Cellular & Molecular Biology
Cell. Mol. Biol. 2015; 61 (2): 98-103
Published online May 28, 2015 (http://www.cellmolbiol.com)
Received on April 22, 2015, Accepted on May 18, 2015.
doi : 10.14715/cmb/2015.61.2.18

siRNA-mediated silencing of MDR1 reverses the resistance to oxaliplatin in SW480/OxR colon cancer cells

N. Montazami[1], M. Kheirandish[2], J. Majidi[1], M. Yousefi[1], B. Yousefi[3], L. Mohamadnejad[1], D. Shanebandi[1], M. A. Estiar[4], V. Khaze[1], B. Mansoori[1], E. Baghbani[5] and B. Baradaran[1]

[1] Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.
[2] Department of Immunology, Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran.
[3] Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran.
[4] Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
[5] Department of Immunology, Faculty of Medicine, Semnan University of Medical Sciences, Semnan, Iran.

Corresponding author: Behzad Baradaran, Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran. E-mail: behzad_im@yahoo.com

Abstract

One of the most challenging aspects of colon cancer therapy is rapid acquisition of multidrug resistant phenotype. The multidrug resistance gene 1 (MDR1) product, p-glycoprotein (P-gp), pump out a variety of anticancer agents from the cell, giving rise to a general drug resistance against chemotherapeutic agents. The aim of this study was to investigate the effect of a specific MDR1 small interference RNA (siRNA) on sensitivity of oxaliplatin-resistant SW480 human colon cancer cell line (SW480/OxR) to the chemotherapeutic drug oxaliplatin. SW480 cells were made resistant by continuous incubation with stepwise serially increased concentrations of oxaliplatin over a 6-months period. Resistance cell were subsequently transfected with specific MDR1 siRNA. Relative MDR1 mRNA expression was measured by Quantitative real-time PCR. Western blot analysis was performed to determine the protein levels of P-gp. The cytotoxic effects of oxaliplatin and MDR1 siRNA, alone and in combination were assessed using MTT and the number of apoptotic cells was determined with the TUNEL assay. MDR1 siRNA effectively reduced MDR1 expression in both mRNA and protein levels. MDR1 down-regulation synergistically increased the cytotoxic effects of oxaliplatin and spontaneous apoptosis SW480/OxR. Our data demonstrates that RNA interference could down regulate MDR1 gene expression and reduce the P-gp level, and partially reverse the drug resistance in SW480/OxR cells in vitro. Therefore, the results could suggest that MDR1 silencing may be a potent adjuvant in human colon chemotherapy.

Key words: Colon cancer, MDR1, P-gp, Oxaliplatin, SW480 cells.

Introduction

Colon cancer is among the major causes of morbidity and mortality in the world (1). Among available treatment modalities for treatment of cancer in advanced stage, chemotherapy is the best (2-3). Oxaliplatin (LOHP) is the third generation of platinum-based drugs and is a first-line therapy for treatment of colon cancer. Oxaliplatin has been tested as the standard first-line drug in patients with the aggressive forms of colon cancer in a number of clinical trials (4). Although treatment has improved greatly in the recent decades, chemotherapy has often failed in long term due to the development of multi-drug resistance which renders chemotherapy ineffective and also due to the toxicity of the chemotherapeutic compounds that limits the dosage and duration of treatment (5-7). The presence or development of resistance to anticancer drugs remains the main cause of chemotherapy failure not only in colon cancer, but also in the majority of the most common forms of cancer e.g., lung and breast (8).

In cancer cells with congenital or acquired multidrug resistance, the resistant cancer cells survive the chemotherapy and proliferate infinitely, finally resulting in patient death due to the lack of response. Therefore, it is necessary to investigate the mechanism of multiple drug resistance in cancer cells, to be able to possibly provide new methods of overcoming this resistance in clinical setting (9). Multiple drug resistance in cancer cells is mainly associated with an increased cellular drug efflux accumulation and a reduced intracellular drug (10). The overexpression of P-gp is a causing of both acquired or/and intrinsic drug resistance in many tumor cells (11). By inhibiting P-gp, it seems that drug resistance could be reduced and cancer cells eliminated. RNA interference (siRNA) is a specific and straightforward approach for blocking the expression of specific genes in post-transcriptional level and can lead to specific endonucleolytic cleavage of mRNA (12-14).

