RESEARCH ARTICLE

MicroRNA-452 contributes to the docetaxel resistance of breast cancer cells

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Abstract MicroRNA-452 (miRNA-452) was overexpressed in docetaxel-resistant human breast cancer MCF-7 cells (MCF-7/DOC). However, its role in modulating the sensitivity of breast cancer cells to docetaxel (DOC) remains unclear. The aim of this study is to investigate the role of miRNA-452 in the sensitivity of breast cancer cells to DOC.

Real-time quantitative PCR (RT-qPCR) were used to identify the differential expression of miRNA-452 between MCF-7/DOC and MCF-7 cells. MiRNA-452 mimic was transfected into MCF-7 cells and miRNA-452 inhibitor was transfected into MCF-7/DOC cells. The role of miRNA-452 in these transfected cells was evaluated using RT-qPCR, MTT assay, and flow cytometry assay. The relationship of miRNA-452 and its predictive target gene "anaphase-promoting complex 4" (*APC4*) was analyzed by RT-qPCR and Western blot.

MiRNA-452 showed significantly higher expression (78.9-folds) in MCF-7/DOC cells compared to parental MCF-7 cells. The expression of miRNA-452 in the mimic transfected MCF-7 cells was upregulated 212.2-folds ($P < 0.05$) compared to its negative control (NC), and the half maximal inhibitory concentration (IC50) value of DOC (1.98 ± 0.15 μM) was significantly higher than that in its NC (0.85 ± 0.08 μM, $P < 0.05$) or blank control (1.01 ± 0.19 μM, $P < 0.05$). Furthermore, its apoptotic rate (6.3 ± 1.3 %) was distinctly decreased compared with that in its NC (23.8 ± 6.6 %, $P < 0.05$) or blank control (18.6 ± 4.7 %, $P < 0.05$). In contrast, the expression of miRNA-452 in the inhibitor-transfected MCF-7/DOC cells was downregulated 0.58-fold ($P < 0.05$) compared to its NC, the IC50 value of DOC (44.5 ± 3.2 μM) was significantly lower than that in its NC (107.3 ± 6.63 μM, $P < 0.05$) or blank control (102.22 ± 11.34 μM, $P < 0.05$), and the apoptotic rate (45.5 ± 10.8 %) was distinctly increased compared with its NC (9.9 ± 2.2 %, $P < 0.05$) and blank control (9.4 ± 2.5 %, $P < 0.05$). Further, there was an inverse association between miRNA-452 and APC4 expression in breast cancer cells in vitro.

Dysregulation of miRNA-452 involved in the DOC resistance formation of breast cancer cells may be, in part, via targeting *APC4*.

Keywords Breast cancer · MiRNA-452 · Docetaxel · APC4 · Resistance

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Introduction

Breast cancer is one of the most common malignant tumors in women worldwide, and its survival is dramatically improved through earlier detection and improvements in comprehensive treatment [1]. Chemotherapy is an important component in treatment of breast cancer. However, drug resistance is a major obstacle in systemic treatment of advanced or metastasis breast cancer, which leads to incontrollable disease and mortality. Docetaxel (DOC) is one of the most common chemotherapeutic drugs; however, chemoresistance remains the most important obstacle restricting the clinical application of docetaxel [2]. Docetaxel resistance is a complex phenomenon,

with multiple factors and mechanisms contributing to the resistance. However, the underlying mechanisms of acquisition of resistance to chemotherapeutic agents are still poorly understood. Therefore, it should be acknowledged that other avenues must be explored.

MicroRNAs (miRNAs) are a class of small (19–25 nucleotides), noncoding RNA molecules that act to inhibit gene expression post-transcriptionally. MiRNAs regulate the expression of target genes by binding to partially complementary sequences in the 3′ untranslated regions (UTRs) of target messenger RNAs (mRNAs), resulting in the translation repression or the sequence-specific degradation of their target mRNAs [3]. MiRNAs have emerged as key regulatory molecules in a wide range of physiological and pathological processes including tumor growth, differentiation, invasion, metastasis, self-renewal, apoptosis, and so on. Aberrant miRNA expressions may contribute to many types of human disease, and they have been associated with every aspect of tumor biology including acquisition of resistance to various chemotherapeutic agents [3, 4] which may provide a new strategy for cancer therapy.

