Original Paper

MiR-4673 Modulates Paclitaxel-Induced Oxidative Stress and Loss of Mitochondrial Membrane Potential by Targeting 8-Oxoguanine-DNA Glycosylase-1

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Key Words

miR-4673 • ROS • MMP • OGG1 • Paclitaxel

Abstract

Background: Our previous study identified a novel microRNA, miR-4673, which is upregulated in A549 cells exposed to paclitaxel (PTX). In this study, we investigated the role of miR-4673 in PTX-induced cytotoxicity. **Methods:** 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, apoptosis assay, 5,5',6,6'-Tetrachloro-1,1',3,3'-tetraethylimidacarbocyanine iodide (JC-1) staining and 2',7'-Dichlorofluorescein (DCFH) staining were used to evaluate cell viability, apoptosis, mitochondrial membrane potential (MMP) loss and reactive oxygen species (ROS) generation in A549 and H1299 cells. Bioinformatics analysis and Luciferase reporter assay were used to explore whether 8-oxoguanine-DNA glycosylase-1 (OGG1) is a target gene of miR-4673. **Results:** Enforced expression of miR-4673 decreased cell viability and increased PTX-induced apoptosis, MMP loss and reactive oxygen species (ROS) generation in A549 and H1299 cells. Bioinformatics analysis, which was used to identify potential target of miR-4673, revealed a binding site of miR-4673 in 3'UTR of OGG1. Luciferase reporters assays showed that miR-4673 specifically binds to 'CUGUUGA' in 3'UTR of OGG1. Enforced expression of miR-4673 decreased accumulation of OGG1. In addition, silencing OGG1 enhanced inhibitory effects of PTX on apoptosis, MMP loss and ROS generation, which is similar to effects of miR-4673. Moreover, enforced expression of OGG1 compromised promoting effects of miR-4673 on PTX-induced apoptosis, MMP loss and ROS generation. **Conclusion:** miR-4673 modulates PTX-induced apoptosis, MMP loss and ROS generation by targeting OGG1.

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Introduction

Drug resistance is one of the reasons caused failure of chemotherapy [1]. Increasing efforts had been made to reveal mystery of drug resistance, which is a complicated process and involves multiple regulation mechanisms [2, 3]. Unfortunately, the knowledge obtained from previous studies still cannot explain all the unsolved problems about drug resistance.

MicroRNAs (miRNAs) is a class of noncoding RNAs, whose length is about 18-22nt [4]. MiRNAs function as regulatory molecules in many physiological and pathological processes. Accumulating evidences indicates miRNAs play a critical role in regulating drug sensitivity of tumor cells [5]. They also involves in regulation of mitochondria.

Recent advances indicate mitochondria are a target for chemotherapy [6, 7]. Mitochondria are closely related to apoptosis. Changes of mitochondrial membrane potential (MMP) contribute directly to apoptosis. MMP loss is considered as an irreversible event during early apoptosis. Chemotherapy reagents cause reactive oxygen species (ROS) generation in tumor cells, which lead to oxidative damages, including DNA fragmentation and MMP loss [8].

According to the deleterious network hypothesis of apoptosis, ROS caused oxidative damages to mitochondrial DNA and critical proteins, which leads to dysfunction of electron transport chain and tricarboxylic acid cycle, deficiency of ATP, MMP loss, opening of mitochondrial permeability transition pore and release of cytochrome c. In the other hand, damages of mitochondria cause accumulation of ROS in cells [9].

8-oxoguanine-DNA glycosylase-1 (OGG1) is a base excision repair (BER) DNA glycosylase [10]. OGG1 involves in repair of oxidative DNA damage [11, 12]. Dysregulation or loss of OGG1 causes accumulation of oxidative DNA damage [13-15], which is strongly associated with various diseases, including cancer [16]. OGG1 polymorphism have been reported to associate to susceptibility of lung cancer [17-20], pancreatic cancer [21-24], bladder cancer [25-28], breast cancer [29-31], esophageal cancer, colorectal cancer. However, the mechanism about how OGG1 functions in lung cancer has not been reported yet.

