Treatment of PC3 prostate cancer cells with mitoxantrone, etoposide, doxorubicin and carboplatin induces distinct alterations in the expression of kallikreins 5 and 11

Hellinida Thomadaki¹; Konstantinos Mavridis¹; Maroulio Talieri²; Andreas Scorilas¹

¹Department of Biochemistry and Molecular Biology, Faculty of Biology, University of Athens, Panepistimiopolis, Athens, Greece; ²“G. Papanicolaou” Research Center of Oncology “Saint Savas” Hospital Athens, Greece

Summary
Several of the novel kallikrein-related peptidases (tissue kallikreins; KLKs) are emerging new serum and/or tissue biomarkers for prostate cancer (CaP) diagnosis, prognosis and monitoring. In the present research approach, our objective was to investigate the possible alterations in the mRNA expression levels of KLK5 and KLK11 genes in prostate cancer cells PC3 as a response to treatment with Mitoxantrone, Etoposide, Doxorubicin and Carboplatin. Viability was assessed with the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay after cell treatment with either mitoxantrone (2 µM), etoposide (20 µM), doxorubicin (1 µM), or carboplatin (15 µM), for 24, 48 and 72 hours. Additionally, trypan blue staining revealed that in PC3 cells all drugs displayed almost the same limited necrotic effects which appeared mainly at 72 hours of treatment. PC3 prostate cancer cells showed a concentration- and time-dependent increased cytotoxicity to the drugs under study which was mainly due to reduction of cell proliferation efficiency. Distinct modulations of KLK5 and KLK11 genes, at the mRNA level, were observed, supporting a drug-dependent cell response. Our experimental data demonstrate that the molecular profile mainly of KLK5 gene may serve as a new potential molecular biomarker predicting treatment response in CaP cells.

Keywords
Kallikrein-related peptidases, kallikreins, KLK11, KLK5, prostate cancer, PC3, cytotoxic drugs, mitoxantrone, etoposide, doxorubicin, carboplatin

Introduction
Prostate cancer remains the most frequently diagnosed cancer in men of the Western countries (1). In the US, prostate cancer has reached epidemic proportions, since more than one in six men is diagnosed with prostate cancer and one in thirty-five men die because of the disease (2, 3). Although the pathogenesis of prostate cancer remains a mystery, several risk factors, such as alterations in the androgen biosynthesis and metabolism, diet and chronic inflammation, have been associated with the disease (4, 5). The androgen receptor (AR) plays a very important role in the survival and growth of prostate cells, as well as in the initiation and progression of prostate tumors. Consequently, the regulation and manipulation of the AR pathway has been the main target of most therapeutic approaches for several years. However, AR function evolves as the cell changes towards a clinically androgen depletion-independent state (6, 7). Additionally, recent findings implicate normal stem/progenitor cells in the prostate tumor initiation and progression (8). As far as the diagnosis of prostate cancer is concerned, human kallikrein 3, widely known as PSA, remains the core of prostate cancer detection (9), despite the fact that it lacks diagnostic specificity, knowing that the levels of prostate-specific antigen (PSA) can be also increased in pathological conditions such as benign prostatic hyperplasia and prostatitis (10).

The human tissue kallikreins can be defined as a subgroup of 15 secreted serine proteases that occupy a cluster of approximately 300 kbp on human chromosome 19q13.4, which is the...
largest contiguous cluster of protease genes in the human genome (11, 12). In addition, the occurrence of alternative splicing within the kallikrein family is extremely frequent and this is evident by the fact that up to date, approximately 70 alternative splice variants of kallikrein genes have been characterized (13).

The expression pattern of human tissue kallikreins is interestingly wide, as well as their physiological functions, which encompass from electrolyte balance and regulation of blood pressure to tissue remodeling and prohormone processing (14, 15). Consequently, if kallikreins are to play these sophisticated roles in the human organism, it is essential that kallikrein function should be regulated at many and distinct levels, including transcriptional, translational and post-translational. Furthermore, the proteolytic activity of kallikreins is believed to be cascade-mediated and kallikreins may crosstalk with other proteases (10–12).