Based on miRNA microarray data in our previous work, microRNA-452 (miRNA-452) was found to be significantly upregulated in the docetaxel-resistant breast cancer MCF-7 cells (MCF-7/DOC) compared with the parental MCF-7 cells [5]. However, the role or function of miRNA-452 has yet to be explored. Whether the abnormal expression of miRNA-452 contributes to acquisition of resistance? Basing on the finding and question, we investigated the association of miRNA-452 expression with the sensitivity of breast cancer cells to DOC and analyzed its potential mechanism. Here, we found that miRNA-452 involved in the acquired DOC resistance in breast cancer MCF-7 cells in vitro may be, at least in part, dysregulated by targeting "anaphase-promoting complex 4" (*APC4*).

Materials and methods

Cell culture

Human breast cancer cell line MCF-7 was purchased from the Institute of Biochemistry (IBCB) and Cell Biology of Chinese Academy of Sciences (Shanghai, China). The MCF-7/DOC cells were established by a stepwise increase of docetaxel concentrations in the culture over 12 months to achieve statistically significant degrees of resistance relative to parental MCF-7 cell line in vitro in our laboratory. The initial and final concentrations of docetaxel were 0.5 and 200 nM in the culture medium. Half maximal inhibitory concentration (IC50) values of DOC in MCF-7/DOC and MCF-7 cells were 97.74 and 0.98 μM, respectively.

Parental MCF-7 cultured synchronously in the absence of drug was used as a control. The drug-resistant derivative cell lines were cultured in drug-free medium for 2 weeks before subsequent experiments to avoid the influence of drug. All the cells were cultured in Dulbecco's modified Eagle's medium (DMEM) high glucose (HyClone) supplemented with 10 % fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37 °C and 5 % CO_2 in a humidified atmosphere.

Transfection experiment

MiRNA-452 mimic, miRNA-452 inhibitor, and their negative control (NC) were synthesized by Shanghai GenePharma (Shanghai, China). MiRNA-452 mimic and its NC were used in MCF-7 parental cells with low miRNA-452 expression, and miRNA-452 inhibitor and its NC were used in MCF-7/DOC cells with high miRNA-452 expression.

Cells were plated onto a six-well plate at a density of 3×10^5 cells/well. After 24 h, cells were separately transfected with miRNA-452 mimic (10 nM), inhibitor (40 nM) and their NC using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. Cells were collected for further analysis after 24 h.

RT quantitative PCR

Total RNA was extracted from MCF-7 and MCF-7/DOC cells, transfected or untransfected, using TRIzol Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Total RNA (1 μg) including miRNA from each sample was reversely transcribed using the BU-Script RT Kit (Biouniquer Technology, Nanjing, China) according to the manufacturer's protocol. Real-time quantitative polymerase chain reaction (RT-qPCR) was performed using the LightCycler 480II (Roche, Switzerland) with SYBR Green PCR Master Mix (Biouniquer Technology). The sequence-specific reverse transcription primers for miRNA-452 and U6 small nuclear RNA (snRNA) were 5′-GTCGTATCCAGTGC GTGTCGTGGAGTCGGCAATTGCACTGGATACGACTC AGTTT-3′ and 5′-GCGCGTGAGCAGGCTGGAGAAATT AACCACGCGCGGAACG-3′, respectively. The PCR primers for miRNA-452 were 5′-GCGAACTGTTTGCAGA GG-3′ and 5′-CAGTGCGTGTCGTGGAGT-3′ and those for U6 snRNA were 5′-CGCAAGGATGACACG-3′ and 5′-GAGCAGGCTGGAGAA-3′. All the primers were purchased from Springen Biotechnology (China). Every run experiment was performed along with negative controls (nuclease-free water or the extracted RNA without RT as a template). At the end of the 45 cycles, based on melting curve analyses which showed a single peak at the product melting temperature, PCR specificity was judged. The Ct values for miRNA-452 were normalized to endogenous control U6 snRNA, and the relative fold change values were calculated using the

ΔΔCt method [6]. All reactions were run in triplicate and all experiments were carried three independent times.

