Cisplatin and Paclitaxel Alter the Expression Pattern of miR-143/145 and miR-183/96/182 Clusters in T24 Bladder Cancer Cells

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Abstract

Although cisplatin-based chemotherapy is considered to be the treatment of choice for metastatic bladder cancer, its efficacy and tolerability has proven to be limited. MicroRNAs are small noncoding RNAs, whose genes are frequently organized in clusters. These molecules constitute posttranscriptional regulators of mRNA expression and are claimed to be deregulated in cancer. miR-143/145 and miR-183/96/182 clusters have been extensively studied in bladder cancer cells. Herein, we tried to add up to this knowledge by assessing the expression levels of the five mature microRNAs derived from the aforementioned clusters in T24 bladder cancer cells exposed to either cisplatin or paclitaxel. For both compounds, the viability of treated T24 cells was estimated via the MTT colorimetric assay and the Trypan Blue exclusion method, while a fraction of the cells was left to recover. The expression levels of all mature microRNAs were finally quantified both in treated and in recovered cells by performing real-time PCR. According to our data, cisplatin and paclitaxel strongly decreased T24 cells' viability, showing in parallel the ability to significantly down-regulate miR-143 levels, and up-regulate the expression levels of miR-145, miR-183, miR-96, and miR-182, which, in their total, demonstrated case-specific variations after recovery period. Clin Trans Sci 2015; Volume 8: 668–675

Keywords: cisplatin, microRNAs, paclitaxel, real-time PCR, T24 bladder cancer cells

Introduction

American Cancer Society has recently estimated that 74,000 new cases of urinary bladder cancer will be diagnosed during 2015 in U.S., while the respective number of deaths expected for the same year will overcome 15,500.[1] The most frequently diagnosed histological type of bladder cancer is transitional cell carcinoma which accounts for approximately 90% of all new cases.[2] About 25% of bladder malignant tumors belong to muscle-invasive bladder cancer (MIBC) which represents all tumors classified as T2 or higher according to the current tumor node metastasis (TNM) classification system.[3] The therapeutic approach applied for the management of MIBC is mainly determined according to the spread of the disease.[4,5] The use of chemotherapy and especially that of cisplatin-containing combinations is considered to be the standard of care for patients with metastatic bladder carcinoma. In particular, according to the latest clinical guidelines, metastatic urinary bladder cancer patients with good performance status and renal function should be treated with either the methotrexate, vinblastine, adriamycin, and cisplatin regimen, known as MVAC, or that of gemcitabine/cisplatin (GC) either alone or together with paclitaxel (PGC).[4-6] Cisplatin, also known as cisplatinum or *cis*-diamminedichloroplatinum (II), is a DNA-damaging agent, which demonstrates the ability to bind to purine residues on DNA and accordingly create intrastrand adducts. The latter interfere with the DNA repair mechanisms of the cells leading to lethal damage to DNA and ultimately cell death via various mechanisms.[7] The most recent addition to the combinatory therapeutic regimens recommended for the treatment of metastatic bladder cancer is paclitaxel.[4-6] This drug belongs to a group of mitotic inhibitors known as taxanes, which share the capability of inhibiting cell proliferation by binding to microtubules and thus blocking their disassembly reactions during mitosis.[8]

MicroRNAs (miRNAs) comprise a novel class of small noncoding RNAs implicated in a wide range of physiological and pathological conditions, including cancer.[9] These small molecules of approximately 19–25 nucleotides length are processed from longer primary RNA transcripts which frequently contain the sequences of more than one mature miRNA. In the latter case, the primary microRNA transcript resembles the polycistronic messenger RNAs (mRNAs) detected in bacteria and is considered to be the transcription product of several microRNA genes organized in clusters. MicroRNA biogenesis eventually results in the loading of the functional small RNA molecules onto a ribonucleoprotein complex known as RISC (RNA-induced silencing complex).[10] RISC uses the miRNA as a guide seeking mRNAs whose sequence shares partial complementarity with that of the miRNA. Several mRNAs can be recognized as targets per miRNA, while the outcome of this interaction is the suppression of translation or the degradation of the mRNA, which might occur either alone or in combination.[11]

Upon a search conducted in the literature, the mature miRNAs derived from the miR-143/145 and miR-183/96/182 clusters were found to be among the few miRNAs that their role is well documented in bladder cancer cells.[12-18] Thus, we herein sought to determine whether T24 bladder cancer cells respond to treatment with cisplatin and paclitaxel, indicated for bladder cancer therapy, by modulating the expression levels of these miRNAs.