In this study, we specifically investigated the effects of MDR1 silencing in oxaliplatin-resistant human colon cancer cell line SW480. We have shown that MDR1 silencing can increase the sensitivity of colon cancer cells to oxaliplatin and can be considered as a potential strategy for overcoming drug resistance in cancer patients.

Materials and methods

Cell culture

RPMI1640 culture medium and fetal bovine serum (FBS) were purchased from Gibco BRL Company. SW480 cell line was obtained from Pasture Institute, Tehran, Iran. SW480 cells were grown in RPMI1640 supplemented with 10% *(v/v)* FBS, penicillin (100 U/ml), Simga-Alderich,st.louis, MO ,USA, and streptomycin (100 µg/ml) and maintained at 37°C in humidi-

fied 5% CO_2 atmosphere.

Oxaliplatin-resistant (OxR) SW480 cells generation

SW480/OxR cell line was generated by continuous incubation of SW480 cells with stepwise increases in oxaliplatin (Cayman Chemicals, Ann Arbor, MI, USA) concentration over a 6-months period. SW480 cells were seeded into two flasks, 1×10^6 cells per 25 cm^2 flask. When the cells were in logarithmic growth phase, oxaliplatin was added to the medium to a final concentration of 0.1 μM. After 24 h incubation, the old medium was discarded and fresh medium was added. Cells were subcultured when they were 80% confluent and oxaliplatin of 0.1 μM was then added. The concentration of oxaliplatin was gradually increased after the cells had stably grown. Finally, cells resistant to oxaliplatin in IC50 concentration were generated. It is important to subculture the untreated parental cells alongside the treated cells as a control, since continuous cell culture can result in alterations in cellular characteristics, including drug resistance. After 6 months of treatment, the resistant cells formed.

siRNA transfection

siRNA was purchased from Thermo Sientefic Company. Before siRNA transfection, the SW480/OxR were seeded at a density of 2×10^5 cells/well in 6-well plates (untraetment cells were kept as controls) in serum- and antibiotic-free RPMI-1640 medium. All transfections were performed using Lipofectamine™2000 reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's recommendations. Briefly, lipofectamine (4 μl/ml of transfection medium) and siRNA (at a final concentration of 80 nM) were diluted in Opti-MEM I medium (Invitrogen) separately and mixed gently. After incubation for 20 min at ambient temperature, the diluted solutions were combined and incubated for another 15 min at ambient temperature. Afterwards, the mixtures were added to each well (the cells incubated only with lipofectamine were considered as controls). The cell culture plates were then incubated for 6 hours at 37°C in a CO_2 incubator. The cells incubated under the above mentioned conditions were added in RPMI1640 medium containing FBS antibiotics (concentration of 2X normal growth medium) and oxaliplatin with IC50concentration without removing the transfection mixture.

qRT-PCR

MDR1 mRNA levels were measured by qRT-PCR Total RNA was isolated from cells using AccuZolTM reagent (Bioneer, Daedeok-gu, Daejeon, Korea) according to the manufacturer's instructions. Complementary DNA (cDNA) was synthesized by cDNA synthesis Bioneer kit from 1 μg of total RNA. Following on, qRT-PCR was performed in the Rotor-GeneTM 6000 system (Corbett Life Science, Australia). The PCR reaction conditions: 3 μl of cDNA template, 0.5 μM of each primer, 12 μl of SYBR green reagent and 9 μl of nuclease-free distilled water. The MDR1 primer sequences were as follows: forward, 5'-AGC TAT CGT GGT GGC AAA C-3', reverse, 5'-ATG GTC AGT GTT GAT GGA CAG-3'. Cycling conditions were as follows: 94°C for 5 min for cDNA and primer denaturing, followed by 35 cycles at 94°C for 30 sec, 56°C for 30 sec, and 72°C for 40 sec. Relative MDR1 mRNA expression was determined with the $2^{-(\Delta\Delta Ct)}$ method using 18s rRNA as the reference gene.