Cell survival analysis

The cells were reseeded in 96-well plates at a density of 8×10^3 per well 24-h post-transfection. Then these cells were treated with DOC at a range of concentration of 0.32 to 100 μM (double wells per condition) in the medium for 48 h. MTT assay was performed to analyze the cell viability. In each well, 20 μl of a 5 mg/ml solution of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium-bromide (MTT; Sigma, Germany) was applied and incubated at 37 °C. After 4 h, 150 μl of dimethyl sulfoxide (DMSO; Amresco, America) was added to per well followed by measuring the absorbance at 550 nM in Clinibio128 (ASYS-Hitech, Austria). The IC50 value of DOC was calculated using SPSS 16.0. Three independent experiments were performed.

Flow cytometric analysis of apoptosis

Cell apoptosis was determined using an Annexin-V-FITC apoptosis detection kit (BD Biosciences, Franklin Lakes, NJ). Briefly, MCF-7 cells and MCF-7/DOC cells with transfection of miRNA-452 mimic and inhibitor were incubated with 0.5 μM docetaxel and 30 μM docetaxel for 24 h, respectively. Then 1×10^6 cells were washed twice with ice-cold PBS and incubated with Annexin-V-FITC and propidium iodide (PI) for 15 min in the dark at room temperature. A FACScan flow cytometer (BD Biosciences) was used to analyze cellular apoptosis. Three independent experiments were performed.

RT-qPCR analysis for target gene expression

Predicted target gene *APC4* and internal control β-actin were detected using RT-qPCR as described above. The primers of RT for APC4 and β-actin were random primers. The sets of primers for APC4 were 5′-TGCTCGAGTCACAGGGATTG-3′ and 5′-ACTTTCTGGCCATCCGAGTT-3′, and those for β-actin were 5′-CACCTTCTACAATGAGCTGCGTGTG-3′ and 5′-ATAGCACAGCCTGGATAGCAACGTAC-3′. Endogenous control β-actin was used for normalization. Three independent experiments were performed.

Western blot

Western blot was used to confirm the expression of protein. Briefly, according to the protocol from manufacturer, total protein was extracted using radio immunoprecipitation assay (RIPA) lysis buffer (Biouniquer Technology). Equal amounts of total protein were applied to 10 % polyacrylamide gradient gels. After electrophoresis, the samples were transferred to polyvinylidene fluoride (PVDF) membrane (Sigma). After

blocking in 5 % skim milk, APC4 proteins (92 kDa) were detected with an anti-APC4 rabbit polyclonal antibody (1:2000; Abcam, America). Then the membranes were incubated with horseradish peroxidase-conjugated secondary antibody (Wuhan Boster Biological Technology, Wuhan, China) for 2 h at room temperature. The membranes were developed with an ECL Substrate (Biouniquer Technology). The levels of each protein were normalized to the level of β-actin (Sigma, Germany). The experiments were carried three independent times.

Statistical analysis

SPSS 16.0 software was used for statistical analysis. Data were presented as mean±SD (standard deviation) of at least three independent experiments. Student's *t* test was used for comparisons between two independent groups. All tests were two-tailed and $P < 0.05$ (*) were considered to be statistically significant.

Results

MiRNA-452 was upregulated in human MCF-7/DOC cells

RT-qPCR analysis demonstrated that the expression of miRNA-452 in the MCF-7/DOC cells was upregulated 78.9-folds compared to its parental MCF-7 cells (Fig. 1a), which showed good consistency with the previous microarray results.

MiRNA-452 modulated chemo-sensitivity of breast cancer cells to DOC

To study the function of miRNA-452 in MCF-7/DOC cells, miRNA-452 mimic was transfected into sensitive cells (MCF-7). The results showed that the expression of miRNA-452 in the transfected MCF-7 cells was upregulated 212.2-folds compared to its NC ($P < 0.05$; Fig. 1b), and the IC50 value of DOC (1.98±0.15 μM) was significantly higher than that in its NC (0.85±0.08 μM) or blank control (1.01±0.19 μM; $P < 0.05$; Fig. 2a and c).