We reported miR-4673 is up-regulated in A549 cells exposed to paclitaxel (PTX). This finding inspired us that miR-4673 may involve in PTX-induced cell death in lung cancer. We investigated the effects of miR-4673 on cell viability and apoptosis. The effects of miR-4673 on ROS generation and oxidative damage on mitochondria were also examined. In order to reveal molecular mechanism of miR-4673 in mitochondrial function, we further investigated the target gene of miR-4673. Our results indicate OGG1 is a direct target of miR-4673. MiR-4673 modulates chemotherapy-induced cell death and oxidative damage on mitochondria by targeting OGG1.

Materials and Methods

Cell culture

Human lung cancer cell line A549 and H1299 were obtained from Shanghai Cell Institute Country Cell Bank, (Shanghai, China). A549 cells were grown in DMEM medium with 10% fetal bovine serum (FBS) (GIBCO/BRL, MD), supplemented with 100U/ml penicillin G and 100µg/ml streptomycin (Sigma-Aldrich Corp., St. Louis, MO). H1299 cells were grown in 1640 medium with 10% fetal bovine serum, supplemented with 100U/ml penicillin G and 100 µg/ml streptomycin. Cells were maintained at 37°C in a humidified 5% CO₂ incubator, and all cell lines were characterized as mycoplasma free.

Quantitative real-time reverse transcription PCR (QPCR)

Total RNA was extracted from clinical samples using the TRIzol reagent (Ambion®) according to the manufacturer's protocol as previously described [32]. For OGG1 detection, RNA samples were then reverse transcribed into cDNA using a FastQuant RT Kit (with gDNase) (TIANGEN, CHINA) in a total volume of 20 µl according to the manufacturer's protocol. Equal amounts of cDNA samples were used as a template for real-time PCR to detect the level of OGG1 expression. GAPDH was used as an endogenous reference, and each

sample was normalized to its GAPDH content. All experiments were performed in duplicate and repeated twice. Results are represented the fold induction using the $2^{-\Delta\Delta Ct}$ method. Primers of OGG1 and GAPDH are as follow. OGG1-F: 5'-CTGTGTACCGAGGAGACAAGAGC-3', OGG1-R: 5'-CCCAGTGGTGATACAGTTGAGC-3'; GAPDH-F: 5'-AGAAGGCTGGGGCTCATTG-3', GAPDH-R: 5'-AGGGGCCATCCACAGTCTTC-3'.

For miRNA detection, RNA samples were reverse transcribed into cDNA using a miRcute miRNA First-Strand cDNA Synthesis Kit (with gDNase). Equal amounts of cDNA samples were used as a template for real-time PCR. The miRcute miRNA qPCR detection kit (SYBR Green) was used for real-time PCR to detect the level of miR-4673 expression. U6 was used as an endogenous reference, and each sample was normalized to its U6 content. Quantitative PCR was performed using the ABI7500 PCR instrument (Applied biosystems). All experiments were performed in duplicate and repeated twice. Results are represented as fold induction using the ΔCt method. Primers of miR-4673 and U6 were obtained from GenePharma Co, Ltd (Suzhou, China).

Cell viability assay

MTT assay was performed according to the manufacturer's protocol as previously described [32]. Briefly, 5,000 cells/well were plated in triplicate in 96-well plates and allowed to attach overnight. Cells were exposed to PTX (5 μ M) for the indicated times. The MTT reagent was prepared at 5 mg/ml in PBS. This MTT stock solution was then added to each well at a 1:10 dilution. Cells were incubated for 4 h and the resulting crystals were dissolved in 100 μ l DMSO. The absorbance at 490 nm was measured using a multi-well plate reader.