Western blot analysis

Following treatments, the cells were washed twice with PBS and resuspended in lysis buffer (50 mMTris-HCl, pH 7.4, 1% SDS, 1% Triton X-100, 150 mM-NaCl, 1mM EDTA, pH 8) containing protease inhibitor (Roche Diagnostics GmbH) for 20 min, then the lysates were centrifuged at 12,000 rpm for 15 min at 4°C. Subsequently, 50 μg of each protein sample was separated on 10% SDS-PAGE and electrotransferred to PVDF membranes GE Healthcare, Amersham, Buckinghamshire, UK). The membranes were blocked with 5% nonfat milk in PBS for 1 h at room temperature, then incubated overnight at 4˚C with monoclonal antibodies against MDR1/ABCB1 (1:500, Abcam, Cambridge, MA, UK) and β-actin (1:5,000, Abcam, Cambridge, MA, UK) overnight. After three 5 min washes with PBS/T, membranes were incubated with appropriate horseradish peroxidase-linked goat anti-mouse secondary antibody (1:2,000, Abcam) for 1 h at room temperature. After rinsing, the protein bands were visualized using the ECL chemiluminescence system (GE Healthcare). Protein bands intensity were measured by ImageJ 1.6 software (National Institues of Health, Maryland, USA) and normalized to its β-actin.

Cell cytotoxicity assay

The effect of MDR1 siRNA on the chemosensitivity of cells was measured using 3-(4, 5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay.Briefly, SW480/OxR cells and SW480 as control were seeded in 96-well plates (8,000 cells/well), each containing 0.2 ml of medium. After 24 h, siRNA transfection was performed and oxaliplatin was added 6 h after transfection. Then cells were incubated at 37˚C in 5% CO_2. After 24h or 48h, MTT solution (5 mg/mL in PBS) was added to the wells and incubated for 4 h at 37 °C. Live cells reduce the tetrazulium-based compound to a purplish formazan. Optical density (OD) values were read on a Synergy HT multi-detection microplate reader (Bio-Tek Instruments, Inc., Winooski, VT) at λ=570 nm. The amount of formazan dye was determined by quantifying its absorbance (A) at 570 nm by microplate reader (Awareness Technology, Palm City, FL, USA). The survival rate (SR) was assessed by following equation: SR(%)=(A Treatment /A Control)×100%. The concentration that produced 50% cytotoxicity (IC50) was determined using GraphPad Prism 6.01 software.

Apoptosis assay

The TUNEL assay (Roche Molecular Biochemicals) was carried out following the manufacturer's instruction for assessment of apoptosis. Briefly, cells were cultivated in 96-well plates (8,000 cells/well), and subsequently, transfection and drug treatment were performed as described above. After 48h, cells were fixed with 4% paraformaldehyde solution (Sigma-Aldrich, St. Louis, MO, USA) at room temperature in PBS, then treated with 0.3% H_2O_2-methanol solutionandpermeabilized with 0.1% Triton X-100 in 0.1% sodium citrate solution for 2 min on ice.

Combination effect analysis

To explore the interaction between survivin siRNA and etoposide, combination effect analysis was performed, based on the principles described by Chou and Talalay (Chou and Talalay, 1984). The value of coefficient of drug interaction (CDI) was calculated using the following formula: $CDI=S_{AB}/(S_A \times S_B)$, where S_A and S_B are the survival rate of oxaliplatin and MDR1 siRNA relative to the corresponding control, S_{AB} is the survival rate of the combination treatment relative to the control. CDI<1, CDI=1 and CDI>1 indicate synergistic, additive and antagonistic effects, respectively.

Results

Oxaliplatin IC50 in SW480 cells

The IC50 values were determined by MTT assay. SW480 cells that could tolerate up to 3.5 μM oxaliplatin were obtained by induction of resistance with increasing the concentration of oxaliplatin in a period of 3 month. The IC50 for oxaliplatin in SW480 cells was 3.8 ± 0.6 μM in 48 h. Figure 1, demonstrates that MDR1 mRNA levels in SW480/OxR cells was increased 2.6 fold compared with parental cells.

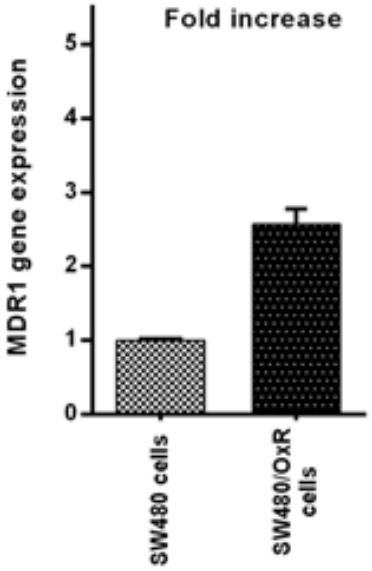

Figure 1. MDR1 mRNA levels in parental and oxaliplatin-resistant cells (resistant to 3.5 μM).