Similarly, miRNA-452 inhibitor was transfected into MCF-7/DOC cells. The results showed that the expression of miRNA-452 in the transfected MCF-7/DOC cells was down-expressed 0.58-fold compared to its NC ($P < 0.05$; Fig. 1c), and the IC50 value of DOC (44.5±3.2 μM) was significantly lower than that in its NC (107.3±6.63 μM) or blank control (102.22±11.34 μM; $P < 0.05$; Fig. 2b and d). These findings suggested that upregulation of miRNA-452 expression could decrease the sensitivity of MCF-7 cells to DOC, and downregulation of miRNA-452 could mitigate drug
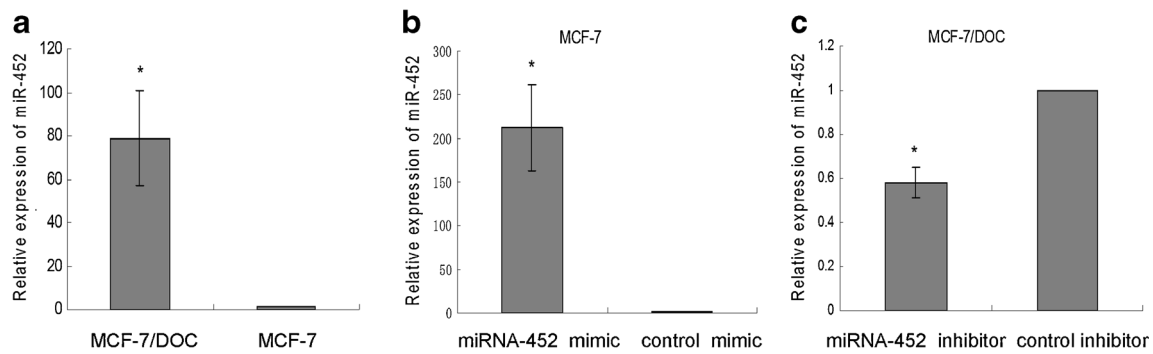
Fig. 1 Relative expression level of miRNA-452 in the breast cancer cells. **a** Relative expression of miRNA-452 in MCF-7 cells and MCF-7/DOC cells ($^*P<0.05$). **b** Relative expression of miRNA-452 in MCF-7 cells transfected with miRNA-452 mimic and its control mimic ($^*P<0.05$). **c** Relative expression of miRNA-452 in MCF-7/DOC cells transfected with miRNA-452 inhibitor and its control inhibitor ($^*P<0.05$)

resistance of the MCF-7/DOC cells to DOC in some degree.

MiRNA-452 was involved in DOC-induced apoptosis

Flow cytometry assay showed that the apoptotic rate of MCF-7 cells transfected with miRNA-452 mimic was distinctly decreased compared with that of the cells transfected with its NC or blank control (6.3±1.3 % vs. 23.8±6.6 % or 18.6±4.7 % Fig. 3a, $P<0.05$). On the contrary, the apoptotic rate of MCF-7/DOC transfected with miRNA-452 inhibitor was significantly increased compared with that of the cells transfected with its

NC or blank control (45.5±10.8 % vs. 9.9±2.2 % or 9.4± 2.5 %, Fig. 3b, $P<0.05$). These results suggested that miRNA-452 could confer DOC resistance in breast cancer MCF-7 cells by inhibiting DOC-induced apoptosis.

MiRNA-452 regulated the expression of *APC4*

To explore the potential mechanisms of miRNA-452 in the drug resistance of breast cancer cells to DOC, a number of Web tools are available for the prediction and identification of target miRNAs. First, prediction of miRNA-452 target gene was performed by in silico analysis based on the computer-aided
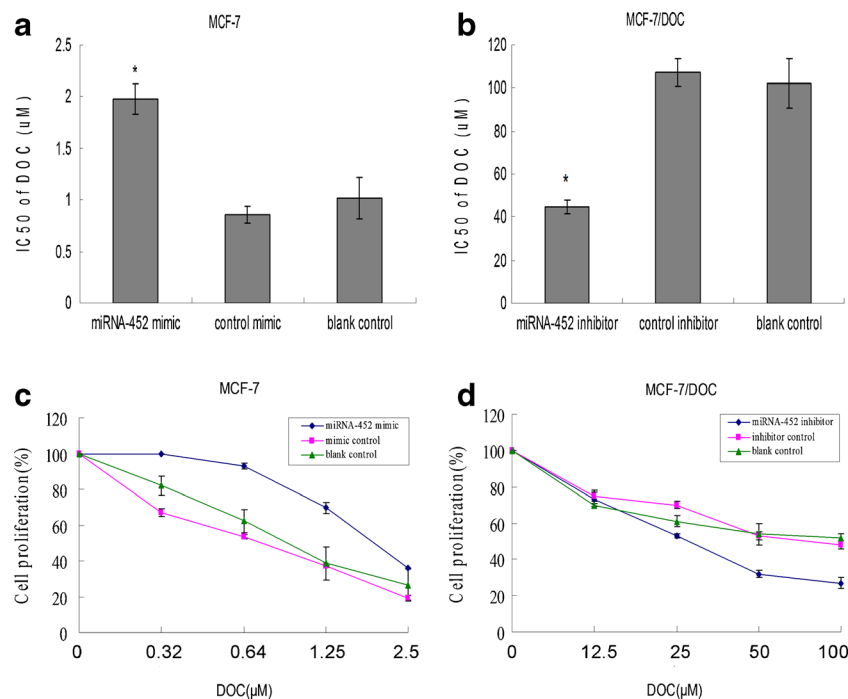