Methods

Cell culture

The human bladder cancer cell line T24 was cultured in McCoy's 5A Medium Modified with 1.5 mM L-glutamine (PAA Laboratories GmbH, Pasching, Austria) and supplemented with 10% fetal bovine serum (FBS), 100 kU/L penicillin, and 0.1 g/L streptomycin. Cells were grown in an incubator at 37 °C in 5% CO_2.

MTT colorimetric assay

The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (Sigma-Aldrich, St. Louis, MO, USA) colorimetric assay

was performed as follows: 100 µL of cell suspension containing 1,000 cells was transferred to each well of a 96-well plate and cells were left overnight inside the incubator in order to adhere and adapt to culture environment. Various concentrations of cisplatin (Cisplatin DBL; Faulding Pharmaceuticals, Warwickshire, United Kingdom) and paclitaxel (Taxol, Bristol-Myers Squibb, Princeton, NJ, USA) were then added to culture medium of T24 cells in quadruplicate and three different incubation periods of 24, 36, and 48 hours followed. At the end of the aforementioned time intervals, 10 µL of MTT solution (5 mg/mL in phosphate-buffered saline [PBS]) was added to each well and subsequently plates were transferred inside the incubator for 4 hours. Afterwards, the supernatant of each well was removed and replaced by 100 µL of a lysis solution [12.5% (w/v) SDS and 45% (v/v) formamide] containing a suitable organic solvent for the produced formazan. Finally, the absorbance of all cell lysates was measured at 550 nm (reference wavelength set at 630 nm) with the assistance of a microtiter plate reader (HumaReader; Human GmbH, Wiesbaden, Germany).

Drug treatment and Trypan Blue dye exclusion test
In full proportion to MTT assay protocol, 10^4 cells/mL were seeded in cell culture flasks and cells were left overnight to adapt to culture environment, inside the incubator. Afterwards, selected concentrations of paclitaxel and cisplatin were added to the culture medium of each vessel, in triplicate, and cancer cells were incubated with either drug for 24, 36, and 48 hours. Following the completion of the 48-hour incubation period, T24 cells were left to recover in the incubator for 24 hours after drug-containing culture medium was replaced by fresh drug-free medium. For the 24-, 36-, and 48-hour treated cells, as well for their respective control counterparts, a Trypan Blue exclusion assay was performed. In brief, cancer cells were first trypsinized with (1×) trypsin-EDTA (PAA Laboratories GmbH) for 5 min at 37 °C and then pelleted via centrifugation. Subsequently, pelleted cells were resuspended in fresh medium and 50 µL of the produced cell suspension was diluted 1:10 in (1×) PBS and then subjected to Trypan Blue exclusion test. For the execution of the latter, a volume of the diluted cell suspension was mixed with Trypan Blue (Sigma-Aldrich, St. Louis, MO, USA) following manufacturer's recommendations and sequentially loaded onto a hemocytometer so as to perform the cell count.

RNA extraction
Total RNA from cultured cancer cells was extracted using TRI Reagent (Ambion Ltd, Huntingdon, United Kingdom) according to the manufacturer's instructions. A spectrophotometric analysis at 260 and 280 nm followed the extraction of total RNA so that its concentration and purity to be assessed.

microRNA levels quantification via real-time PCR
miRNA quantification was achieved by developing a highly sensitive quantitative real-time PCR protocol for each miRNA included in our study based on the basic principles of the methodology described by Shi and Chiang.[19] In particular, 1 µg of total RNA was initially subjected to polyadenylation in a 10 µL-reaction mixture composed of 2 U of *Escherichia coli* Poly(A) polymerase (PAP; New England Biolabs, Hertfordshire, United Kingdom), 1 mM ATP, and (1×) PAP buffer, which was incubated at 37 °C for 1 hour. Polyadenylated RNA was then reverse transcribed to cDNA within the same tube (final reaction volume of 20 µL) by adding 0.5 µM of a poly(T) adapter (5'-GCGAGCACAG AATTAATACGACTCACTATAGGTTTTTTTTTTTTVN-3',