siRNA down-regulated MDR1 mRNA in cells

MDR1 mRNA expression was measured by qRT-PCR. MDR1 mRNA expression was investigated in oxaliplatin-resistant colon cancer transfected with MDR1 siRNA in 24 and 48 h as well as in oxaliplatin-resistant cells. The MDR1 gene expression was inhibited in oxaliplatin-resistant cellstransfected with MDR1 siRNA both after 24 h and 48 h. Overall, MDR1 siRNA induced a 50% inhibition of the MDR1 mRNA expression as assessed by qRT-PCR 24 h after transfection in the oxaliplatin-resistant cells. MDR1 siRNA caused a 65% inhibition in MDR1 mRNA 48 h after transfection. Therefore, the inhibition rate of MDR1 in SW480 was time dependent (Figure 2).

siRNA suppressed P-gp protein levels in cells

The P-gp protein expression in siRNA transfected

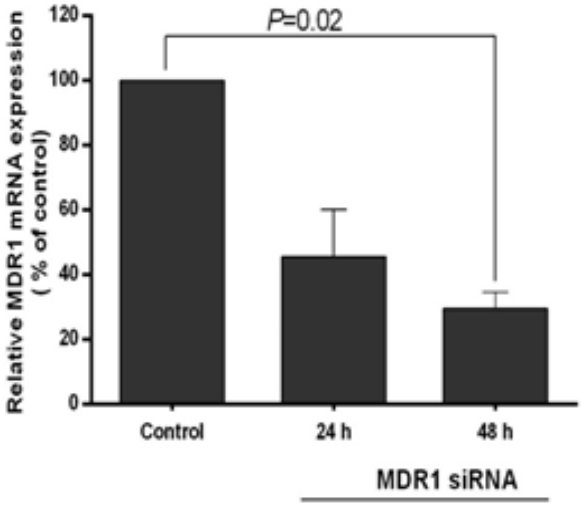

Figure 2. MDR1 siRNA could down-regulate the expression of MDR1 in oxaliplatin-resistant SW480 cells. Cell line was transfected with siRNA and treated with oxaliplatin. Subsequently, MDR1 mRNA expression was measured 24 h and 48 h after addition of oxaliplatin and siRNA. The inhibition of MDR1 mRNA expression was more significant in 48h. p=0.02.

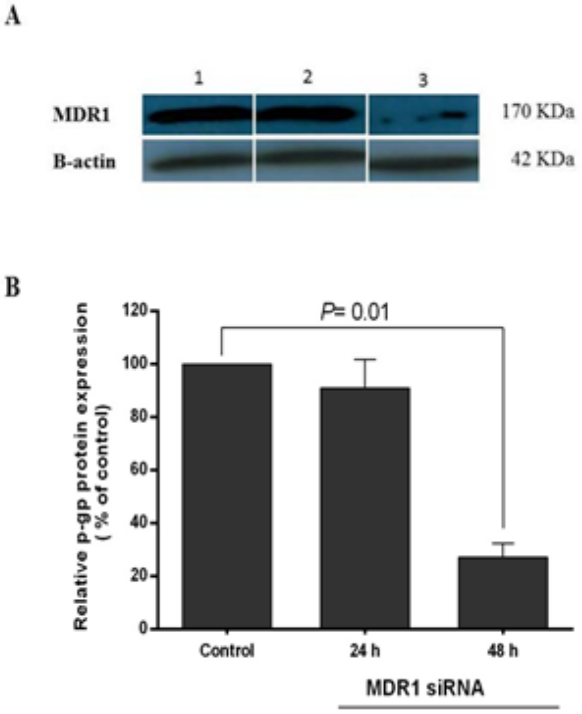

Figure 3. **A.** Western blotting analysis showing p-gp expression in SW480/OxR cells as control (1), SW480/OxR cells transfected with MDR1 siRNA after 24 h (2), and SW480/OxR cells transfected with MDR1 siRNA after 48 h (3). **B.** The inhibition of MDR1 protein expression was more significant 48 h after treatment of SW480/OxR cells with siMDR1.

resistant SW480 cells was evaluated by Western blot analysis. As shown in Figure 3, treatment with siRNA could successfully reduce the levels of ABCB1 protein in 24 h. The effect was even more prominent after 48 h.