Fig. 2 Effect of miRNA-452 expression on the sensitivity of MCF-7 cells and MCF-7/DOC cells to DOC. **a** After MCF-7 cells were transfected with miRNA-452 mimic and its negative control for 24 h, the IC50 value of DOC was determined by MTT assay ($^*P<0.05$). **b** After MCF-7/DOC cells were transfected with miRNA-452 inhibitor and its negative control for 24 h, the IC50 value of DOC was determined by

MTT assay ($^*P<0.05$). **c** The dose response cell survival curve after MCF-7 cells, transfected or without transfected, were treated with increasing concentrations of DOC for 48 h. **d** The dose response cell survival curve after MCF-7/DOC cells, transfected or without transfected, were treated with increasing concentrations of DOC for 48 h
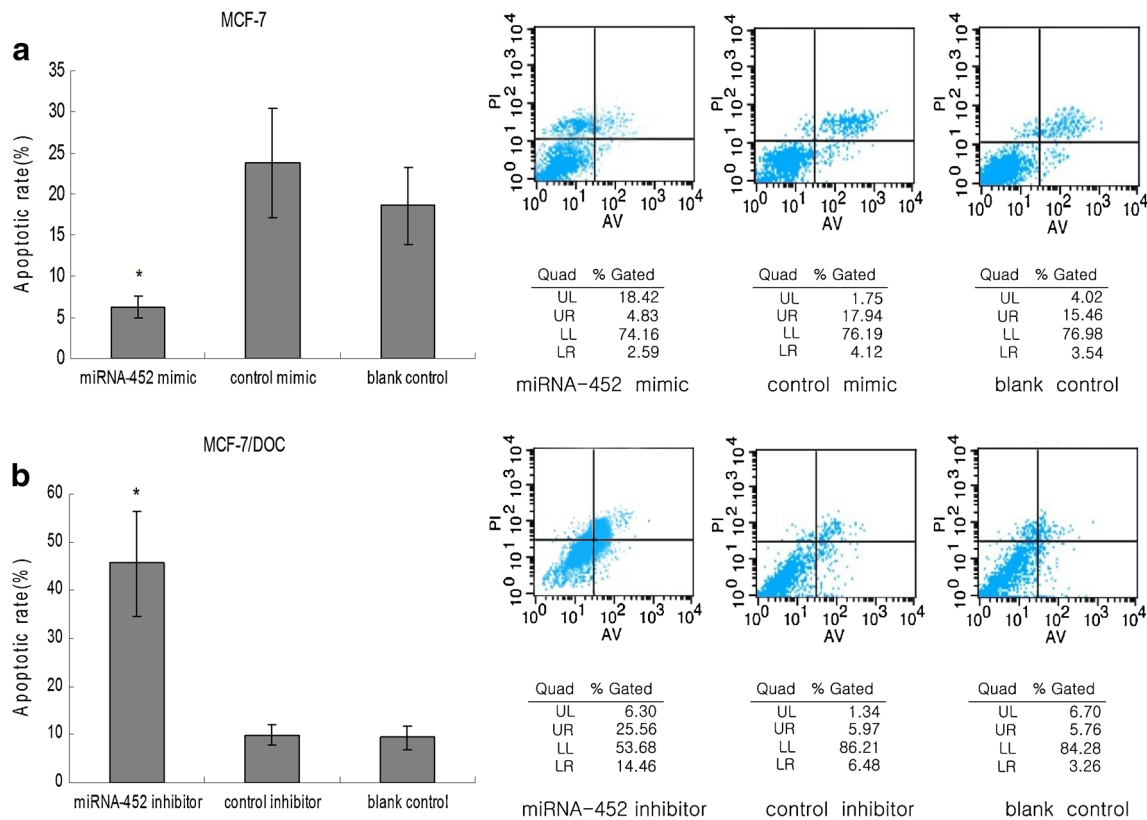
Fig. 3 Flow cytometry assessment of apoptotic MCF-7 cells and MCF-7/DOC cells induced by DOC. **a** The representative FACS figures and apoptotic rate of MCF-7 cells transfected with miRNA-452 mimic was distinctly decreased compared with control mimic and blank control ($^*P<0.05$). **b** The representative FACS figures and apoptotic rate of MCF-7/DOC cells transfected with miRNA-452 inhibitor was significantly increased compared with its control inhibitor and blank control ($^*P<0.05$)