Kallikreins are implicated in various pathological and mainly cancer conditions. As far as prostate cancer is concerned, KLK4, KLK11, KLK14, and KLK15 genes have been shown to be highly expressed at the mRNA level, although most members of the kallikrein gene family have been found to be modulated at the mRNA in distinct, mainly endocrine related cancer conditions, supporting and enhancing their implication in carcinogenesis (12, 16–19). Research data have also shown that PSA, one of the most important and well studied members of the kallikrein family of genes as far as prostate diagnosis is concerned, possesses tumor-suppressor functions through the induction of cellular mechanisms such as apoptosis, as well as mitogenic cellular characteristics (liberation of insulinlike growth factor-1, activation of transforming growth factor-β) (20).

KLK11 was originally isolated from the human hippocampus. The protein product, of KLK11 (hk11) which was previously referred to as trypsinlike serine protease (TLSP), hippostasin and protease serine 20 (PRSS20) encodes for a secreted serine protease of 260 amino acids. The KLK11 gene occupies a region of 5.3 kb of the genomic DNA and it consists of six exons and five introns (21, 22). KLK11 expression appears to be regulated by steroid hormones in endocrine-related malignancies, such as ovarian and prostatic carcinoma (22–26). In addition, KLK11 expression in breast cancer cell lines is up-regulated by estradiol (21). The KLK11 protein is most abundant in the prostate and testis. The highest expression levels of KLK11 protein are found in seminal plasma and CVF (27, 28). As far as its physiological function is concerned, KLK11 is involved, in collaboration with other kallikreins, in a proteolytic cascade in seminal plasma. These kallikreins are critical in semen liquefaction through a cascade-mediated processing of semenogelins I and II (12). However, KLK11 is also involved in pathological conditions, as elevated serum levels of the kallikrein 11 protein (hk11) were found in women with ovarian cancer and in men with prostate cancer. In addition, the ratio of KLK11 to total PSA (tPSA) is significantly lower in the patients with prostate cancer than in those with benign prostate hyperplasia providing a potentially useful tool in the differential diagnosis of prostate cancer (22, 24–26).

Kallikrein 5 (KLK5), formerly known as kallikrein-like gene-2 (KLK-L2) or human stratum corneum trypsic enzyme (HSCTE), is a newly discovered member of the kallikrein gene family. It consists of five coding exons and four intervening introns, spanning an area of 9,349 bp of genomic sequence on chromosome 19q13.3-q13.4 [29]. KLK5 gene is primarily expressed in the brain, mammary gland, and testis, but lower levels of expression are found in a variety of other tissues. It is up-regulated by estrogens and progestins in BT-474 breast carcinoma cell line (29, 30) and it is expressed at the mRNA level in the K11 bladder carcinoma cell line, in the OVCA3 ovarian cancer cell line and the gastric cancer cell lines MKN28 and MKN74 (31). KLK5 is also highly expressed in the brain, possessing a potential role in the central nervous system, although the biological role of KLK5 has not been unravelled yet (29). Scientific data have revealed overexpression of KLK5 in breast cancer, where it constitutes an independent indicator of poor prognosis (30). In addition, differential expression of KLK5 gene and the hk5 protein as well, takes place in normal ovaries and ovarian carcinomas, with particularly high expression levels in epithelial-derived SER carcinomas (32). Furthermore, a statistically significant association is found between KLK5 overexpression and invasive potential in the case of primary bladder carcinomas (31). Finally, KLK5 is highly expressed, at the mRNA level, in testicular (33) and lung cancer (34) and, in controversy it is downregulated in prostate cancer, where it is also negatively correlated with cancer aggressiveness (35).

In the present research approach, we studied the cellular response at the mRNA level of two members of the kallikrein gene family, KLK5 and KLK11, which are readily modulated and therefore implicated in distinct types of cancer, after treatment of the prostate cancer cell line PC3 with distinct anticancer drugs, such as etoposide, carboplatin, doxorubicin and mitoxantrone.