MDR1 silencing increased the apoptotic cells

The TUNEL assay is extensively used to detect apoptosis in individual cells and this method enables enzymatic labeling and detection of genomic DNA breaks. Subsequently, apoptotic cells can be quantified by fluorescence or light microscopy. Since the TUNEL assay is

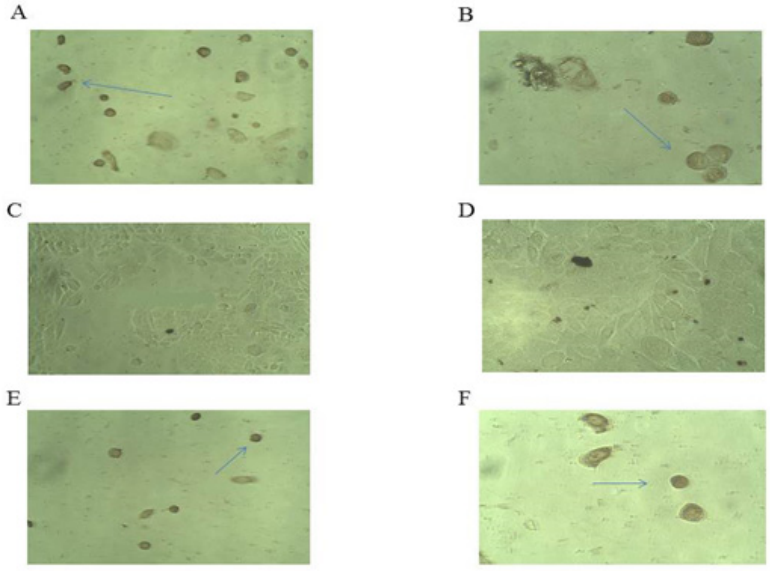

Figure 4. A. 20x magnification and **B.** 40x magnification; in posetive control group (SW480 cells treatmented with oxaliplatin), a higher number of brown-stained cells were observed. **C.** 20x magnification and **D.** 40x magnification; in negative control group (SW480/OxR cells), a higher number of cells without brown-staining were found. **E.** 20x magnification and **F.** 40x. magnification; In SW480/OxR cells transfected with MDR1 siRNA after 48 h.

prone to false negative and positive results, TUNEL test interpretation was done with the color reaction. Based on TUNEL staining, a higher number of brown-stained cells were found in cells transfected with MDR1 siRNA group compared to those of the control group ($P<0.01$) (figure 4).

Down-regulation of MDR1 sensitizes cells to oxaliplatin

The MDR1 siRNA-mediated reversal of the oxaliplatin-resistant phenotype was evaluated by comparison of oxaliplatin cytotoxicity using MTT assay. Cytotoxicity was measured 48 h after treatment with siRNA and oxaliplatin in siRNA treated and untreated resistant control cells. As measured by MTT assay, the cytotoxicity of oxaliplatin was higher in siRNA-treated oxaliplatin-resistance SW480 cells than in untreated resistant cells, suggesting that MDR1 siRNA inhibits P-gp expression and partially restores the sensitivity of these resistant cell lines to oxaliplatin (Figure 5).

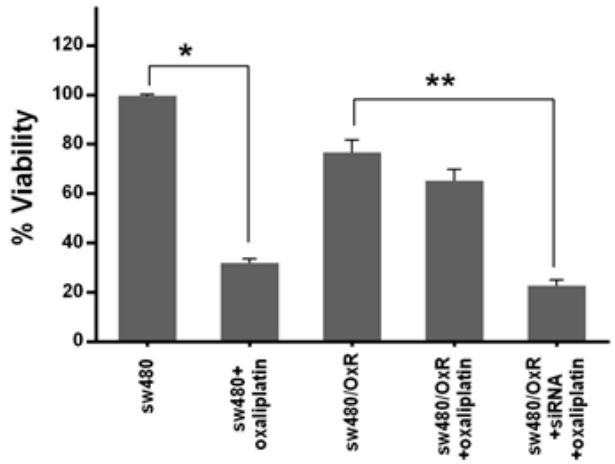

Figure 5. Effect of MDR1 down-regulation on cell viability (48 h). * p= 0.0001,**p<0.001, versus control.