algorithms: TargetScan (http://www.targetscan.org), PicTar (http://pictar.mdc.berlin.de/), and Miranda (http://www.microrna.org), respectively. Second, these miRNA-target prediction algorithms were commonly based on a base-pairing rule, and other features, such as evolutionary conservation, thermodynamics of mRNA–miRNA duplexes, and nucleotide composition of target sequences, were often integrated to improve the accuracy, so we chose initial screening of predicted genes which can be predicted by at least two web tools above to analyze. At last, KEGG pathway enrichment analysis using Web-based tool DAVID (http://david.abcc.ncifcrf.gov/) were used to screen further for the initial screening of the predicted genes. Because one miRNA can target multiple mRNAs and one target can be repressed by multiple miRNAs, we thus chose one target to explore the potential mechanisms at present. At last, *APC4*, a specific target, had never been reported in breast cancer and a complementary site of miRNA-452 in the APC4 3′ UTR (position 94-101; Fig. 4a) was found, so we chose *APC4* as a candidate target gene of miRNA-452.

To investigate whether the expression of APC4 was regulated by miRNA-452, the APC4 mRNA and protein expression were determined using RT-qPCR and Western blot. The results showed that both APC4 mRNA (Fig. 4b, $P<0.05$) and

protein (Fig. 4e, $P<0.05$) expression were reduced significantly in MCF-7/DOC cells compared to MCF-7 cells. After transfection, the relative level of APC4 mRNA (Fig. 4c, $P<0.05$) and protein (Fig. 4f) expression in MCF-7 cells transfected with miRNA-452 mimic were significantly decreased compared with that in MCF-7 cells transfected with control mimic. Reversely, APC4 mRNA (Fig. 4d, $P<0.05$) and protein (Fig. 4g) expression in MCF-7/DOC cells transfected with miRNA-452 inhibitor were significantly increased compared with that in MCF-7/DOC cells transfected with its control inhibitor.

