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ORIGINAL ARTICLE

Paclitaxel resistance and the role of miRNAs in prostate cancer cell lines

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Abstract

Purpose To investigate the expression profiles of 86 miRNAs in paclitaxel-resistant prostate cancer cell lines and to identify the genes that have a role in the development of drug resistance.

Methods Three prostate cancer cell lines, androgen-dependent VCaP, androgen-independent PC-3 and DU-145, were used to obtain paclitaxel-resistant cells by progressively increasing the concentration of paclitaxel in the culture medium. Viability assays with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium and sulforhodamine B were used to assess the cell resistance level and cytotoxic effects of paclitaxel treatment. Total RNA was isolated from both prostate cancer cell lines and their resistant versions, and cDNA samples were reverse transcribed from total RNA. Selected target genes of miRNAs that showed differences in expression and were estimated to be effective on drug resistance mechanism were analyzed with western blot analysis.

Results Expression study of 86 miRNAs by RT-PCR demonstrated that several of the miRNAs were expressed at different levels in paclitaxel-resistant cells compared to wild-type cells. Moreover, the expression profiles of these miRNAs varied among different prostate cancer cell line types, with 13 miRNAs being up-regulated in the resistant cells. Among these, miR-200b-3p, miR-34b-3p and miR-375 exhibited a marked up-regulation. Further, miR-100-5p showed a prominent increase in paclitaxel-resistant VCaP-R and DU145-R cells. Western blot and RT-PCR studies showed that only the LARP1 and CCND1 genes were over-expressed up to 2–5 times in all paclitaxel-resistant cell lines compared to the other investigated genes.

Conclusions In this study, the three paclitaxel-resistant prostate cancer cell lines examined showed remarkably different miRNA expression profiles.

Keywords miRNA · Prostate cancer · Paclitaxel · Drug resistance

Introduction

Prostate cancer is the second most common type of cancer that causes death in men. Prostate cancer is a multifactorial disease for which a number of associated risk factors are under investigation. Beyond environmental factors, it is widely accepted that genetic factors are the main risk factors for prostate cancer [1].

MicroRNAs (miRNAs) are among the molecules that are responsible for carcinogenesis. miRNAs, which were discovered by Lee et al. in 1993, have been known to play an important role in cancer development and treatment [2]. miRNAs are biomolecules of 22–24 nucleotides in length that control post-transcriptional gene translation [3]. In addition, they have roles in pathways such as development, apoptosis and differentiation. Distortions in the regulation of miRNAs cause either an increase or decrease of gene translation processes leading to carcinogenesis. Accordingly, many gene expression studies have shown that different miRNA expression profiles are present on different cancers [4].

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A number of chemotherapy protocols are in clinical trials to achieve palliative improvement and increase life expectancy in prostate cancer patients. Paclitaxel (PAX) shows anti-cancer effects with its cytotoxic effects and induction of apoptosis. PAX stabilizes the microtubule structures by connecting to tubulin. Therefore, this compound is able to arrest the cell cycle and promote the accumulation of cells in the G2 and M phase [5]. In addition to these cytostatic properties, PAX controls intercellular signaling by regulating gene expression. According to two large studies on the effectiveness of taxanes as a hormone-refractory prostate cancer (HRPC) treatment, the PSA response rate rarely exceeds 50%, and the mean survival time is less than 20 months. Therefore, the chemotherapy of HRPC remains an ongoing clinical research subject [5, 6].

As the role of miRNAs in metastasis and drug resistance is being understood, the main focus of the current research is to explain the mechanism of drug resistance [7]. The role of miRNAs in the development of PAX resistance has been demonstrated in prostate cancer tissue samples and cell cultures; the down-regulation of miR-34a, miR-148a [8, 9], miR-200c, miR-205 [10] and miR-130a [11]; and the up-regulation of miR-21 [12]. Although the role of miRNAs in the development of resistance to taxanes has been demonstrated, the exact underlying mechanism could not be elucidated.

In this study, we used wild-type (VCaP, PC-3, DU-145) and paclitaxel-resistant prostate cancer cell lines (VCaP-R, PC-3-R, DU-145-R) to investigate the expression profiles of 86 miRNAs. RT-PCR and western blot techniques were used to analyze the expression of 10 genes that were potential targets of these miRNAs and were believed to have roles in the development of drug resistance.