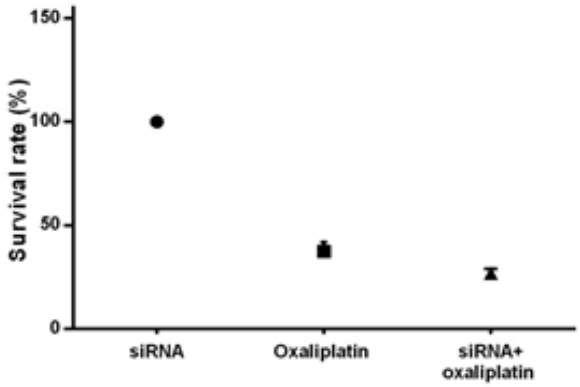

Figure 6. **Effect of siRNA on the chemosensitivity of the SW480 Cells**. The cells were treated with MDR1 siRNA, oxaliplatin and their combination (oxaliplatin IC50) for 48 h and then the cytotoxicity was measured with MTT assay. The interaction effect between siRNA and oxaliplatin was determined by calculating the CDI values. CDI< 1.

MDR1 siRNA enhanced the cytotoxic effect of oxaliplatin

To investigate whether reduced MDR1 expression could enhance the sensitivity of SW480 cells to oxaliplatin, a combination treatment with MDR1 siRNA and oxaliplatin was performed. As shown in Figure 6, single treatment with oxaliplatin reduced survival. Also, results showed that combination therapy synergistically lowered the cell survival with the CDI values of less than 1 ($p<0.05$).

Discussion

Despite the recent improvements in the production of new anticancer agents, chemotherapy has often failed to treat certain cancer types.One of the major reasons can be the overexpression P-gp on the membrane of cancer cells (14), which makes them chemo-resistant by pumping the drug out of the cancer cell cytoplasm. siRNA technology has proved to be very promising in gene knockdown studies. Tuschl and colleagues (2001) have

shown that siRNAs enables a complete knockdown of a specific protein in numerous mammalian cell lines (15).

In this study, we evaluated whether siRNAs specific for MDR1mRNA could reverse the chemo-resistance phenotype in oxaliplatin-resistant colon cancer cells SW480 by reducing the P-gp expression. The results showed that MDR1siRNA significantly down-regulated the MDR1 mRNA expression in SW480/OxR cells. The effectiveness of siRNA in reducing the expression of P-gp resulted in the loss off chemo-resistant phenotype in SW480/OxR cells. The results were confirmed both in the RNA and protein levels using Real-time PCR and Western blot, respectively. Furthermore, our data confirms that MDR1 siRNA enhances the cytotoxicity of the anticancer drug oxaliplatin after 24 and 48 h in SW480/OxR cells, as shown by MTT assay.

In a previous study, Jennifer Perez and colleagues have shown that MDR1 siRNA significantly inhibits the proliferation of human MNNG/HOS osteosarcoma cells, embryonic kidney 293T cells, human adenocarcinoma cell line MDA-MB549 and neuroblastoma cell line Sk N-Be, in the presence of doxorubicin or methotrexate. This inhibition of cell proliferation was associated with decreasing the MDR1 mRNA levels. Moreover, in cells treated with MDR1 siRNA, an increase in the efficacy (reduction of IC50 values) of drugs was shown. These results have also shown that MDR1 siRNA reduced the innate chemo-resistance and prevented the acquisition of chemo-resistance (14).

In another study, Xia et al. investigated the effect of silencing MDR1 mRNA in the human COLO 320DM colon cancer cell line (multidrug resistant due to expression of P-gp) with HT-29 cells (not expressing P-gp) as the control. Targeted COLO 320DM cells with #4123 and #4029 MDR1 siRNAs reduced MDR1/P-gp-dependent multidrug resistance. However, the siRNAs had no effects in HT-29 control cells (16). Subsequently, the authors determined the effect of siRNAs on the sensitivity of COLO 320DM cells to doxorubicin and vincristine and showed that the siRNAs could stably reverse the multidrug resistance phenotype in colon cancer cells (16,17). In a study conducted by Duan et al. demonstrated the efficacy of both vector-based and synthetic expression of siRNA in specific reduction of the ABCB1(MDR1) and ABCB4 expression levels in paclitaxel-resistant SKOV-3TR and OVCAR8TR ovarian cancer cells. Although ABCB4 and ABCB1 share similar genetic characteristics (18), inhibiting ABCB1 expression played the major role in reversal of resistance to paclitaxel and ABCB4 siRNA could only induce a minor reduction in the paclitaxel-resistant phenotype (19). Furthermore, in two previous studies, similar synthetic siRNA-based suppression of ABCB1 gene expression in multidrug resistance cancer cell lines has successfully inhibited ABCB1 mRNA and P-gp expression (20,21) Peng and et al. have previously shown that MDR1 siRNA can effectively inhibit the expression of MDR1 gene in drug-resistant K562/A02 leukemia cells. However, the siRNA-induced inhibition was not complete and the maximal suppression was around 60%. Overall, the siRNA inhibited the P-gp expression and resulted in an increase in the intracellular accumulation of chemotherapeutic drugs such as doxorubicin, etoposide and vincristine in the leukemia cells (22) Finally, according to Dong and colleagues, siRNA treatment could enhance the therapeutic effect of neoadjuvant chemotherapy using paclitaxel or epirubicin in the MCF-7 cells, by significantly enhancing the sensitivity of the cells to drugs and apoptosis (23).