V = A, G, C; N = A, T, G, C) and M-MuLV Reverse Transcriptase RNase H⁻ (Finnzymes, Espoo, Finland) according to manufacturer's instructions. Regarding real-time PCR reactions, SYBR Green I was used as the chemical detection system in a 10 µL-reaction mixture consisted of 0.1 µL of cDNA, (2×) KAPA SYBR FAST qPCR Master Mix (Kapa Biosystems, Charlestown, MA, USA), 200 nM of a universal reverse primer, and 200 nM of a miRNA-specific forward primer. All sequences of primers used for the execution of PCR reactions can be found in *Table 1*. The thermal protocol applied for all reactions included one step at 95 °C for 3 min and 40 sequential cycles of two steps performed at 95 °C for 15 s and at 60 °C for 1 min. Each sample was assayed in triplicate, while all PCR reactions ran in an ABI 7500 thermal cycler (Applied Biosystems, Foster City, CA, USA). PCR products specificity was confirmed by conducting melting curve analysis at the end of each reaction. For miRNA levels assessment, a relative quantification (RQ) approach was performed by applying the comparative threshold cycle (C_T) ($2^{-\Delta\Delta C_T}$) method after all prerequisite conditions have been met.[20] In brief, the relative expression levels of all mature miRNAs analyzed per sample were measured by firstly determining C_T of each miRNA and then normalizing its value to that corresponding to the C_T of the small nucleolar RNA, C/D box 48 (*SNORD48*; also known as *RNU48*) selected as the appropriate reference gene. *RNU48* expression was analyzed by using the same experimental conditions mentioned above. Afterwards, the RQ units of each miRNA per sample, expressed in copies of target gene to copies of reference gene (miRNA copies/*RNU48* copies), were calculated by applying the $2^{-\Delta\Delta C_T}$ formula, in which $\Delta\Delta C_T$ stands for the difference between

Small RNA-specific forward primers		Accession Number (Database)
RNU48	5'-TGATGATGACCCCAGGTAACTCT-3'	NR_002745.1 (GenBank)
miR-183	5'-TATGGCACTGGTAGAATTCACT-3'	MIMAT0000261 (miRBase)
miR-96	5'-TGGCACTAGCACATTTTTGCTAAA-3'	MIMAT0000095 (miRBase)
miR-182	5'-TTTGGCAATGGTAGAACTCACA-3'	MIMAT0000259 (miRBase)
miR-143	5'-TGAGATGAAGCACTGTAGCTCAAA -3'	MIMAT0000435 (miRBase)
miR-145	5'-CCAGTTTTCCCAGGAATCCCTAA-3'	MIMAT0000437 (miRBase)
Universal reverse primer	5'-GCGAGCACAGAATTAATACGAC-3'	

Table 1. Sequences of primers used in real-time PCR reactions.

the above normalized C_T value ($\Delta C_T = C_{T\,miRNA} - C_{TRNU48}$) and the respective ΔC_T value calculated for the same miRNA in the sample set as the calibrator. The untreated (control) cells included in our study per incubation period served as the calibrator in all drug treatment experiments, whereas regarding recovery experiments two distinct RQ units were measured per miRNA either by using the 48-hour treated cells as a calibrator or by taking the average ΔC_T of the three control samples and applying their value to the $2^{-\Delta\Delta C_T}$ formula. In parallel, in order to verify the validity of our data, the amplification efficiencies for the five miRNAs and *RNU48* were estimated by constructing a standard curve per molecule and afterwards applying the formula $E(\%) = [-1 + 10^{(-1/\alpha)}] \times 100$, where E is the PCR amplification efficiency and α is the slope of the RNA-specific standard curve constructed.[20]

Statistical analysis

The statistical significance of all variations observed upon the pairwise comparisons performed between the differentially treated cells was estimated using a 2-tailed Student's *t*-test ($p < 0.05$).

Results

Cytotoxic activity of cisplatin and paclitaxel against T24 cells
Exposure of T24 cells to different concentrations of either cisplatin or paclitaxel for 24, 36, and 48 hours revealed the capability of both drugs to decrease the viability of these cells in a time- and dose-dependent manner (*Figure 1*). Upon the same process
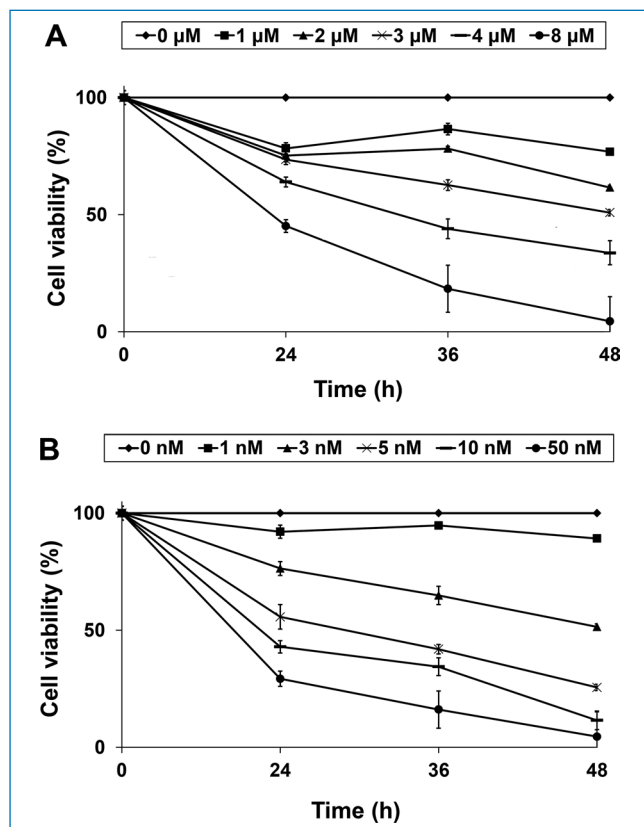