Materials and methods

Cell culture and generation of paclitaxel-resistant cells

In this study, we used three prostate cancer cell lines: androgen-dependent VCaP, androgen-independent PC-3 and DU-145. Cells were cultured in DMEM/Ham's F-12 and RPMI 1640 culture media containing 10% fetal calf serum (Invitrogen, Paisley, UK) and supplemented with 1% L-glutamine, penicillin (100 U/mL), and streptomycin (100 µg/ml). Cells were incubated at 37 °C, in a 95% humidified atmosphere containing 5% CO_2.

Paclitaxel-resistant cancer cells were obtained from parental VCaP, PC-3 and DU-145 cells by progressively increasing the concentration of PAX (Sigma-Aldrich, T7402) in the culture medium. Cells were left for 24 h and then incubated with 5 nM of PAX for 48 h. Then, the culture medium was replaced with fresh medium without PAX, which was maintained until the cells showed proliferation. The same procedure was repeated until resistant and viable cell lines were obtained using 100 nM PAX [9]. The resistant prostate cancer cell lines VCaP-R, PC-3-R and DU-145-R were obtained. PAX cytotoxicity tests were performed, and the viability of VCaP, PC-3 and DU-145 cells and their paclitaxel-resistant versions were confirmed by microscopic examination.

Assessing the viability and number of cells

The number of cells and their viability were determined with trypan blue stain, which evaluates the membrane integrity (Biological Industries, 03-102-1B). Viability assays with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) and sulforhodamine B (SRB) were used to assess the cell resistance level and cytotoxic effects of PAX in VCaP, PC-3, DU-145 cells and their resistant variants. Cells were seeded at a density of 5000 cells per well. After 24 h of incubation, increasing concentrations of PAX were added to each well and incubated for 24-h, 48-h and 72-h periods. MTT and SRB tests were done after incubation. Finally, the plates were read using a 96-well plate reader with working wavelength of 570 nM and the optical density was recorded. The viability of PAX-treated cells versus untreated control cells was calculated using the following formula:

Cell viability (%) = [100 × (Sample Absorbance)/(Control Absorbance)].

Total RNA extraction and evaluation of miRNA/mRNA expression by RT-PCR

Cultured VCaP, PC-3, DU-145 cells and their resistant variants were counted to be 10^6 cells in the suspension. Total RNA isolation was performed using the High Pure RNA Isolation Kit, (Roche, Cat.No:11828665001). cDNA samples were obtained by reverse transcription of the total RNA using the Universal cDNA Syntesis Kit, (Exiqon, Cat. No:203301) and 86 miRNAs were analyzed on a Roche Lightcycler 480 using the Exilent SYBR Green Master Mix (Exiqon, Cat No:203403). To evaluate the miRNA expression RNU6-2, SNORD48, SNORD61, SNORD66, and SNORD95 were used as reference genes. All analyzes were calculated automatically by Roche LightCycler 480 software. All of the reference genes for normalization of the expression ratios were used in the calculation of relative quantification. In addition, all reference genes displayed correlation and similarity each other. Therefore, reference crossing points were calculated by geo-mean.

In relative quantification, we normalized to the miRNA of interest with the mean of reference genes in the same sample. This reference gene provides a basis for

normalizing sample–sample differences. After finding target/reference ratios, we reached the fold change expression values.

Target genes were explored based on an international data source, MiRTarBase. In addition, MiRBase, MiRDB, TargetScan, and RNA22 were examined to evaluate the target genes of selected miRNAs. The Human Protein Atlas was used for detecting the genes that are expressed specifically in prostate tissue. Hence, miRNAs that showed differences in expression were determined and were presumed to be effective based on the resistance mechanism. Ten genes were detected that played some role in resistance mechanisms (Table 1).

Table 1 List of primers and genes that were investigated for expression

Gene	Forward primer sequence	Reverse primer sequence
ZEB1	ggcagaaaatctcgagaagg	ctgagcttcctcctgatcca
ZEB2	tgaagaagagactggagatcactc	cgtggtctgatttggtttcc
E-Cadherin (CDH1)	aagttttccaccaaagtcacg	tgcttggattccagaaacg
PHLPP1	aaacttcctaaggcagaaccccta	tggtgaacctttgcagttcat
EphA2	tgtgcaacgtgatgtctgg	tctcctcggtacacccagtt
c-MYC (MYC)	tccacctccagcttgtacct	tgagagggtaggggaagacc
Cyclin D1 (CCND1)	gaagatcgtcgccacctg	gacctcctcctcgcacttct
LARP1	agatcaaagggtctgagtctgc	gctccggtttgatctctgg
CAND1	cacatttccaatttgctgga	ttctgcagttccgtcatcaa
Beclin-1 (BECN1)	cggaaaccattcatatctggag	tcccagaaaaaccgcaac
Beta-actin[a]	tgagagggaaatcgtgcgtg	tgcttgctgatccacatctgc

[a]Reference gene

Western blot analysis

The identified miRNA target genes (ZEB1, ZEB2, CDH1, PHLPP1, EphaA2, MYC, CCND1, LARP1, CAND1, BECN1) that showed differences in their expression and were believed to play a role in drug resistance mechanisms were analyzed by western blot. It is worth to notice that PHLPP1 antibody has been known to cross-react with β-catenin as suggested by Lobert et al. [13].