The stability of the complexed formed between siRNA and mRNA is a determinant factor influencing siRNA efficacy (24,25). The clinical application of MDR1 siRNA could be limited by the fact that non-malignant normal cells such as the peripheral blood cells and hematopoietic progenitors found in human bone marrow also have a relatively low level of MDR1 expression and P-gp protects these rapidly dividing cells from accumulation of toxic compounds inside the cells. Thus, if the MDR1 siRNA is to be used on humans in a clinical setting, it would be necessary to confine siRNA exposure only to MDR1/P-gp-expressing multidrug-resistant cancer cells. One proposed strategy is the delivery of MDR1 siRNA using targeted vector systems such as functionalized liposomes (26).

Although the clinical application of MDR1 siRNA has a number of obstacles to overcome, MDR1 siRNAs have shown efficacy in sensitizing various resistant cell lines to different chemotherapeutics. In this study, we demonstrated that a MDR1 siRNA can sensitize SW480 colon cancer cells to the first line chemotherapeutic agent oxaliplatin.

References

1. World Health Organization. Cancer Incidence in Five Continents. Lyon: The World Health Organization and The International Agency for Research on Cancer. 2002

2. Esteva, F.J., Valero, V., Pusztai, L., Boehnke-Michaud, L., Buzdar, A.U., Hortobagyi, G.N., Chemotherapy of metastatic breast cancer: what to expect in 2001 and beyond. *Oncologist*. 2001, **6**(2): 133–46. doi: 10.1634/theoncologist.6-2-133

3. Hortobagyi, G.N., Treatment of breast cancer. *N. Engl. J. Med.* 1998, **339** (18): 974–84. doi: 10.1056/NEJM199810013391407

4. Tourniqand, C., Andre, T., Achille, E., FOLFIRI followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer: a randomized GERCOR study. *Clin. Oncol.,* 2004, **22** (2): 229 -237. doi: 10.1200/JCO.2004.05.113

5. Harris, A.L., Hochhauser, D., Mechanisms of Multidrug Resistance in Cancer Treatment. *Acta. Oncologica*. 1992, **31**(2): 205-13. doi: 10.3109/02841869209088904

6. Kellen, J.A., The reversal of multidrug resistance: an update. *Exp. Ther. Onco*. 2003, **3** (1): 5-13. doi: 10.1046/j.1359-4117.2003.01067.x

7. Krishna, R., Mayer, L.D., Multidrug resistance (MDR) in cancer: Mechanisms, reversal using modulators of MDR and the role of MDR modulators in influencing pharmacokinetics of anticancer drugs. *Eur. J. Pharma. Sci.* 2000, **11**(4): 265-83. doi: 10.1016/S0928-0987(00)00114-7

8. Giaccone, G., Pinedo, H., Drug Resistance. *The Oncologist*. 1996, **1**(1 & 2):82-87.

9. Liu, Z., Qiu, M., Tang, Q.L., Liu, M., Lang, N., Bi, F., Establishment and biologic characteristics of oxaliplatin-resistant human colon cancer cell lines. *Chin. J. Cancer.* 2010, **29** (7): 661-7.

10. Cao, D.R., Yu, S.Y.X., P.H., Deng, Y.M., Method for Preparing Protopanoxadiol and Protopanaxatriol by Using Synergistic Oxidation and Alkaline Bydrolysis of Oxide and Hyperoxide. 2005. CN 200510100735.