Figure 1. Impact of chemotherapy agents on cell viability of T24 cancer cells. The effect of (A) cisplatin and (B) paclitaxel on the viability of T24 cells was assessed via the colorimetric MTT assay. Each point represents the mean value (± standard error) of the (%) cell viability relative to the untreated cells (0 nM) as assessed from four biological replicates.

the maximal inhibitory concentration (IC50) of each drug was calculated and found to be equal to 2 μM for cisplatin and 3 nM for paclitaxel, respectively. By adding the aforementioned IC50 doses of these drugs to the culture medium of T24 cells and sequentially performing Trypan Blue assay, we found that the decrease noticed in cell viability was accompanied by almost no disruption of T24 cells' membrane as regardless of the incubation period that preceded, the percentage of Trypan Blue-positively stained cancer cells did not exceed 3.5% in either case.

Cisplatin- and paclitaxel-induced modulations in miR-143/145 and miR-183/96/182 clusters expression of T24 cells
Alterations in the expression of miR-143/145 and miR-183/96/182 clusters of T24 cells upon treatment with cisplatin or paclitaxel, as well as following their removal from the culture medium of the cells were estimated by analyzing the relative levels of the corresponding mature miRNAs via quantitative real-time PCR. The amplification plots generated per assay, as well the analytical specificity of each reaction, as attested by melting curve analysis, are illustrated in *Figure 2*, while the individual PCR efficiency of each amplicon was estimated and found to range from 96.0% to 97.9%, ensuring the validity of the variations recorded. In particular, cisplatin treatment had a negative impact on miR-143 expression leading to a significant down-regulation of 2.8-fold at 36 hours of treatment and another one of 1.8-fold in 48-hour treated cells (*Figure 3*A). Upon recovery though, the latter was reversed as miR-143 levels were found to be elevated by 2.4-fold compared with those of the 48-hour treated cells, reaching miR-143 expression levels detected in control cells (*Table 2*). Contrary to miR-143, cisplatin had a positive influence on miR-145 levels, whose expression was found to be up-regulated by 3.4-fold at 24 hours of incubation and by 2.2-fold at the respective time interval of 48 hours (*Figure 3*B). However, withdrawal of cisplatin from the culture medium of the cells further augmented miR-145 expression as illustrated in *Table 2*. Moving to the analysis performed for miR-183/96/182, cisplatin showed the ability to up-regulate miR-183 expression levels, which were found to be elevated at the end of all three indicated incubation