Materials and methods

Cell culture conditions

The cell line PC3, derived from human prostate cancer, was maintained in an atmosphere of 95% air / 5% CO2, with 100% humidity, at 37°C and cultured in RPMI 1640 medium, supplemented with 10% fetal bovine serum (FBS), 100U/ml penicillin, 100 μg/ml streptomycin, 0.3 g/ml L-glutamine and 2 mM NaHCO3. Cells were seeded, incubated at 37°C for 30 hours (h) to adhere and then they were treated with each drug, while in exponential growth phase, for the indicated time periods. Five drugs were used, which included mitoxantrone (Wyeth-Ayerst Lederle Inc., Puerto Rico), etoposide (Ebewe, Pharma m.b.H. Nfg.KG, Unterach, Austria), carboplatin (Ebewe, Pharma m.b.H. Nfg.KG, Unterach, Austria) and doxorubicin (Ebewe, Pharma m.b.H. Nfg.KG, Unterach, Austria). Drugs were added to the cell medium, where they remained constantly for the indicated time periods.

Cell viability by MTT

The effect of each drug to PC3 cells was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric assay, as previously described (36). Briefly, exponentially growing, human prostate cancer cells were plated in 96-well plates at a density of 2 x 104 cells/100 μl of medium/well, in triplicate, and allowed to adhere. Thirty hours later cells were treated with each drug, at 37°C in a humidified 5% CO2 atmosphere, for the indicated time periods, followed by addition of
3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT; Sigma Chemical Co, St. Louis, MO, USA), at a final concentration of 0.5 mg/ml. Cells were further incubated for 4 h at 37°C, in a humidified 5% CO2 atmosphere, to allow the yellow compound MTT to form blue formazan crystals in metabolically active cells. The medium was carefully aspirated and solubilization of formazan crystals was achieved by overnight incubation at 37°C, in a solution containing 12.5% (w/v) SDS (Sigma Chemical Co) and 45% (v/v) Formamide (ACROS Organics, Geel, Belgium). The absorbance of each cell lysate solution was read at 545 nm, with a reference wavelength of 690 nm. The results were expressed as the percentage (%) of treated cells versus untreated cells.

**Trypan blue staining**

Exponentially growing, prostate cancer cells were seeded at a density of 2 x 10^4 cells/ml (4 x 10^5 cells/flask), in triplicate, and 30 h later they were treated with each drug, at 37°C in a humidified 5% CO2 atmosphere, for the indicated time periods. Cells were detached by trypsinisation and a 1:10 dilution of the cell suspension was prepared in phosphate-buffered saline (PBS) (1x) and 2 µl of 0.4% (w/v) trypan blue stain (Sigma Chemical Co) were added to 18 µl of 10x diluted cell suspension. The total number of cells as well as the number of trypan blue positive cells was counted on a haemocytometer under a microscope (37). The results were expressed as the concentration of trypan blue-stained cells (cells/ml) and as the percentage of the total cell number of cells. Each data point represents the mean for two separate experiments (mean ± standard error of the mean [SEM]).

**Cell proliferation assay**

The effect of the drugs under study on the proliferative capacity of PC3 prostate cancer cells was studied. For that purpose, exponentially growing, human prostate cancer cells were seeded at a density of 2 x 10^4 cells/ml (4 x 10^5 cells/flask), in triplicate, and 30 h later they were treated with each drug, at 37°C in a humidified 5% CO2 atmosphere, for the indicated time periods. Then a 50-µl aliquot from each sample was mixed thoroughly with 450 µl of complete medium without serum (1:10 dilution) and total cell number was counted under a light microscope, using a haemocytometer. The results were expressed as the total cell number of cells, with each data point representing the mean of two separate experiments (mean ± SEM).