Apoptosis assay

As stated in the KGI Annexin V-FITC apoptosis detection kit instruction manual, the cells were digested with trypsin, and centrifuged at 1,000 rpm for 5 min. After collection, the cells were washed twice with PBS, and centrifuged at 1,000 rpm for 5 min. A total of 5×10^5 cells were collected and suspended in 500 μ l binding buffer. Subsequently, 5 μ l Annexin V-FITC and 5 μ l propidium iodide (PI) were added followed by mixing at room temperature, in the dark for 15 min. Within 1h, the apoptotic cells were detected by flow cytometry. The excitation wavelength was 488 nm and the emission wavelength was 530 nm. Green fluorescence of Annexin V-FITC was detected using the FITC channel (FL1); PI red fluorescence was detected using the PI channel (FL2 or FL3). For fluorescent compensation adjustment, normal cells not treated with HG to induce apoptosis were used as the controls for fluorescence compensation settings adjustment.

Detection of mitochondrial membrane potential (MMP)

The MMP detection kit (JC-1; Beyotime Biotechnology Research Institute) was used to detect MMPs. Cells were washed once with PBS and then 1 ml cell culture medium containing serum and phenol red was added. JC-1 dye working solution (1 ml) was then added followed by mixing. The cells were subsequently incubated in a cell incubator at 37°C for 20 min, and then washed with JC-1 staining buffer (1X) twice. Cell culture medium (2 ml) was then added and the cells were analyzed by FACS Calibur flowcytometry (Becton Dickinson, San Jose, CA, USA). The aggregate JC-1 (red fluorescence) was detected at an emission wave length of 590 nm, and the monomeric JC-1 (green fluorescence) was monitored at 529 nm. The ratio of aggregated and monomeric JC-1 was used to quantify changes in MMP, and a decreased JC-1 ratio represented the depolarization of the mitochondria, indicating a decrease in MMP.

Detection of reactive oxygen species (ROS) generation

DCF-DA was used to estimate ROS generation. PTX-treated and untreated cells were washed with PBS, treated with 10mM DCF-DA, and incubated at 37°C for 15 min. DCF-DA is cleaved by ROS to produce the fluorescent 2', 7'-dichlorofluorescein (DCF), which was analyzed by FACS Calibur flowcytometry (Becton Dickinson, San Jose, CA, USA) with excitation at 480 nm and emission at 515 nm.

Prediction of miRNA binding sites in 3'UTR of OGG1

Online program Targetscan was used to predict potential binding sites of miRNA in 3'UTR of OGG1. Result of Targetscan was verified by luciferase reporter assay.

Creation of OGG1 3'UTR luciferase reporter constructs

Wild type OGG1 3'UTR was amplified from blood cDNA and sub-cloned into psiCHECK-2 vector (Promega). The primers used to amplify 3'UTR of OGG1 was synthesized by Sangon Biotech (Shanghai,

China). The binding sites of miR-4673 in OGG1 3'UTR was mutated using QuikChange® Site-Directed Mutagenesis Kit (Stratagene). The primers used to create the mutant of OGG1 3'UTR was listed as followed. All constructs were verified by sequencing.

Luciferase reporter assays

A549 cells were seeded in 96-well plates at 6,000 cells per well the day before transfection. A mixture of 100 ng luciferase reporter constructs (psiCHECK-OGG1-WT and psiCHECK-OGG1-mutant) and 200 ng of NC or miRNA mimics was transfected into A549 cells with Lipofectamine 2000. Forty-eight hours later, firefly and renilla luciferase activities were measured with a Dual-Luciferase Reporter System (Promega) according to the manufacturer's protocol.

Western blot analysis

Western blot analysis was performed according to standard western blot procedures as previously described [32]. Briefly, proteins were separated by 10% SDS-PAGE and then transferred to nitrocellulose membrane (Bio-Rad). After blocking in 5% nonfat milk, the membranes were incubated with the following primary antibodies: rabbit anti-OGG1 polyclonal antibody, (1:300; Novous), mouse anti-GAPDH mAb (1:1,000; Abcam). The proteins were visualized with enhanced chemiluminescence reagents (Pierce).