periods by 2.6-, 2.1-, and 3.0-fold at 24, 36, and 48 hours, respectively (*Figure 3*C), while they were even more enhanced following recovery period (*Table 2*). As for miR-96 (*Figure 3*D) and miR-182 (*Figure 3*E), they exhibited a similar expression pattern, which consisted of lack of variation at 24 hours, an initial up-regulation by 2.4-fold for miR-96 and 2.9-fold for miR-182 at 36 hours, and a further increase at 48 hours of exposure equal to 3.8- and 5.0-fold for miR-96 and miR-182, correspondingly. Likewise, upon recovery both miRNAs were above the levels of control but exhibited the propensity to decline (*Table 2*). Analysis of paclitaxel-induced modulations in the expression of the aforementioned miRNAs disclosed an analogous expression pattern. In particular, miR-143 levels exhibited a 1.6-fold down-regulation at 24 and 48 hours of incubation but only the latter reached a statistically significant level (*Figure 4*A). Interestingly, the decline observed in miR-143 levels was not stopped even when paclitaxel was removed from the culture medium and T24 cells were left to recover (*Table 2*). The expression pattern of miR-145, though, was quite the opposite as it was found to be up-regulated by 2.2-, 3.0-, and 2.0-fold at 24, 36, and 48 hours of exposure (*Figure 4*B), respectively, whereas recovery process did not manage to further impact on its expression levels (*Table 2*). Likewise, all mature miRNAs of the miR-183/96/182 cluster were overexpressed upon treatment
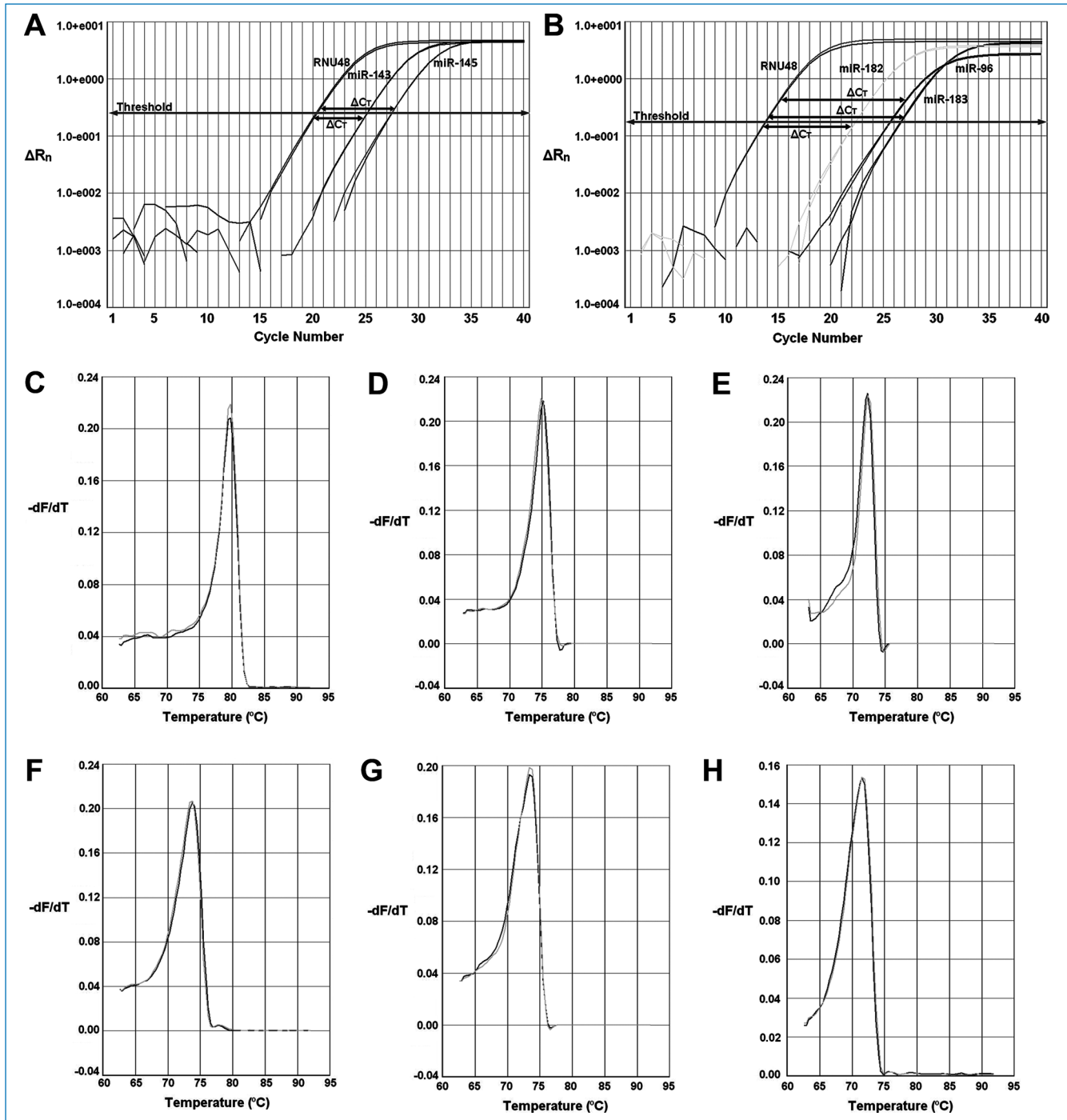
Figure 2. Ilustration of the amplification plots produced by real-time PCR for the reference gene *RNU48* and the mature miRNAs of the (A) miR-143/145 and (B) miR-183/96/182 clusters of T24 cells, along with the respective dissociation curves generated for (C) *RNU48*, (D) miR-143, (E) miR-145, (F) miR-183, (G) miR-96 and (H) miR-182. ΔRn displayed in amplification plots represents the normalized and baseline-corrected fluorescence intensity, while ΔC_T stands for the difference between the threshold cycles (C_T) of target and reference genes. In dissociation curves, the term -dF/dT, shown in *y* axis of the plot, corresponds to the first negative derivative of fluorescence over temperature.

(*Figure 4* C–E) but their miRNA levels dropped at the level of control cells after recovery period (*Table 2*). In detail, miR-183 levels were found be elevated at all three indicated time periods (24, 36, and 48 hours) by 1.7-, 4.5-, and 4.9-fold, correspondingly. Regarding miR-96, a 2.0-fold increase was noticed for both 36 and 48 hours of exposure, while miR-182 expression was found to be enhanced by 2.3- and 2.5-fold, at the respective time points.