To prepare the protein lysates, the cells were first washed with PBS that was at + 4 °C and then treated with a solution containing 50 mM Tris–HCl (pH 7.5), 1 mM EDTA, 150 mM NaCI, 5 mM $MgCl_2$, 0.5% NP-40 and 0.5% Triton-X-100, and protease/phosphatase inhibitors. Lysates were centrifuged at 10,000*g* in a refrigerated centrifuge and removed from artifacts other than the protein. The concentration of proteins in the supernatants was analyzed with a spectrophotometer using the BCA method (Pierce, Cat No: 23,225). The samples were then mixed 1:1 with SDS–PAGE loading buffer and incubated at 95 °C for 5 min. Equal amounts of protein (25 µg) were separated by SDS–PAGE and transferred to a PVDF membrane. The proteins of interest were detected by chemiluminescence after incubation with specific antibodies and HRP-conjugated secondary goat anti-rabbit or goat anti-mouse IgGs. The alpha-tubulin gene was used as internal reference. The primary antibody list is shown in Table 2.

Results

Paclitaxel-resistant prostate cancer cells showed better proliferation performance at the 100 nM PAX concentration than did wild-type cells (Fig. 1).

MTT and SRB viability tests were used to evaluate the cytotoxic effect of PAX treatment in both wild-type and resistant cells. Resistant cells showed higher IC_{50} doses than did wild-type cells (Fig. 2, Table 3). The paclitaxel-resistant

Table 2 Primary and secondary antibodies used in western blot analysis

Gene name	Antibody	Product/cat no
ZEB1	TCF8/ZEB1 (D80D3) rabbit mAb	CST (cell signaling technology) 3396P
ZEB2	Anti-Zeb2	MILLIPORE ABE573
CDH1	E-cadherin (24E10) rabbit mAb	CST (cell signaling technology) 3195P
PHLPP1	Anti-PHLPP1	MILLIPORE07-1341
EpnA2	EphA2 (D4A2) XP® rabbit mAb	CST (cell signaling technology) 6997P
MYC	Myc-tag (71D10) rabbit mAb	CST (cell signaling technology) 2278P
CCND1	Cyclin D1 (92G2) rabbit mAb	CST (cell signaling technology) 2978P
LARP1	LARP1 antibody	CST (cell signaling technology) 14763S
CAND1	CAND1 (D1F2) rabbit mAb	CST (cell signaling technology) 8759S
BECN1	Beclin-1 (D40C5) rabbit mAb	CST (cell signaling technology) 3495P
TUBA1A	Alfa-tubulin (DM1A) mouse mAb	CST (cell signaling technology) 3873S

