
GSK3ß	+	+	++	++
P-GSK3ß	+	+	++	+++
MKK4	++	++	+	+
JNK1	+	++	+	++
JNK2	+	++	+	++
p38	+	+	+	+
P-p38	+	+++	-	++
MEK1	+	+	+	+
ERK1	++	++	+	+
ERK2	+	+	+	+
P-ERK1	+	++	-	-
P-ERK2	+	++	-	-

Signal intensities from Western blot analysis (Fig. 6) of the cisplatin-treated DU-145 and PC-3 cells are evaluated. Signals for phosphorylated proteins reflect the phosphorylation status of the following residues: P-CHK2, phospho-Thr68-CHK2; P-PKC, phospho-ßIISer660-PKC; P-AKT*, phospho-Thr308-AKT; P-AKT**, phospho-Ser473-AKT; P-GSK3ß, phospho-Ser9-GSK3ß; P-p38, phospho-Thr180/Tyr182-p38; P-ERK1/2, phospho-Thr202/Tyr204-ERK1/2.

cisplatin (Figs. 2 and 3). Data from Uzgare and Isaacs (42) show phospho-p38 detection in DU-145, but also in LNCaP and PC-3 cells. We do not have any explanations for these disagreements.

MEK1 expression levels were the same in all four cell lines. As in the case of JNK1/2, the expression levels of ERK1/2 are highest in LNCaP. Phosphorylation of ERK1/2 at Thr202 and Tyr204 was observed in DU-145 and 22RV1 cells. There was no phospho-ERK1/2 in LNCaP and PC-3 cells, which is in agreement with the findings of Fahy *et al* (18). However, Uzgare and Isaacs (42) detected phospho-ERK1/2 in LNCaP and PC-3 cells, although with a significantly lower expression level than in DU-145 and CWR22R cells. Thus, the general tendency of a low ERK1/2 phosphorylation in LNCaP cells in comparison to DU-145 and 22RV1 cells has been verified by these groups.

The ERK pathway is also associated with increased proliferation, as observed by the higher ERK1/2 expression in cancerous prostate tissue compared to normal prostate tissue (43). Others report only a slight upregulation of the level of ERK1/2 but a dramatic increase in the phosphorylation of ERK1/2 in prostate cancer compared to normal prostate (44,45). Analogous to p38, the cisplatin-sensitive cell lines, i.e. DU-145 and 22RV1, exhibit stronger phosphorylation of ERK1/2 (Fig. 4, Table II). The ERK1/2 phosphorylation in DU-145 and 22RV1 cells is indicative of the higher proliferation status and may, therefore, explain why they are more responsive to cisplatin.

Comparison of profiling results in DU-145 and PC-3 cells before and after cisplatin treatment. Two of the four profiled cell lines, DU-145 and PC-3, were used for a comparison with respect to cisplatin-induced alterations of the profiled molecules. Both cell lines are AR negative (simulating the hormonal refractory state of progressed prostate cancer) and harbor a non-functional p53 (as observed at the final stages of many tumors). Hence, a comparison of these prostate cell lines, which are both derived from prostate metastases, i.e. brain (DU-145) and bone (PC-3) respectively, might help to answer the question of why some cell lines enter apoptosis more readily than others, especially since these cell lines show marked differences in their responses towards cisplatin-induced apoptosis (Figs. 2 and 3).

Fig. 6 shows the profiling of the panel of signaling molecules in DU-145 and PC-3 cells in the absence and presence of cisplatin treatment. Cisplatin concentrations of 10 and 40 μM are applied for treatment of DU-145 and PC-3 cells respectively, since these concentrations yield comparable percentages of dead cells (~13% for both cell lines) (Fig. 2). The profile of the untreated DU-145 and PC-3 cells has already been shown in Fig. 4; however, when performing the profiling of cisplatin-treated DU-145 and PC-3 cells, we have run a second profile of untreated cells in parallel in order to directly compare the two cell lines.

Testing for CHK2 showed no alterations in the immunoblots (Fig. 6, Table III). Yet, when looking for Thr68 phosphorylation of CHK2, a clear increase is seen in PC-3 cells, supporting the notion that some cell lines (e.g. DU-145) may have already reached a threshold phosphorylation of this amino-acid residue and, therefore, undergo apoptosis more readily. Indeed, DU-145 is more prone to apoptosis induction by cisplatin than PC-3 cells by a factor of 20.