Discussion

Metastatic bladder cancer is currently treated via the administration of chemotherapy regimens, which in their vast majority are platinum-based.[4–6] These combination chemotherapy schemes have managed to increase survival of bladder cancer patients but still their efficacy and tolerability require significant improvement.[21] Toward this direction, several studies have
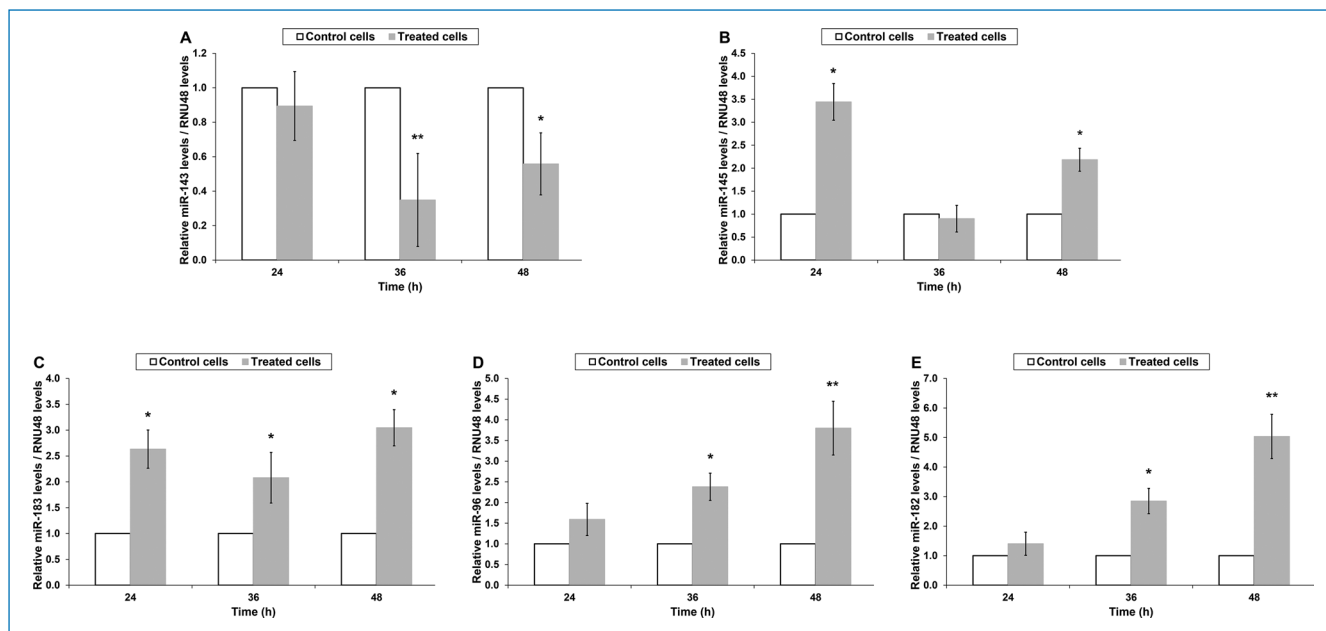
Figure 3. Cisplatin-induced modulations in microRNAs expression. The expression levels of the mature miRNAs derived from the (A, B) miR-143/145 cluster, as well as those of the (C, D, and E) miR-183/96/182 cluster of T24 cancer cells were estimated via real-time PCR. Each column represents the mean relative levels of each miRNA normalized to the expression levels of *RNU48* ± standard error according to the results of three biological replicates. Statistically significant variations have been marked by stars. (*$p < 0.05$; **$p < 0.01$).

sought to identify the molecular mechanisms implicated in the action of these agents in order to locate therapeutic targets that could enhance their antineoplastic activity.[22,23] Upon a search conducted in the literature, we found that a lot of research has been performed, at a preclinical level, regarding the potential impact of synthetic microRNA sequences on bladder cancer cells' viability. Even more, lots of these studies have especially focused on the mature miRNAs derived from the miR-143/145 and miR-183/96/182 clusters, whose function has been also extensively studied in bladder cancer cells.[12–18,24–26] Specifically, several protein-coding genes implicated in the process of carcinogenesis in urinary bladder have been identified as targets of the above miRNAs, as shown in *Table 3*. Nevertheless, the research conducted so far does not provide any information with regard to the effect that chemotherapy agents indicated for the management of bladder cancer exert on the expression levels of

these miRNAs. Thus, aiming to cover this missing link, we herein estimated how cisplatin and paclitaxel impact on the expression levels of the mature miRNAs generated from the above clusters both upon manifestation of cytotoxicity and following removal of these drugs from bladder cancer cells' environment.