RNA interference

Small-interferingRNA (siRNA) oligos for OGG1 and general negative control were synthesized and annealed by GenePharma (Shanghai, China). The sequences of OGG1 siRNA were 5'-GCGCAAGUACUCCAGCUATT-3' (sense) and 5'-UAGCUGGAAGUACUUGCGCTT-3' (antisense). Each siRNA duplex was transfected into A549 cells using Lipofectamine® 2000 (Invitrogen) following the manufacturer's protocol. siRNA-NC, siRNA-NC-FAM and siRNA-GAPDH served as negative control, transfection control and siRNA positive control targeting GAPDH gene, respectively. Cells were exposed to 5 μ M PTX after transfection and harvested for indicated analysis.

Statistical analysis

Data were shown as mean $\pm$ SD unless otherwise noted. The two-tailed Student's t-test was used to analyzing the difference between two groups. The P value of <0.05 was considered statistically significant.

Results

MiR-4673 enhance PTX-induced cell death

We investigate effects of miR-4673 on PTX induced cell death. A549 and H1299 cells were used in this study. QPCR analysis showed the expression of miR-4673 was normal level in A549 and H1299 cells (Fig. 1A). Cells were transfected with mimics of miR-4673 or NC and exposed to PTX (5 μ M) for 24 h. Transfection efficiency was determined by QPCR (Fig. 1B). MTT assays and Annexin V/PI double staining method were used to evaluate cell viability and apoptosis, respectively. Our data showed A549 and H1299 cells transfected with miR-4673 exhibited decreased cell viability (Fig. 1C) and increased apoptosis (Fig. 1D) compare with those transfected with NC. These data indicates miR-4673 enhance inhibitory effects of PTX on cell viability and PTX-induced apoptosis.

MiR-4673 enhance MMP loss and ROS generation induced by PTX

MMP loss is considered as an irreversible event during early apoptosis. ROS is one of the factors contributed to MMP loss. We next investigate effects of miR-4673 on PTX-induced MMP loss and ROS generation. The MMP of A549 and H1299 cells was measured by JC-1, an indicator mitochondrial function. Red fluorescence represents the mitochondrial aggregate JC-1 and green fluorescence indicates the monomeric JC-1. Our data suggest that enforced expression of miR-4673 increased MMP loss induced by PTX (Fig. 2A). In addition, increased ROS generation was observed in A549 cells transfected with miR-4673 compare with those transfected with NC (Fig. 2B).

Fig. 1. miR-4673 enhances PTX-induced apoptosis. The cells were transfected with miR-4673 or NC and exposed to PTX (5 μ M) after transfection. The expression of miR-4673 was normal level in A549 and H1299 cells (A). Transfection efficiency was determined by expression of miR-4673 using QPCR (B). MTT assays were performed to evaluate cell viability (C) Annexin V/PI double staining was used to examine effects of miR-4673 on PTX-induced apoptosis (D). Data represents means $\pm$ SD. The Students' t test was used to determine difference of data between group NC and group miR-4673 mimics. *, $p < 0.05$. ***, $p < 0.001$.

MiR-4673 down-regulates OGG1 by binding to 3'UTR of OGG1

miRNAs function as regulatory molecules by downregulating downstream targets. We investigated downstream targets using online program TargetScan. Hundreds of genes were found (Table 1). We narrowed the number of potential targets by comparing several parameters including context++ score, context++ score percentile and conserved branch length. A few targets were selected based on conserved branch length and context++ score percentile. OGG1 is one of the genes we identified with putative binding sites of miR-4673 in its 3' untranslated region (3'UTR). OGG1 involves in reaction of oxidative stress, which intrigue us to investigate whether OGG1 is a direct target in regulation of PTX-induced apoptosis.

Reporter constructs containing 3'UTR of OGG1 was created by sub-cloning full length 3'UTR of OGG1 into psiCHECK-2 vector. The psiCHECK-OGG1 reporter constructs was transfected with miRNA mimics or NC into A549 cells. NC served as control. Our data showed relative luciferase activities of group mimics were decreased compare to group NC (Fig. 3A),

Fig. 2. miR-4673 enhances PTX-induced MMP loss and ROS generation. The cells were transfected with miR-4673 or NC and exposed to PTX (5 μ M) after transfection. JC-1 staining was performed to evaluate MMP loss of A549 and H1299 cells (A). DCFH staining was performed to evaluate ROS generation of A549 and H1299 cells (B). Data represents means $\pm$ SD. The Students' t test was used to determine difference of data between group NC and group miR-4673 mi-mics. ***, $p < 0.001$.