Of interest is the expression of the two cyclins. Cisplatin treatment leads to a reduction of the early G1-phase cyclin,

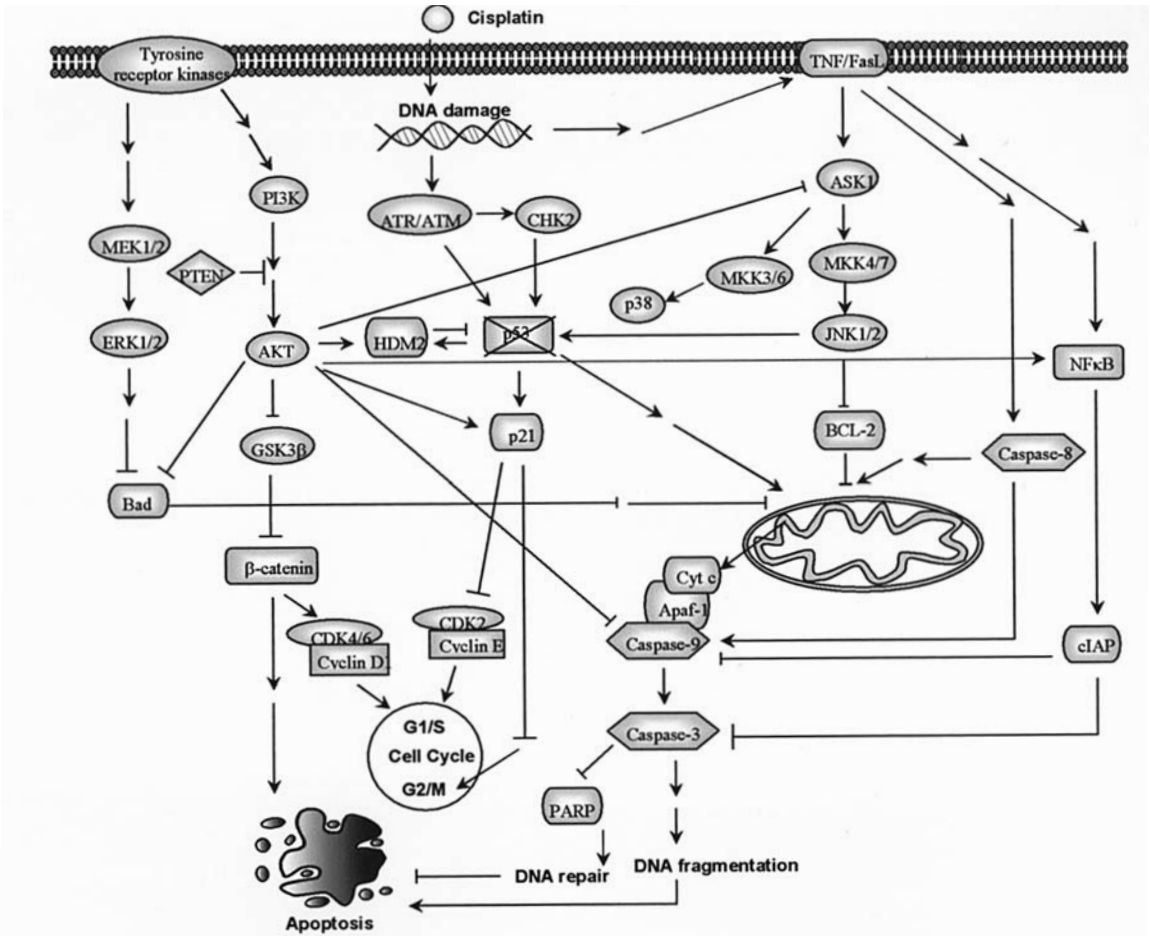

Figure 7. Overview of signaling pathways. Important signaling pathways related to apoptosis induction and regulation of cell-cycle progression have been included in the figure in a simplified version. The X placed over p53 indicates that this protein is non-functional in DU-145 and PC-3 cells.