So far, several studies have been conducted regarding the implication of miR-143 and miR-145 in different malignancies. Among them, the role of mi-143 and miR-145 has been extensively studied in colorectal cancer, prostate cancer, and esophageal squamous cell carcinoma, where both miRNAs are claimed to act as tumor suppressors, while their expression levels are found to be down-regulated.[31–34] The expression analysis performed herein disclosed a number of interesting and in some cases unexpected variations. The latter refers especially to the alterations noticed in miR-143 expression levels that, despite its also well documented antitumor activity in bladder cancer cells[12–14,24] and

| | Cisplatin | | Paclitaxel | |
| | Fold change* (*p*-value+) | | Fold change* (*p*-value+) | |
	Treated cells‡	Untreated cells§	Treated cells‡	Untreated cells§
miR-143	2.4 (0.031)	1.4 (n.s.)	−1.4 (n.s.)	−2.2 (0.019)
miR-145	3.5 (0.028)	7.6 (0.009)	1.0 (n.s.)	2.0 (0.044)
miR-183	3.5 (0.013)	10.5 (0.001)	−3.5 (0.013)	1.4 (n.s.)
miR-96	−1.5 (n.s.)	2.5 (0.021)	−1.9 (0.048)	1.3 (n.s.)
miR-182	−1.9 (0.039)	2.6 (0.008)	−2.0 (0.034)	1.0 (n.s.)

*Positive and negative values reflect increase and decrease in miRNA expression, respectively.
+n.s. stands for not significant variations (*p*-value > 0.05).
‡Fold changes in comparison with the relative levels of miRNAs detected in the 48-hour treated cells.
§Compared with the average of the relative miRNA levels measured in untreated (control) cells.

Table 2. Fold changes in the expression levels of microRNAs after recovery period.
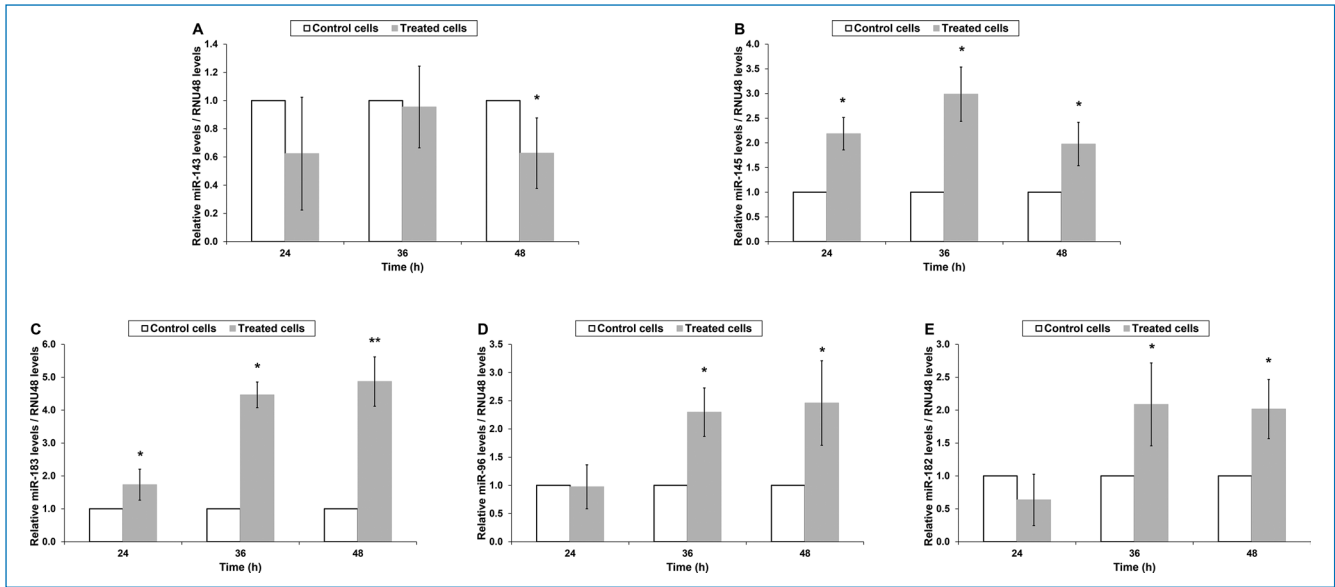
Figure 4. Alterations in microRNA levels of T24 cells upon treatment with paclitaxel. Relative levels (mean± standard error) of (A) miR-143, (B) miR-145, (C) miR-183, (D) miR-96, and (E) miR-182 of T24 cells were estimated by performing three independent biological experiments. Observed variations were accordingly subjected to statistical analysis and those demonstrating significance are illustrated as follows: *$p < 0.05$; **$p < 0.01$.