Table 1. Parts of targets for miR-4673 were predicted using online program TargetScan

Target gene	Number of 3P-seq tags supporting UTR + 5	Representative miRNA	Cumulative weighted context++ score	Total context++ score	Aggregate P _{CT}
FAM83F	5	hsa-miR-4645-5p	-3.33	-3.33	N/A
FGFRL1	1114	hsa-miR-4645-5p	-2.13	-2.16	N/A
FBXL18	92	hsa-miR-4673	-1.63	-1.91	N/A
GPR37L1	5	hsa-miR-4673	-1.36	-1.36	N/A
STAC2	123	hsa-miR-4673	-0.66	-0.66	N/A
KCNJ11	282	hsa-miR-4673	-0.65	-0.66	N/A
AIRE	23	hsa-miR-4673	-0.64	-0.71	N/A
TNNI1	5	hsa-miR-4673	-0.64	-0.72	N/A
SIX5	47	hsa-miR-4673	-0.64	-0.71	N/A
CDIP1	1857	hsa-miR-4673	-0.63	-0.77	N/A
DNAH100S	89	hsa-miR-4673	-0.63	-0.63	N/A
TMEM126B	529	hsa-miR-4673	-0.63	-0.67	N/A
TEX22	9	hsa-miR-4673	-0.62	-0.78	N/A
RXRA	609	hsa-miR-4673	-0.62	-0.86	N/A
RIMS4	30	hsa-miR-4673	-0.61	-0.61	N/A
POMGNT1	2123	hsa-miR-4673	-0.61	-0.61	N/A
ENHO	124	hsa-miR-4673	-0.6	-0.6	N/A
NXPH1	5	hsa-miR-4673	-0.6	-0.6	N/A
ADCYAP1R1	9	hsa-miR-4673	-0.6	-0.76	N/A
AC079612.1	5	hsa-miR-4673	-0.59	-0.59	N/A
SDC1	3152	hsa-miR-4673	-0.59	-0.7	N/A
OGG1	364	hsa-miR-4673	-0.37	-0.37	N/A
NT5C1A	4	hsa-miR-4673	-0.37	-0.37	N/A
PRODH	5	hsa-miR-4673	-0.37	-0.37	N/A
F11R	2844	hsa-miR-4673	-0.37	-0.45	N/A
UBE2G2	681	hsa-miR-4673	-0.37	-0.38	N/A
C2orf91	5	hsa-miR-4673	-0.37	-0.37	N/A
RPL37	1785	hsa-miR-4673	-0.37	-0.45	N/A
TNFRSF13B	5	hsa-miR-4673	-0.37	-0.37	N/A
ZNF69	5	hsa-miR-4673	-0.37	-0.37	N/A

Fig. 3. miR-4673 down-regulates OGG1. (A). binding sites of miR-4673 was marked with red character. Mutated strategy of OGG1 3'UTR. (B). luciferase assays of A549 cells transfected with psiCHECK-OGG1 and miR-4673. (C) luciferase assays of A549 cells transfected with miR-4673 and reporter constructs containing mutated binding site of miR-4673. (D) expression of OGG1 in A549 and H1299 cells transfected with NC or miR-4673. Data represents means $\pm$ SD. The Students' t test was used to determine difference of data between group NC and miR-4673 mimics. **, $p < 0.01$.

which suggest miR-4673 binds to 3'UTR of OGG1. miR-4673 was considered as potential regulator of OGG1.

In order to further verify our finding, 'CUGUUGA' binding site of miR-4673 in reporter constructs containing 3'UTR of OGG1 was mutated (Fig. 3B). Luciferase assays were performed to investigate whether miRNA binds to mutated 3'UTR of OGG1. Our data showed there was no significant difference between relative luciferase activities of group mimics and group NC (Fig. 3C), which suggest miR-4673 doesn't bind to mutated 3'UTR of OGG1. These data indicate that miR-4673 specifically binds to 'CUGUUGA' in 3'UTR of OGG1.