D1, which is especially noticeable in the case of the PC-3 cells (Fig. 6, Table III). The opposite was observed for Cyclin E, a late G1-phase cyclin; cisplatin treatment led to a significant increase in signal intensity, which was most pronounced in PC-3 cells. Similar changes in Cyclin D1 and E expression were reported by Agner *et al* (46) and Fu *et al* (47), who induced apoptosis either in human osteocarcinoma cells or PC-3 cells using cisplatin or licochalcone-A respectively. Furthermore, Fu *et al* (47) also detected decreased Retinoblastoma protein (Rb) phosphorylation at Ser780 (target residue of CDK4/Cyclin D1) but unchanged phosphorylation at Ser721 (CDK2/Cyclin E target). This was accompanied by a reduction in the level of the E2F transcription factor associated with growth suppression. A similar mechanism may explain the effect of cisplatin on Cyclin D1 and E expression in DU-145 and PC-3 cells. Contradictory to our results, Li *et al* (48) report that 5-fluorouracil, but not cisplatin, treatment of oral cancer cells results in downregulation of Cyclin D1 and upregulation of Cyclin E.

A reduction was observed in the level of pro-caspase-3 expression (Fig. 6, Table III), which is consistent with enhanced cleavage and, thus, activation of this effector caspase upon execution of apoptosis.

No significant changes were seen for BCL-2, c-SRC and PKA expression following cisplatin treatment of the two cell lines (Fig. 6, Table III). CK2 can be considered a housekeeping protein and, hence, no differences are expected upon apoptosis induction (Fig. 6, Table III). However, it has been proposed that CK2 is regulated by association into inactive higher molecular mass structures or active free protomers (49). Thus, the CK2 protein level may not be the only determinant for CK2 activity.

In the case of PKC (pan detection) and the individual PKC isoenzymes (PKCα, PKCδ and PKCζ) no changes in the expression levels were observed; however, Ser660 phosphorylation of PKC was reduced in both cell lines after cisplatin treatment (Fig. 6, Table III). As this phosphorylation is involved in activation of cPKCs (e.g. PCKα) and nPKCs (e.g. PKCδ) (20), this implies that cisplatin treatment results in less activation of one or both of these PKC species. As mentioned earlier, PKCα and PKCδ have been reported to exhibit pro-apoptotic functions in prostate carcinoma cell lines (21-23); however, this is not supported by the reduced phosphorylation of these PKC species upon cisplatin treatment in DU-145 and PC-3 cells (Fig. 6).

The PTEN status was unaltered in the case of DU-145 (as mentioned earlier, PC-3 cells are negative for PTEN). The same was found for AKT1 and GSK3ß, while AKT2 levels were reduced in both cell lines (Fig. 6, Table III). Phosphorylation of AKT at Thr308 is unaltered following cisplatin induction, whereas a minor decrease is observed for AKT phosphorylation at Ser473. As AKT is a pro-survival signaling

kinase, the reduced activation level of AKT (indicated by reduced phosphorylation at Ser473) may reflect the growth-suppressing effect of cisplatin. A minor, albeit significant, induction of GSK3ß-Ser9 phosphorylation is seen in the case of PC-3 cells, when these cells are treated with 40-μM cisplatin (Fig. 6, Table III). This residue is not only a target of AKT but also of PKA (50,51) and integrin linked kinase (ILK) (52). The modest increase in GSK3ß phosphorylation is, at a first glance, not in line with the unaffected (Thr308)/reduced (Ser473) phosphorylation of AKT. Yet, it is possible that the activity of PKA or ILK is altered following cisplatin treatment, which may explain the enhanced GSK3ß phosphorylation.

MKK4, p38, MEK1, and ERK1/2 levels were all unaltered after cisplatin treatment, whereas JNK1/2 levels increased (Fig. 6, Table III). Although the p38 protein levels were unchanged, a drastic increase in phosphorylation of p38-Thr180/Tyr182 was seen in both cell lines (Fig. 6). This upregulation supports a pro-apoptotic role of p38 in these prostate carcinoma cell lines.

It has been shown that various cytotoxic stresses can induce activation of the MKK4-JNK1 and MKK3/MKK6-p38 MAP kinase pathways, resulting in the activation of caspases and onset of apoptosis (53,54). Chen *et al* (54) have shown that cisplatin has these effects in human ovarian carcinoma and kidney cells.