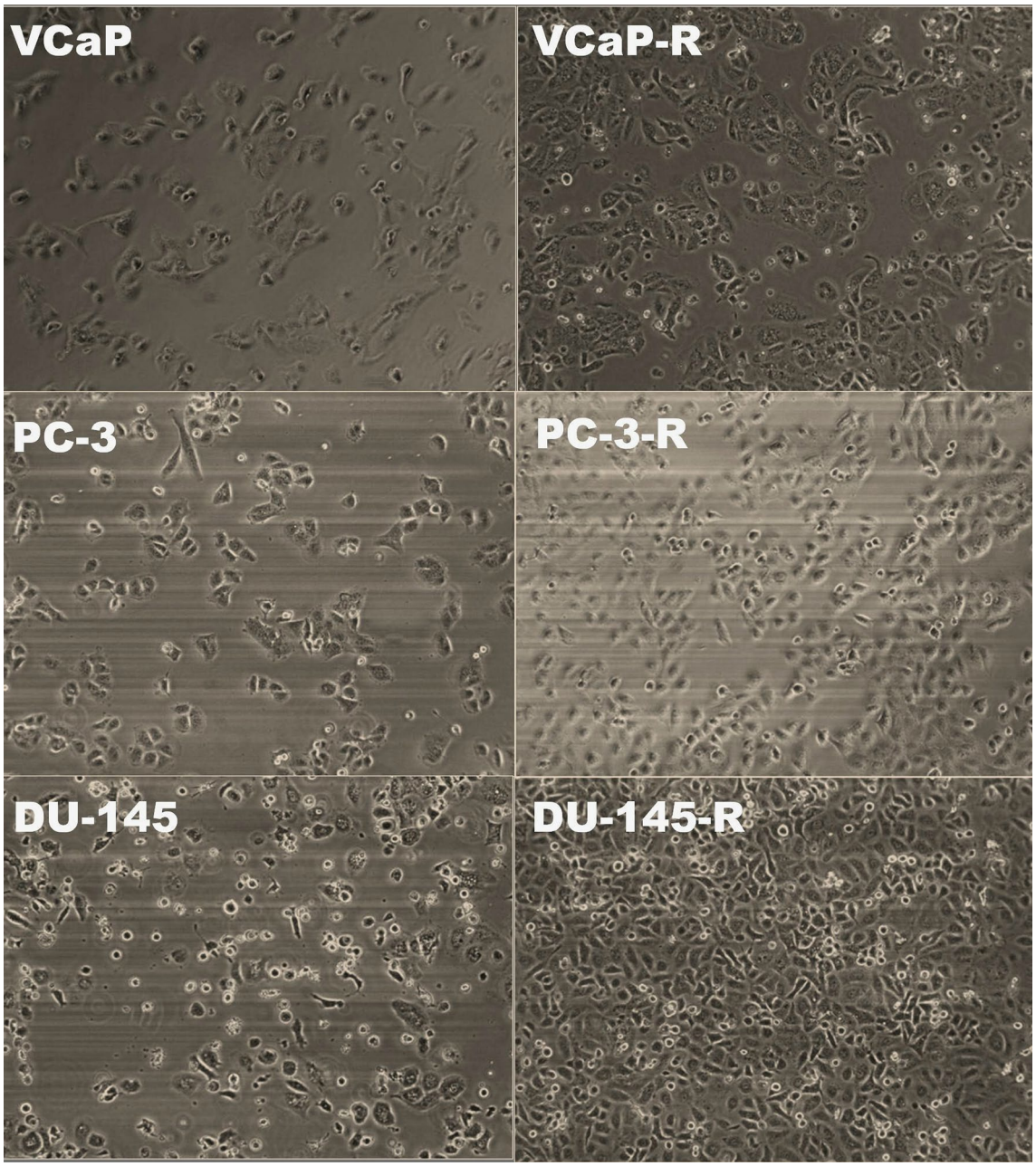

Fig. 1 20X microphotographs of wild-type and paclitaxel-resistant cells (at 100 nM paclitaxel concentration)

prostate cancer cell lines, VCaP-R, PC-3-R and DU145-R exhibited a higher viability percentage than did wild-type cells by 36, 62 and 101 times, respectively.

An expression study of 86 miRNAs demonstrated that some of the miRNAs were expressed at different levels compared with the wild-type cells. Results revealed that, 14 miRNAs were up-regulated 2- to 8000-fold in paclitaxel-resistant cancer cell lines. Of these miRNAs, hsa-miR-100-5p, hsa-miR-125b-5p, and hsa-miR-182-5p were increasingly expressed in both VCap-R and DU145-R cell lines compared to non-resistant prostate cancer cell lines. In addition, hsa-miR-200b-3p was significantly increased in the VCap-R cell line, whereas, hsa-miR-34b-3p and hsa-miR-375 showed a remarkable up-regulation in DU145-R cell line.

Thirty-nine out of 86 miRNAs were down-regulated 2- to 92-fold in paclitaxel-resistant prostate cancer cell lines. Of these down-regulated miRNAs, hsa-miR-17-5p, hsa-miR-19b-3p, hsa-miR-20b-5p, hsa-miR-26b-5p, hsa-miR-374b-5p, and hsa-miR-616-3p showed a considerable down-regulation (11.4- to 92.14-fold) in DU145-R cell line. Moreover, hsa-miR-19b-3p, hsa-miR-26b-5p,