**DNA fragmentation assay**

Samples (10^6) intact cells/sample) were subjected to electrophoresis on 2% agarose (Sigma Chemical Co) gel, according to Eastman protocol (38). High-molecular-weight (HMW) DNA fragments were trapped in or near the well, whereas the DNA fragments of low molecular weight ran and separated through the gel. The gel was stained with ethidium bromide and photographed by a Nikon F-801 SLR camera with accompanying UV filter and AGFA APX, ISO 25, B/W professional film.

**Total RNA isolation and reverse transcription**

Total RNA was extracted from PC3 cells, using TRI reagent (Ambion Inc., Austin, TX, USA), according the manufacturer’s instructions. The concentration and purity of RNA were assessed spectrophotometrically at 260 nm and 280 nm. Two micrograms of total RNA were used for reverse-transcription into first-strand cDNA using Finnzymes M-MuLV Reverse Transcriptase (Finnzymes Oy, Espoo, Finland).

**Reverse transcriptase-polymerase chain reaction**

Based on the published information for KLK5, KLK11 and GAPDH gene sequences (GenBank accession numbers AF135028, AF243527 and AY340484, respectively) three pairs of gene-specific primers were designed and utilized (Table 1). PCR reaction was carried out in a reaction mixture containing 1 µl of cDNA, 10 mM Tris- HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 200 mM dNTPs, 150 ng of primers and 1.25 units of GoTaq® DNA polymerase (Promega Corporation, Madison, WI, USA) in a LabNet thermocycler. The conditions of PCR for each individual gene were optimized in order to detect each amplified product in the linear range of amplification, resulting in the cycling conditions as follows: a denaturation step at 95°C for 2 minutes (min), followed by 38–40 cycles (GAPDH: 38 cycles, KLK5: 38 cycles, KLK11: 40 cycles) of 95°C for 30 seconds (s), 59–60°C for 30 s, 72°C for 1 min and a final extension step at 72°C for 5 min. Equal amounts of PCR products were electrophoresed on 1.5% agarose gels and visualized by ethidium bromide staining. Gels were photographed under UV light, and images were analyzed for gene expression levels by the NIH Image program, developed at the U.S, National Institute of Health (http://rsb.info.nih.gov/nih-image/). GAPDH was amplified as a housekeeping gene (internal control) for the integrity of the mRNA, as well as to normalize the initial content of total cDNA. Each PCR reaction was performed twice to evaluate reproducibility of data. Expression was calculated as the relative expression ratio between each gene under study and GAPDH and was compared for each condition relatively to the control sample. As a negative control, reverse transcription-PCR reactions without cDNA template were also made.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Annealing temperature (°C)</th>
<th>Sequence of primers (5’-3’)</th>
<th>Size of PCR product (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAPDH</td>
<td>60</td>
<td>CCA CAT CGC TCA GAC ACC AT</td>
<td>240</td>
</tr>
<tr>
<td>KLK5</td>
<td>60</td>
<td>CCA AAG GTG ACA TTC CCT AA</td>
<td>260</td>
</tr>
<tr>
<td>KLK11</td>
<td>59</td>
<td>CCT TTC AGC CTG TG CCA AA</td>
<td>139</td>
</tr>
</tbody>
</table>

Table 1: Nucleotide sequence of the primers utilized and conditions for RT-PCR analysis of the genes GAPDH, KLK5 and KLK11.
Results

Effect of the drugs under study on PC3 prostate cancer cells’ growth and viability

To evaluate the effect of each anticancer drug under study on PC3 prostate cell line, cells were exposed to increasing doses of each drug, as individual entities, for increasing periods of time (24, 48 and 72 h), and cell viability was assayed by the MTT assay. As shown in Figure 1, cells exhibited sensitivity to all four drugs, in a time- and concentration-dependent manner. However, apart from the MTT method which assess quantitatively cell viability but cannot distinguish between growth arrest or necrosis, the effect of the compounds under study on cell growth as well as the induction of necrotic phenomena, were evaluated, as well as the induction of DNA fragmentation (apoptosis). The cell growth curves exhibited a concentration- and time-dependent important reduction in cell proliferation capacity, since 24 h of treatment, with important differences among the chemotherapeutic drugs used (Fig. 2). Additionally, trypan blue staining revealed that in PC3 cells all drugs displayed almost the same limited necrotic effects which appeared mainly at 72 h of treatment (Fig. 3, Table 2), whereas no DNA fragmentation was observed at the conditions studied (data not shown).