Losa *et al* (55) also reported a specific activation of p38 MAPK upon cisplatin treatment in various cell lines. Furthermore, they showed that inhibition of p38 MAPK activation or low activation of p38 correlated with a resistant phenotype, indicating an important role of this kinase in cisplatin-induced apoptosis. In contrast, Persons *et al* (56) demonstrated cisplatin-induced activation of ERK1/2 and JNK but not activation of p38 MAPK.

In DU-145 cells, ERK1/2 phosphorylation at Thr202 and Tyr204 occurs in untreated cells. This phosphorylation signal is slightly but clearly increased following cisplatin treatment (Fig. 6, Table III). In PC-3 cells, however, no phosphorylation is observed, neither in untreated cells nor in cisplatin-treated cells. Disagreements exist concerning the role of ERK activation upon cisplatin treatment. Wang *et al* (57) showed that ERK activation induced apoptosis, whereas Persons *et al* (56) and Cui *et al* (58) demonstrated that activation of the ERK1/2 pathway in ovarian carcinoma cells partially protects against cisplatin-induced cytotoxicity.

In HeLa cells, Wang *et al* (57) found that all MAP kinases were activated in response to cisplatin. These findings are in agreement with our results demonstrating a slightly enhanced ERK1/2 and a strong p38 phosphorylation (Fig. 6). It appears that MAP kinase pathways play crucial roles upon cisplatin induction of apoptosis in androgen-independent prostate cell lines; however, further experiments are required in order to assess the role of the increased ERK1/2 phosphorylation in DU-145.

In conclusion, our results demonstrate that the AR and p53 status is not of primary importance for the observed differences in the sensitivity of prostate carcinoma cell lines to cisplatin-induced apoptosis. Instead, our profiling implies that non-functional PTEN and, thus, constitutive active AKT render the cells more resistant to cisplatin; hence, the PTEN/AKT pathway (Fig. 7) may be a suitable marker for predicting the sensitivity of prostate tumor cells to cisplatin. Other possible markers may be the activation levels of MAP kinase pathways (Fig. 7).

Cisplatin treatment led to a significant increase in p38 phosphorylation in both DU-145 and PC-3 cell lines. In DU-145 cells, there was already a basal level of phosphorylated p38, which was significantly increased upon cisplatin treatment. No basal phospho-p38 is visible in PC-3 cells (Fig. 6, Table III). It is remarkable that treatment of PC-3 cells with a four times higher cisplatin concentration, as used for DU-145 cells, only led to p38 activity, which was comparable to that found in untreated DU-145 cells. Since both cells do not harbor functional p53, it is unlikely that the detected apoptosis is p53-dependent. p53-independent apoptosis has been described in PC-3 cells treated with phenethyl isothiocyanate (PEITC), a well established inducer of apoptosis (59). These authors found high levels of phosporylated p38 and ERK1/2 after treatment of PC-3 cells with PEITC. The mechanism of PEITC on apoptosis induction is not well understood and cannot be compared with the mechanism behind cisplatin action, since PEITC does not exert its apoptosis-inducing action through DNA damage. Despite this, we have to exclude a role for p53 in the activation of p38 and ERK1/2 kinases after cisplatin treatment in DU-145 and PC-3 cells. We have shown that there is only a minor increase of activated ERK1/2 in DU-145 cells and that PC-3 cells are totally devoid of phospho-ERK1/2 in untreated and cisplatin-treated cells. A low ERK1/2 activity favors the pro-apoptotic function of Bad (Fig. 7). Moreover, a lack of activated AKT, as present in DU-145 cells, also favors the pro-apoptotic function of Bad. In contrast, constitutively activated AKT phosphorylates Bad and interferes with its pro-apoptotic function in PC-3 cells. Therefore, it is unlikely that ERK1/2 activity accounts for the observed difference in cisplatin sensitivity between the two cell lines.

We believe that the significantly higher p38 kinase activity in conjunction with a functional PTEN/AKT pathway accounts for the higher sensitivity towards apoptosis induction by cisplatin in DU-145 cells. Therefore, we conclude that lack of a functional PTEN/AKT pathway together with low p38 activity may be the cause of the observed resistance of PC-3 cells towards cisplatin-induced apoptosis.

Acknowledgements

This research work was supported by the Danish Cancer Society (grant no. 00252 110 9210) and the Natural Science Foundation (grant no. 21-04-0517).

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