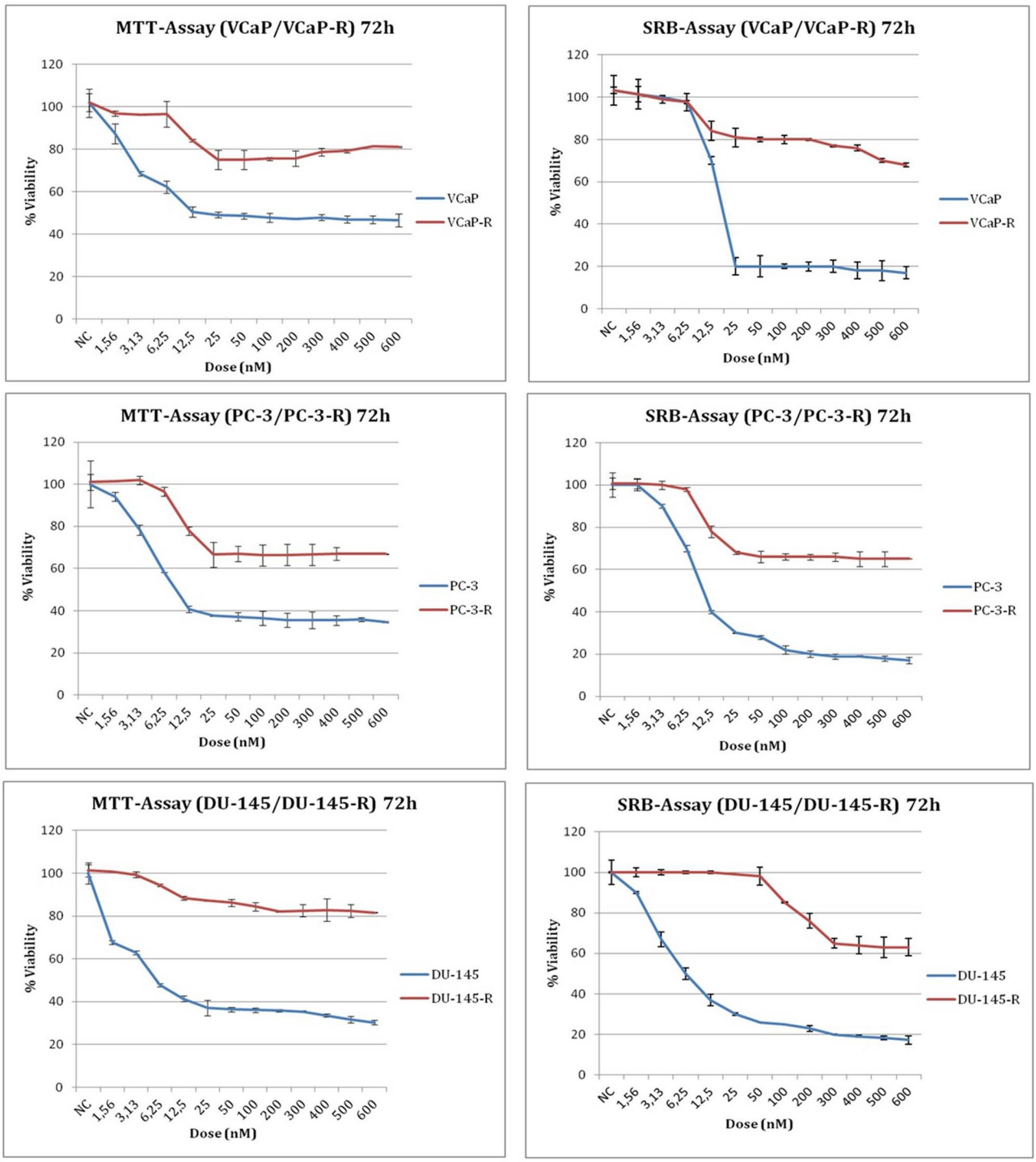

Fig. 2 MTT and SRB assays demonstrated the cytotoxic effect of paclitaxel on resistant and wild-type cells

hsa-miR-374b-5p were also down-regulated in all paclitaxel-resistant prostate cancer cell lines. Table 4 shows the altered miRNA expression profiles across prostate cancer cell line types.

Western blot and RT-PCR experiments showed that only the LARP1 (La-Related Protein1) and CCND1 (Cyclin D1) genes were over-expressed up to 2–5 times in all paclitaxel-resistant cell lines compared to the other genes investigated (Fig. 3). The differences in expression levels and the fold change values are given in Table 5. Expression levels of miR-26a-5p, miR-26b-5p and miR-374-5p, which are relevant to LARP1 gene, showed a down-regulation in all three

Table 3 Paclitaxel IC_{50} doses calculated in cell lines tested with MTT and SRB viability assays

Cell line	MTT-assay (72 h) (nM paclitaxel)	SRB-assay(72 h) (nM paclitaxel)
VCaP	16.55	17.66
VCaP-R	> 600	> 600
PC-3	9.19	10.01
PC-3-R	> 600	> 600
DU145	5.79	6.03
DU145-R	> 600	> 600

paclitaxel-resistant prostate cancer cell lines, as shown in Table 6.