mRNA expression profile of the genes KLK5 and KLK11 in chemotherapeutic drug-treated PC3 prostate cancer cells

Following screening of the cytotoxic effect of the drugs mitoxantrone, etoposide, doxorubicine and carboplatin on PC3 cells, one effective, non-inducing early extensive necrotic phenomena concentration was chosen for each drug and the expression levels of KLK5 and KLK11 gene transcripts were studied.

Only in the case of the antiandrogen mitoxantrone (Fig. 4, Table 3), no modulations in KLK5 gene transcript were observed, at any timepoint of cell exposure to it. However, cells treated with any of the other three drugs (etoposide, doxorubicin, carboplatin) resulted in pronounced increase of KLK5 mRNA levels, in
most cases since 24 h of treatment (Fig. 4, Table 3), except in carboplatin-treated cells where the increase in KLK5 mRNA levels appeared after 48 h. Moreover, the highest mRNA level of KLK5 appeared as a response to drug treatment in PC3 cells were evident at 48 h, prior and quite earlier than the appearance of necrotic phenomena.

As far as KLK11 gene transcript is concerned, interestingly, it showed very low mRNA expression levels in PC3 untreated cells in comparison to KLK5 mRNA levels (Fig. 4, Table 3). Exposure of cells to etoposide, doxorubicin or carboplatin resulted in no apparent modulations at the mRNA levels of the gene. However, in the case of mitoxantrone, an initial increase in KLK11 mRNA levels at 24 h and 48 h of treatment was observed (Fig. 4, Table 3), followed by a decrease at 72 h, concomitantly with the appearance of necrotic phenomena (Fig. 4, Table 3). The highest KLK11 mRNA modulations were observed prior to and in the absence of necrotic phenomena.

Our results indicate and support the hypothesis that the kalikrein gene family members under study (KLK5 and KLK11) are modified in PC3 prostate cancer cells in a time- and drug-specific manner.

Discussion

The only solid fact that has been established and enlisted in a variety of clinical trials, concerning the changes in PSA levels after therapy, is that these alterations have been associated with improved survival in prostate cancer patients with castrate metastatic disease, where rates of rise in PSA, expressed as PSA doubling times, provide important prognostic information regarding risk of metastatic progression or death from the disease (39). The state of a rising PSA signals treatment failure, and changes in PSA levels over time can determine whether an intervention should be considered (39, 40). Another significant benefit of measuring post-therapy PSA levels is that this methodology remains easily assessable, quantitative, reproducible, and inexpensive. Hence, a screening for treatment effects has been suggested in prostate cancer clinical trials on the basis of the hypothesis that PSA declines reflect significant cell kill in response to agents that cause reduction in overall tumor burden (39). Moreover, finasteride appears to lower total and free PSA levels equally in men with benign prostate hyperplasia but does not appear to change the ratio of free to total serum PSA (41). Furthermore, the most commonly used surrogate biomarker in monitoring prostate cancer recurrence after radiotherapy for prostate cancer is PSA. First-year post-radiotherapy PSA kinetics is predictive of overall survival for prostate cancer patients, who have undergone radiotherapy (40, 42).

In the contrary and while these analyses indicate an association between post-therapy PSA levels and an improvement in survival, this association is not strong enough. There are many situations where reliance on PSA kinetics alone is misleading, as declining PSA values may not indicate that an intervention is working, and rising values may not indicate that it is failing (39, 43).