11. Sharom, F.J., ABC multidrug transporters: structure, function

and role ichemoresistance. *Pharmacogenomic*. 2008, **9** (1):105-27. doi: 10.2217/14622416.9.1.105
12. Raoul, C., Abbas-Terki, T., Bensadoun, J.C., Lentiviral-mediated silencing of SOD1 through RNA interference retards disease onset and progression in a mouse model of ALS. *Nat. Med.* 2005, **11**(4):423-8. doi: 10.1038/nm1207
13. Hill, J.A., Ichim, T.E., Kusznieruk, K.P., Immune modulation by silencing IL-12 production in dendritic cells using small interfering RNA. *Immunol.* 2003, **171**(2):691-6. doi: 10.4049/jimmunol.171.2.691
14. Perez, J., Bardin, C., Rigal, C., Anthony, B., Rousseau, R., Dutour, A., Anti-MDR1 siRNA restores chemosensitivity in chemoresistant breast carcinoma and osteosarcoma cell lines. *Anticancer. Res.* 2011, **31**(9):2813-20.
15. Elbashir, S.M., Harborth, J., Lendeckel, W., Yalcin, A., Weber, K., Tuschl, T., Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature.* 2001, **411**(6836):494-8. doi: 10.1038/35078107
16. Xia, Z., Zhang, L., Chen, Q., Royal, C., Yu, Z., Liu, Z., Adam, B.L., Stable reversal of multidrug resistance in colon cancer cells by RNA interference targeting the MDR1 gene. *Mol. Med. Rep.* 2009, **2** (4):579-84. doi: 10.3892/mmr_00000140
17. Xia, Z., Zhu, Z., Zhang, L., Specific reversal of MDR1/P-gpdependent multidrug resistance by RNA interference in colon cancer cells. *Oncol. Rep.* 2008, **20** (6):1433-9. doi: 10.3892/or_00000163
18. Lincke, C.R., Smit, J.J., van der, Velde-Koerts, T., Borst, P., Structure of the human MDR3 gene and physical mapping of the human MDR locus. *Biol. Chem.* 1991, **266** (8):5303-10.
19. Duan, Z., Brakora, K.A., Seiden, M.V., Inhibition of ABCB1 (MDR1) and ABCB4 (MDR3) expression by small interfering RNA and reversal of paclitaxel resistance in human ovarian cancer cells. *Mol. Cancer. Ther.* 2004, **3** (7):833-8.
20. Wu, H., Hait, W.N., Yang, J.M., Small interfering RNA-induced suppression of MDR1 (P-glycoprotein) restores sensitivity to multidrug-resistant cancer cells. *Cancer. Res.* 2003, **63** (7):1515-9.
21. Nieth, C., Priebsch, A., Stege, A., Lage, H., Modulation of the classical multidrug resistance (MDR) phenotype by RNA interference (RNAi). *FEBS. Lett.* 2003, **545** (2-3):144-50. doi: 10.1016/S0014-5793(03)00523-4
22. Peng, Z., Xiao, Z., Wang, Y., Liu, P., Cai, Y., Lu, S., Feng, W., Han, Z.C., Reversal of P-glycoprotein-mediated multidrug resistance with small interference RNA (siRNA) in leukemia cells. *Cancer. Gene. Ther.* 2004, **11** (11):707-12. doi: 10.1038/sj.cgt.7700738
23. Dong, H., Yao, L., Bi, W., Wang, F., Song, W., Lv, Y., Combination of survivinsiRNA with neoadjuvant chemotherapy enhances apoptosis and reverses drug resistance in breast cancer MCF-7 cells. *J. Cancer. Res. Ther.* 2014, **26**(5): 310-314
24. U, Z.J., Mathews, D.H., Fundamental differences in the equilibrium considerations for siRNA and antisense oligodeoxynucleotide design. *Nucleic. Acids. Res.* 2008, **36** (11):3738-45. doi: 10.1093/nar/gkn266
25. Bohula, E.A., Salisbury, A.J., Sohail, M., Playford, M.P., Riedemann, J., Southern, E.M., Macaulay, V.M., The efficacy of small interfering RNAs targeted to the type 1 insulin-like growth factor receptor (IGF1R) is influenced by secondary structure in the IGF1R transcript. *Biol. Chem.* 2003, **278** (18):15991-7. doi: 10.1074/jbc.M300714200
26. Holm, P.S., Lage, H., Bergmann, S., Multidrug-resistant cancer cells facilitate E1-independent adenoviral replication: impact for cancer gene therapy. *Cancer. Res.* 2004, **64** (1):322-8. doi: 10.1158/0008-5472.CAN-0482-2