Discussion

Surgery, radiation therapy and chemotherapy are the currently available treatment options for prostate cancer. Although taxanes are first-line chemotherapeutic agents, they do not provide a definitive cure for cancer patients. A number of genomic and proteomic studies are currently in progress to identify the resistance mechanism(s) against taxanes. A number of mechanisms and effectors have shown to play a role in the development of resistance; these include alpha and beta tubulin mutations, different tubulin isotypes, and P-glycoprotein (pg) and MAP over-expression [14].

miRNAs, which have a role in post-transcriptional regulation, are the among the factors involved in the development of resistance. miRNAs play an important role in cell functioning by regulating cell division, invasion, differentiation, resistance and apoptosis. miRNAs have a diagnostic, prognostic and therapeutic importance that goes beyond the relation of tumorigenesis and tumor metastasis [4]. A number of independent mechanisms may help tumor cells to escape form cell death from PAX [15].

In our study, miR-100-5p was significantly increased in the paclitaxel-resistant VCaP-R and DU145-R cell lines. Leite et al. reported that miR-100 is a context-dependent miRNA that controls BAZ2, mTOR, FGFR3, SMARCA5 and THAP2 and might be involved in prostate cancer progression [16]. miR-100 has been shown to be both underexpressed in metastatic cancer compared to localized disease and over-expressed during biochemical recurrence after surgery. Additionally, the function of miR-100 differs in various cell lines. In our study, we demonstrated a significant up-regulation of miR-100 expression in resistant cell lines. We can, thus, postulate that the over-expression of miR-100 may play an important role in PAX resistance due to post-translational mechanisms.

Our results also showed that miR-375 and miR-34b-3p were significantly up-regulated in resistant DU-145R cells (607.5 and 79.13 times, respectively). miR-375 has been reported to be up-regulated in prostate cancer cells [17]. Validation of this result in two large independent sets of patients demonstrated that miR-375 expression was increased in PCa with higher Gleason score and at an advanced pathological stage, which corresponded to a worse prognosis. It has been also reported that miR-375 is able to down-regulate CCND2 gene expression, which is a key step in cell cycle regulation [17].

Majid et al. reported that miR-34b-3p is able to suppress prostate cancer cell division and invasion. However, this result differs from our findings, possibly due to the number of genes (over 300) that are controlled by miR-34b-3p and to the fact that the dysregulation of this miRNA was caused by the long-lasting development of drug resistance, which lasted about 6 months in their study [18].

We showed that miR-200b-3p was up-regulated 163 times more in VCaP-R cell lines than in wild-type cells. A few clinical and in vitro studies investigating miR-200b-3p claimed that this miRNA has an important function in the regulation of tumor invasion, metastasis and chemoresistance, which is achieved by inhibiting the ZEB1 and ZEB2 genes [4, 19]. miR-200b-3p controls the post-transcriptional regulation of 757 genes, but there is no published report on its expression (up- or down-regulation) after a long period of PAX resistance development. In our study, we detected an up-regulation of miR-200b-3p only in VCaP-R cell lines, which may have been due to a different intracellular process that is controlling the resistance mechanism. One possible connection between the miR-200b-3p and tumor invasion, metastasis and chemoresistance may be through the regulation of the corresponding genes. For instance, it was found that inhibition of miR-200b could enhance the sensitivity of cholangiocarcinoma cells to gemcitabine, probably by modulation of CLOCK, PTEN, PTPN12 and the downstream oncogene products such as c-Abl, Src, and Ras [20]. Furthermore, remarkable evidence exhibited the existence of similarities between drug-resistant and metastatic cancer cells in terms of resistance to apoptosis and enhanced invasiveness.

Six miRNAs showed an approximately 9- to 92-fold down-regulation in paclitaxel-resistant prostate cancer cell lines; these included miR-17-5p, miR-19b-3p, miR-20b-5p, miR-26b-5p, miR-374b-5p and miR-616-3p. Only three of them (miR-19b-3p, miR-26b-5p, and miR-374b-5p) were down-regulated in all three paclitaxel-resistant cell lines. The miR-17-92 miRNA group has been defined as oncogenic a member of this group, miR-19b-3p, was reported to be up-regulated in human breast, melanoma and ovarian cancers [21]. In contrast, some of the miRNAs in the miR-17-92 group (miR-19 and miR-92) were reported to be markedly down-regulated in gastric and acute leukemia