Whether post-therapy decline in PSA has significant clinical value, and whether post-therapy declines can be used as an intermediate endpoint for accelerated drug approval is still highly controversial and bares some risk. There has not been, up to now,
a clear demonstration that a post-therapy PSA change can account for all of the treatment effects. Moreover, no drug for prostate cancer therapy has been approved strictly on the basis of a post-treatment decline in PSA, despite the fact that a cytotoxic drug that does not produce any PSA decline is unlikely to be effective. For example, mitoxantrone, estramustine and etoposide all result in a >50% decline in PSA levels but they have been approved by the Food and Drug Administration (FDA) basing on other criteria such as overall survival and palliation of symptoms and metastatic disease (39, 44). However, this does not mean that an effective cytotoxic drug results always in the decrease of the PSA levels because differentiating agents, angiogenesis and growth factor inhibitors might produce a rise in PSA before a decline is observed, or may act through immunization strategies where the effect on PSA levels might be delayed or not occur at all. It is also well known that a category of drugs acts on different aspects of the malignant process such as angiogenesis or by mechanisms, where PSA measurements are not informative, due to the fact that the biological basis of these mechanisms cannot be delineated by PSA alterations. As such, the clear demonstration that a post-therapy PSA change can account for all of the treatment effects observed is not yet available. As a result, alternative clinical benefits, which are independent of PSA levels, could improve the survival of patients suffering from prostate cancer. Large-scale prospective studies incorporating different post-therapy PSA change definitions, as well as other potential biomarkers, are ongoing (39, 42).

According to our results, concerning PC3 prostate cancer cells, distinct cytotoxic pathways are probably induced as a response to either the antiandrogen mitoxantrone or the rest of the drugs under study (etoposide, doxorubicin or carboplatin), where the mRNAs of the genes examined behave differentially. More specifically, in the case of mitoxantrone no modulations in the mRNA levels of the KLK5 gene transcript was observed, whereas KLK11 showed modulations only in response to mitoxantrone and not in response treatment with any other of the drugs under study. However, treatment of PC3 cells with any of the other three drugs resulted in significant alterations (upregulation) of KLK5 mRNA levels only. Such an effect of drug exposure over the mRNA levels of KLK5 and KLK11 genes was observed in parallel with cell cytotoxicity progression which derived mainly from reduction in cell proliferation capacity, with limited necrotic phenomena being evident only at 72 h of treatment. The necrotic phenomena may be characterized as limited, with the reduction in cell proliferation being the predominant phenomenon, because the % of necrotic cells was calculated as the percentage (%) of trypan blue + cells versus total number of cells, with the total number of cells in each case being much lower than the total number of cells in the control sample, due to important reduction in cell proliferation efficiency. Therefore,

**Figure 4:** mRNA expression profile of the genes KLK5 and KLK11 as a response to drug treatment, in PC3 prostate cancer cells. PC3 cells were treated with either mitoxantrone (2 µM), etoposide (20 µM), doxorubicin (1 µM), or carboplatin (15 µM), for the time periods indicated. RT-PCR analysis of the abundance of mRNAs encoding the genes under study was carried out. Total cellular RNA was subjected to RT and PCR with gene-specific primers. PCR products were separated by agarose gel electrophoresis and stained with ethidium bromide. Lane N: negative control.

**Table 3:** Gene expression modulations in PC3 cells as a response to drug treatment.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mitoxantrone</th>
<th>Etoposide</th>
<th>Doxorubicin</th>
<th>Carboplatin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
<td>24 h</td>
<td>48 h</td>
<td>72 h</td>
</tr>
<tr>
<td>KLK5</td>
<td>1</td>
<td>0.001</td>
<td>1.8</td>
<td>0.021</td>
</tr>
<tr>
<td>KLK11</td>
<td>1</td>
<td>0.001</td>
<td>1.2</td>
<td>0.004</td>
</tr>
<tr>
<td>KLK5</td>
<td>1</td>
<td>0.002</td>
<td>1.1</td>
<td>0.021</td>
</tr>
<tr>
<td>KLK11</td>
<td>1</td>
<td>0.000</td>
<td>1</td>
<td>0.014</td>
</tr>
</tbody>
</table>

* F, average ratio of change (n-fold). The standardized ratios between the treated samples and untreated controls are shown. The data are presented in the following 6 groups (ratio of change in intensity relative to untreated samples, where expression levels were set as 1). The exact fold increase is also displayed. SEM, standard error of the mean.
the existence of limited necrosis and more extensive reduction in cell proliferation explains the amount of existing mRNA in each sample, with the latter being mainly responsible for the observed mRNA modulations, and not limited necrosis. The time point of necrosis appearance, in the case of mitoxantrone, was accompanied by parallel reduction in KLK11 mRNA levels, while no change in KLK5 mRNA levels, and with KLK11 upregulation being observed prior to necrosis.