its proven down-regulation in bladder cancer tissue specimens,[35] demonstrated significant decrease upon treatment of T24 cells with either cisplatin or paclitaxel. This paradox is even more enhanced if we consider that cisplatin,[36] paclitaxel,[37] and miR-143[12,13] have been, separately, reported to be associated with the induction of apoptosis in bladder cancer cells. Data from previous preclinical studies in established bladder cancer cell lines suggest that the synthetic sequence of miR-143 should be considered for clinical investigation in bladder cancer.[12,13,24] Interestingly, in one of the aforementioned studies researchers subjected bladder cancer cells to co-treatment with cisplatin and synthetic miR-143 sequence concluding that this combination may exert additive effect on suppressing cells' growth.[12] Hence, our results seem to substantiate this concept as the tumor suppressor miR-143 appears to be down-regulated in T24 bladder cancer cells exposed to chemotherapy and thus restoring its expression levels might be a promising therapeutic approach. Furthermore, this might be even more interesting in case of paclitaxel, as our data show

that the down-regulation noticed in paclitaxel-treated cells was not reversed upon recovery. Instead, the other tumor suppressive miRNA generated from the miR-143/145 cluster[12,15,25] behaved as expected, as miR-145 levels were found to be up-regulated in T24 cells regardless of the chemotherapy drug added to their culture medium. Moreover, this increase was not affected upon withdrawal of the antineoplastic agents. Therefore, our results imply that miR-145 might be a crucial mediator of cisplatin and paclitaxel cytotoxic activity against bladder cancer cells. Within the frame of these observations, it is worthwhile mentioning that cisplatin and paclitaxel have been reported to trigger apoptotic cell death in bladder cancer cells, in which, likewise, miR-145 has been implicated in the induction of both caspase-dependent and caspase-independent apoptosis.[15,36,37]

Contrary to the mature miRNAs derived from miR-143/145 cluster of T24 cells, those of miR-183/96/182 cluster presented a more consistent expression pattern as they were all found to be up-regulated upon treatment with either drug and, with the

miR-143	miR-145	miR-183	miR-96	miR-182
AKT[12,14]	ILK[14]		IRS1[26]	SMAD4[17]
PAI-1[27]	PAI-1[27]	NR*	MAP4K1[26]	RECK[17]
COX-2[13]	CBFB[15]		FOXO1[18]	
ERK5[12]	CLINT1[15]			
	PPP3CA[15]			
	FSCN1[28]			
	SOCS7[25]			
	PAK1[29]			
	IGF-IR[30]			
*NR = none retrieved.				

Table 3. Identified gene targets of miR-143/145 and miR-183/96/182 clusters reported to be deregulated in bladder cancer.

exception of miR-183 in cisplatin-treated T24 cells, all miRNAs dropped at the level of control cells following the removal of the antineoplastic agents from cells' environment. Alike miR-143, though, these results were not in agreement with the suggested role of miR-183, miR-96, and miR-182 in bladder cancer, which has proven to be oncogenic.[16–18,26] Evidence toward this direction has been provided from different studies conducted by transfecting bladder cancer cells with either the synthetic sequences of the above mature miRNAs or with the antisense inhibitors of their action.[16,17,26] Within the same frame, the expression levels of miR-183, miR-96, and miR-182 have been reported to be up-regulated in bladder cancer upon analysis performed in fresh cancerous and adjacent noncancerous bladder tissue specimens.[35] In addition, the same expression pattern for all three mature miRNAs has been also well documented in colorectal cancer, prostate cancer, lung cancer, and hepatocellular carcinoma,[38] whereas miR-183/96/182 cluster has been claimed to demonstrate oncogenic properties in various malignancies.[39–42] Therefore, the fact that their expression levels are herein found to be elevated as a result of drug treatment might reflect the propensity of T24 bladder cancer cells to resist to the above chemotherapy agents. Although further experimentation is required in order to determine whether miR-183, miR-96, and miR-182 confer chemoresistance to bladder cancer cells, our data add up to current suggestions and moreover present a different perspective on the idea of considering the use of synthetic devices against the miR-183/96/182 cluster[16] for clinical evaluation in bladder cancer, either alone or in combination with conventional chemotherapy.

Conclusion

Overall, we herein present a number of significant modulations in the expression levels of the mature miRNAs derived from the miR-143/145 and miR-183/96/182 clusters of T24 bladder cancer cells upon their treatment with cisplatin or paclitaxel, as well as following the removal of the aforementioned drugs from cells' environment. In their vast majority, the alterations noticed in the expression of the five mature miRNAs of T24 cells were found to be inconsistent with their either oncogenic or tumor suppressive role, as defined by previous studies, reflecting the complexity of the molecular events that occur upon exposure of bladder cancer cells to these anticancer agents. In addition, our data along with those from other preclinical studies argue in favor of considering synthetic miRNA sequences as investigational products for clinical evaluation in bladder cancer.

Acknowledgments

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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