Table 4 Fold change of miRNAs showing differences in expression as obtained by RT-PCR analysis of VCaP-R/VCaP, PC-3-R/PC-3 and DU145-R/DU145 cell lines

miRNA	VCaP-R/VCaP	PC-3-R/PC-3	DU145-R/DU145
hsa-miR-100-5p	8766		11.83
hsa-miR-125b-5p	2.75		5.85
hsa-miR-133a-3p		13.09	
hsa-miR-135b-5p		9.28	
hsa-miR-146a-5p	3.07	5.88	
hsa-miR-148a-3p	29.38		
hsa-miR-182-5p	5.98		2.14
hsa-miR-200b-3p	163.8		
hsa-miR-296-5p		2.09	
hsa-miR-34b-3p			79.13
hsa-miR-3163_1_382096-1		4.42	
hsa-miR-494-3p	3.41		
hsa-miR-616-3p	5.33		
hsa-miR-375			607.5
hsa-let-7a-5p		−2.52	
hsa-let-7c-5p		−2.25	
hsa-let-7f-5p		−4.05	−4.39
hsa-miR-101-3p	−5.27		
hsa-miR-106b-5p		−3.22	−7.59
hsa-miR-128-3p	−2.3	−2.13	
hsa-miR-141-3p			−2.25
hsa-miR-15a-5p	−2.41	−3.02	
hsa-miR-15b-5p		−7.33	−2.8
hsa-miR-16-5p		−2.44	
hsa-miR-17-3p	−5.21		
hsa-miR-17-5p			−11.4
hsa-miR-181b-5p	−2.12		
hsa-miR-183-5p		−2.31	
hsa-miR-194-5p		−3.15	
hsa-miR-196a-5p			−6
hsa-miR-19b-3p	−4.36	−2.14	−13.07
hsa-miR-200c-3p	−2.31	−5.05	
hsa-miR-20a-5p		−3.56	
hsa-miR-20b-5p	−3		−34.58
hsa-miR-21-5p		−2.69	−2.19
hsa-miR-221-3p	−2.43	−3.36	
hsa-miR-222-3p		−2.27	
hsa-miR-24-3p		−2.87	
hsa-miR-25-3p		−5.18	
hsa-miR-26a-5p		−2.06	
hsa-miR-26b-5p	−10.95	−23.52	−14.76
hsa-miR-27b-3p			−5
hsa-miR-29b-3p		−2.05	
hsa-miR-330-3p	−2.05		
hsa-miR-361-5p		−2.57	
hsa-miR-374b-5p	−4.43	−22.22	−20.29
hsa-miR-425-5p		−2.12	
hsa-miR-616-3p			−92.14
hsa-miR-7-5p	−4.72	−2.02	
hsa-miR-92a-3p		−2.09	
hsa-miR-93-5p		−2.12	

Table 4 (continued)

miRNA	VCaP-R/VCaP	PC-3-R/PC-3	DU145-R/DU145
hsa-miR-96-5p			− 3.94

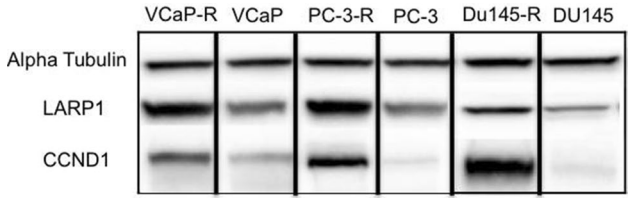

Fig. 3 LARP1 and CCND1 protein expression was evaluated by western blotting

patient samples [22, 23]. We also found that these miRNAs were down-regulated in paclitaxel-resistant prostate cancer cell lines. The difference in the expression of these miRNAs in different tissues was, however, not clear; this contradiction may have been due to a number of reactions that occurred in the different cancer cells due to the stem cell source, or they may have been caused by the active uptake by the cells from blood or the selective digestion of miRNAs in the cytoplasmic environment. The complete picture of the location and source of miRNAs in the chemoresistance system awaits further clarification.

Our study showed that miR-26b-5p was the miRNA most significantly down-regulated in all the three paclitaxel-resistant prostate cancer cell lines. This miRNA plays an important role in the regulation of proliferation, angiogenesis and apoptosis in hepatocellular carcinoma [24] and we suggest that it is relevant in the development of PAX resistance in prostate cancer. Although its biologic function in breast cancer is not known, the over-expression of miR-374b-5p is known to result in the inhibition of FGF and TGF pathways, which limits the invasion of tumor cells [25].