Overall, we may say that KLK11 mRNA levels were modulated only in response to the drug (mitoxantrone) in which KLK5 mRNA levels were not modulated and vice versa. The divergence in mRNA modulations among the different drugs and genes under study may be due to differential implication of these kallikrein genes, according to the cytotoxic or proliferation inhibitory pathway induced by each drug. As far as apoptosis induction is concerned, DNA-fragmentation studies revealed that DNA was not fragmented along drug treatment. However, the induction of alternative DNA-independent apoptotic pathways cannot be excluded, in which case the reduction of cell proliferation capacity will still be the predominant phenomenon.

The drugs used in the present research approach possess similar molecular targets along their anticancer and cytotoxic mode of action, with the antiandrogen and anthracycenedione agent mitoxantrone, the topoisomerase II inhibitor etoposide, the platinum agent carboplatin and the anthracycline antitumour agent doxorubicin, targeting DNA and ultimately leading to DNA adducts. More specifically, mitoxantrone targets and inhibits topoisomerase II (45, 46), as well as etoposide that also binds to tubulin, and disrupts chromosomal dynamics (47, 48), stabilising covalent enzyme-cleaved DNA complexes that accumulate in the cell, ultimately causing permanent DNA breaks (49). Furthermore, platinum agent carboplatin forms inter- and intra-strand helix-deforming DNA adducts. Platinum binding to thiol groups as well as interactions of platinum compounds with proteins and enzymes is probably responsible for the toxic effects of these types of drugs (50–54). Doxorubicin is also a topoisomerase II inhibitor and a DNA intercalator that inhibits DNA and RNA synthesis and produces DNA strand breaks (55).

In previous studies, KLK5 was found to be up-regulated by estrogens and progestins in BT-474 breast carcinoma cell line (29, 30). In addition, KLK5 was found to be expressed at the mRNA level in the KU1 bladder carcinoma cell line, in the OVCAR3 ovarian cancer cell line, as well as in the gastric cancer cell lines MKN28 and MKN74 (31). KLK11 expression appears to be regulated by steroid hormones in endocrine-related malignancies, such as ovarian and prostatic carcinoma (22–26). In addition, KLK11 expression was found to be up-regulated by estrogen in the breast cancer cell line BT-474 (21).

Our results provide valuable information about the potential of the genes KLK5 and KLK11 as tools in cancer chemotherapy response, and reveal the endogenous needs of PC3 cells for certain kallikreins expression in response to anticancer drug treatment. Interestingly, KLK5 mRNA levels were evidently higher in comparison to KLK11, probably implying modulations not only at the mRNA levels of kallikrein gene family members as individual entities, but also at the equilibrium among them. However, further work is required and more kallikrein family members to be studied in order to evaluate the biological role of KLK5 and KLK11 genes in prostate cancer cell response to drug treatment. To the best of our knowledge, this is the first study examining the expression of KLK5 and KLK11 genes, at the mRNA level, under mitoxantrone/etoposide/carboplatin/doxorubicin treatment in human prostate cancer cells.

References

Thomadaki et al. KLK5 and KLK11 in prostate cancer treatment

30.


31.


32.


33.

Luo LY, Yousef G, Diamandis EP. Human tissue kallikreins and testicular cancer. APMIS 2003;111[1]:225–32; discussion 32–33.

34.


35.


36.


37.


38.


39.


40.


41.


42.


43.


44.


45.


46.


47.


48.


49.


50.


51.


52.


53.


54.


55.