Western blot analysis showed endogenous expression of OGG1 in group mimics was decreased compared to group NC (Fig. 3D). These data indicate that miR-4673 is a negative regulator of OGG1.

Silencing OGG1 enhances inhibitory effects of PTX on cell death, MMP loss and ROS generation

We have proved OGG1 is one of the targets of miR-4673, which inspired us that miR-4673 may accomplish its function through down-regulating OGG1. So, we decided to investigate whether the effects of silencing OGG1 are consistent with those of miR-4673. We inhibited expression of OGG1 using siRNA targeted OGG1. Efficiency of siRNA was determined by detecting expression of OGG1 using QPCR (Fig. 4A). Our data showed A549 and H1299 cells transfected with siRNA exhibited decreased cell viability (Fig. 4B) and increased apoptosis (Fig. 4C) compared with those transfected with NC. Results obtained from JC-1 staining suggest that suppression of OGG1 increased MMP loss induced by PTX (Fig. 4D). In addition, increased ROS generation was observed in A549 and H1299 cells transfected with siRNA compared with those transfected with NC (Fig. 4E). In conclusion, these data indicate the effects of silencing OGG1 on PTX-induced cell death, MMP loss and ROS generation are consistent with those of miR-4673.

Enforced expression of OGG1 compromises effects of miR-4673 on cell death, MMP loss and ROS generation

In order to further verify our hypothesis that miR-4673 increased PTX-induced cell death, MMP loss and ROS generation through down-regulating OGG1, we transfected ex-

Fig. 4. OGG1 knockdown enhances PTX-induced apoptosis, MMP loss and ROS generation. The cells were transfected with siRNA targeted OGG1 or NC and exposed to PTX (5 μ M) after transfection. Transfection efficiency was determined by expression of OGG1 using western blot analysis (A). MTT assays were performed to evaluate cell viability (B). Annexin V/PI double staining was used to examine effects of miR-4673 on PTX-induced apoptosis (C). JC-1 staining was performed to evaluate MMP loss of A549 and H1299 cells (D). DCFH staining was performed to evaluate ROS generation of A549 and H1299 cells (E). Data represents means $\pm$ SD. The Student's t test was used to determine difference of data between group NC and group siRNA. *, $p < 0.05$. ***, $p < 0.001$.

pression constructs of OGG1, pcDNA-OGG1, into A549 and H1299 cells, and examined PTX induced cell viability, apoptosis, MMP loss and ROS generation. Transfection efficiency was determined by expression of OGG1 using western blot analysis (Fig. 5A). Our data showed A549 and H1299 cells co-transfected with pcDNA-OGG1 and miR-4673 exhibited increased cell viability (Fig. 5B) and decreased apoptosis (Fig. 5C) compare with those transfected with pcDNA3.1 and miR-4673. Results obtained from JC-1 staining and ROS assays suggest that enforced expression of OGG1 decreased MMP loss (Fig. 5D) and ROS generation (Fig. 5E) induced by PTX. These data suggest enforced expression of OGG1 compromises effects of miR-4673 on cell viability, apoptosis, MMP loss and ROS generation, and support our hypothesis that

Fig. 5. Enforced expression of OGG1 compromised effects of miR-4673 in PTX-induced apoptosis, MMP loss and ROS generation. The cells were cotransfected with miR-4673 and expression constructs of OGG1 or control vector pcDNA3.1 and exposed to PTX (5 μ M) after transfection. Transfection efficiency was determined by expression of OGG1 using WB (A). MTT assays were performed to evaluate cell viability (B). Annexin V/PI double staining was used to examine effects of miR-4673 on PTX-induced apoptosis (C). JC-1 staining was performed to evaluate MMP loss of A549 and H1299 cells (D). DCFH staining was performed to evaluate ROS generation of A549 and H1299 cells (E). Data represents means $\pm$ SD. The Students' t test was used to determine difference of data between group miR-4673 + pcDNA3.1 vector and group miR-4673 + OGG1. *, $p < 0.05$. **, $p < 0.01$. ***, $p < 0.001$.

miR-4673 increased PTX induced cell death, MMP loss and ROS generation through down-regulating OGG1.