Our study showed that all three prostate cancer cell lines became resistant to PAX but showed markedly different expression profiles. Such diversity may have been due to different cancer cell sources, metastatic or not, or to their susceptibility to androgens and capability of becoming metastatic. Moreover, all three cancer cell lines gained PAX resistance over different periods, and the VCaP cell line had the shortest duration. This was demonstrated with the IC_{50} values obtained from the cytotoxicity tests, as shown in Table 3. Because the number of genes controlled by miRNAs is enormous, it reduced the chance of detecting the single gene responsible for developing PAX resistance. RT-PCR and western blot analyses helped to identify two genes that may be responsible for PAX resistance. One of these genes, LARP1, has recently attracted the attention of investigators. LARP1 is a highly evolutionarily conserved RBP and a member of the LARP family, each member of which carries a conserved La domain. LARP1 is a regulator of both mRNA stability and translation [26, 27].

LARP1 protein is highly expressed in hepatocellular carcinoma, squamous cervical cancer, prostate cancer and lung cancer, and the up-regulation of this protein is an independent predictor of adverse prognosis [26]. The expression of LARP1 is elevated in squamous cervical cancer, where it promotes cell motility and invasion, and reduces apoptosis [27, 28].

Moreover, the mammalian (or mechanistic) target of rapamycin (mTOR) complex 1 (mTORC1) kinase is regulated by LARP1, which has identified this protein as a key player in the mTOR pathway and as a regulator of cellular growth and proliferation [29]. Kato et al. also reported that miR-26a/b targets and down-regulates LARP1 and inhibits prostate cancer cell invasion by regulating the ribosome, RNA transport and mTOR signaling pathways [30].

The importance of LARP1 in the chemoresistance mechanism has been reported for the first time in a study in ovarian cancer cell lines. Following LARP1 knockdown, the mRNA levels of pro-survival genes such as BCL2, ERBB3 and AKT3 were significantly reduced, whereas the expression of pro-apoptotic genes, including BIK, TNF and DAPK2, was increased. As a result, treatment with chemotherapeutic drugs such as cisplatin, salinomycin and gemcitabine, increased the apoptosis rate up to four times [28].

Two miRNAs (miR-26a-5p and miR-26b-5p) have been shown to target the LARP1 gene/protein [30] and one miRNA (miR-374a-5p) suggested to have LARP1 as target gene by NGS data [31] were down-regulated in all three paclitaxel-resistant prostate cancer cell lines in our study

Table 5 Fold change of genes that showed differences in expression

Gene	ZEB1	ZEB2	CDH1	PHLPP1	EphaA2	MYC	CCND1	LARP1	CAND1	BECN1
VCaP-R/VCaP	− 1.03	− 1.39	11.43	− 1.27	− 1.27	− 1.35	3.25	4.26	− 1.26	1.02
PC-3-R/PC-3	1.34	1.26	− 1.80	− 2.17	1.82	1.88	3.84	3.65	− 2.78	1.86
DU145-R/DU145	2.65	2.06	0.38	− 1.16	1.22	1.73	2.40	2.25	1.73	1.45

Table 6 Expression levels (expressed in fold change) of miR-26a-5p, miR-26b-5p and miR-374-5p (that exert a post-transcriptional control on the LARP1 gene) in prostate cancer cell lines

miRNA	VCAPR/VCAP	PC3R/PC3	DU145R/DU145
hsa-miR-26a-5p		−2.06	
hsa-miR-26b-5p	−10.95	−23.52	−14.76
hsa-miR-374-5p	−4.43	−22.22	−20.29

(Table 6). These results were supported by western blot (Fig. 3) and RT-PCR results (Table 5), indicating that the expression of LARP1 gene was up-regulated in all paclitaxel-resistant prostate cancer cell lines. Therefore, we believe that the LARP1 gene could play an important role in the development of PAX resistance in all prostate cancer cell lines.

The other protein that we showed to be increased in all the resistant cell lines was CCND1. This protein is coded by the CCND1 gene. The over-expression of this gene in cancer cells results in the down-regulation of Fas expression, which leads to an increase in chemoresistance and a decrease in apoptosis. A number of studies have shown that CCND1 plays a role in the development of chemoresistance in breast, lung, gastric and ovarian cancers [32–35].

Conclusion

In conclusion, miRNAs are well-conserved noncoding RNAs that broadly regulate gene expression through the post-transcriptional silencing of coding genes. Aberrant miRNA expression and activity are commonly observed in clinical prostate cancer. Functional studies have implicated miRNAs or the genes that interact with them in the initiation and progression of PCa and in the development of resistance to chemotherapeutics. With the accumulation of data, miRNAs may be future therapeutics or therapeutic targets.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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