Discussion

In this study, we presented that miR-4673 involves in regulation of chemotherapy sensitivity, which accomplishes its function by modulating MMP loss and ROS generation. PTX resistance is one of the major reasons contribute to chemotherapy failure [33-35]. Our previous study showed miR-4673 is up-regulated in A549 cells exposed to PTX, which intrigued us that miR-4673 may play a role in PTX-induced cytotoxicity. We

investigated the effects of miR-4673 on cell viability and apoptosis. Our data indicates miR-4673 enhances inhibitory effects of PTX on cell viability and increases PTX-induced apoptosis in A549 cells. These findings support our initial speculation that miR-4673 play a role in PTX-induce cytotoxicity.

We further explore the molecular mechanism underlying the role of miR-4673 in PTX-induce cytotoxicity. ROS are constantly generated and eliminated in the biological system, and play important roles in a variety of normal biochemical functions and abnormal pathological processes [36]. Growing evidence suggests that cancer cells exhibit increased intrinsic ROS stress, due in part to oncogenic stimulation, increased metabolic activity, and mitochondrial malfunction [37]. Since the mitochondrial respiratory chain (electron transport complexes) is a major source of ROS generation in the cells, the vulnerability of the mitochondrial DNA to ROS-mediated damage appears to be a mechanism to amplify ROS stress in cancer cells. The escalated ROS generation in cancer cells serves as an endogenous source of DNA-damaging agents that promote genetic instability and development of drug resistance [34, 35, 37]. Malfunction of mitochondria also alters cellular apoptotic response to anticancer agents [36, 37]. Despite the negative impacts of increased ROS in cancer cells, it is possible to exploit this biochemical feature and develop novel therapeutic strategies to preferentially kill cancer cells through ROS-mediated mechanisms [36, 37]. Our data showed enforced expression of miR-4673 increased PTX-induced ROS generation and MMP loss, which indicates miR-4673 involves in PTX-induced cytotoxicity through functioning as a modulator of ROS stress and mitochondrial malfunction.

MiRNAs accomplish their cellular function by down-regulating downstream targets [38]. In order to further explore the role of miR-4673, we investigate its target genes in mitochondrial function. 8-oxoguanine-DNA glycosylase-1 (OGG1) is a DNA damage repair gene [39]. Previous study demonstrated that OGG1 protect epithelial cell from oxidative damage [40-42]. We recently found OGG1 protect lung cancer cells from chemotherapy reagents (data not shown). In the current study, a miR-4673 binding site was identified in 3'UTR of OGG1, which indicates OGG1 may be a potential target of miR-4673. Luciferase reporter assays showed decreased luciferase activities in group miR-4673 compare to its control group NC, which suggest miR-4673 does bind to 3'UTR of OGG1. Moreover, we found enforced expression of miR-4673 caused decreased accumulation of OGG1. These data suggest miR-4673 downregulates OGG1 by binding to 3'UTR of OGG1. We speculate miR-4673 accomplishes its function in mitochondria by targeting OGG1. The effect of OGG1 on promoting effects of miR-4673 on PTX-induced ROS generation and MMP loss was investigated. Results showed enforced expression of OGG1 alleviates promoting effects of miR-4673 on PTX-induced ROS generation and MMP loss. These results support our speculation and prove miR-4673 promotes PTX-induced ROS generation and MMP loss by down-regulating OGG1.

In conclusion, our data indicates miR-4673 promotes PTX-induced ROS generation and MMP loss by down-regulating OGG1. This work investigates ROS stress in cancer cells, its underlying mechanisms and relationship with mitochondrial malfunction and alteration in drug sensitivity, and suggests new therapeutic strategies that take advantage of increased ROS in cancer cells to enhance therapeutic activity and selectivity.

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Disclosure Statement

The authors declare that there are no conflicts of interest.

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