Discussion

Microtubule-poisoning drugs, such as paclitaxel and docetaxel, are powerful and commonly used antineoplastic agents for the treatment of several malignancies including breast cancer. The mechanisms of docetaxel activity include binding to the β-tubulin subunits of microtubules and inhibiting the rate of exchange of free and bound tubulin, which disrupts mitotic spindle formation in cells, inhibits cell division, and leads to cell death [7]. Although up to half of the patients treated with docetaxel achieve a clinical response, development of
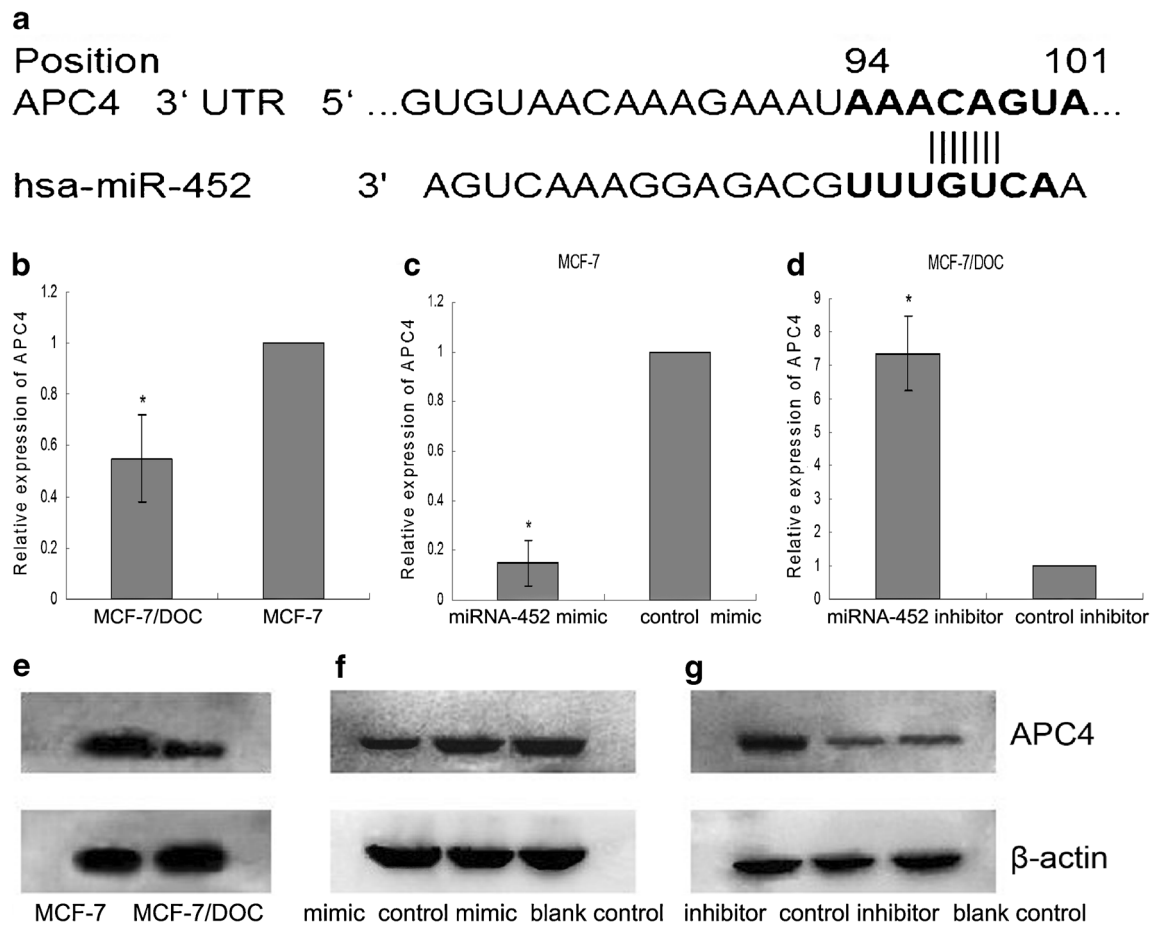
Fig. 4 APC4 is a candidate target of miRNA-452. **a** The complementary site of miRNA-452 in APC4 3′ UTR as predicted by bioinformatics analysis. **b** Relative expression of APC4-mRNA in MCF-7/DOC cells and MCF-7 cells ($^*P<0.05$). **c** Relative expression of APC4-mRNA in MCF-7 cells transfected with miRNA-452 mimic and its mimic control ($^*P<0.05$). **d** Relative expression of APC4-mRNA in MCF-7/DOC cells transfected with miRNA-452 inhibitor and its inhibitor control ($^*P<0.05$). **e** Expression of APC4 protein in MCF-7 cells and MCF-7/DOC cells. **f** Expression of APC4 protein in MCF-7 cells transfected with miRNA-452 mimic and its mimic and blank control. **g** Expression of APC4 protein in MCF-7/DOC cells transfected with miRNA-452 inhibitor and its inhibitor and blank control

acquired resistance to docetaxel often occurs and is a notable clinical problem. The resistant phenotype against docetaxel results from both genetic and epigenetic dysregulation of key genes involving drug transporters, changes in drug metabolism, and pathway alterations of cell cycle and apotosis [2]. However, to data, there is no validated drug response/resistant biomarker available in clinical settings, and the mechanisms of acquisition of resistance to docetaxel are not fully understood. Established human breast cancer cell lines are widely used as experimental models in breast cancer research. In clinical areas, most of breast cancers were ER-positive and MCF-7 cell line was ER-positive, which represented the major type. Importantly, MCF-7 cells and breast tumors shared substantial global similarities in the structures, and molecular events could be reflected truthfully [8]. So, in this study, the established MCF-7/DOC cells were chosen as the subject.

In the last decade, miRNAs have emerged as critical regulators in tumor progression, metastasis, and chemoresistance, resulting in the development of novel approaches to breast cancer management. Recent studies have linked the acquisition of drug resistance to altered expression of miRNAs. Evidence of miRNA-mediated drug resistance is accumulating, and much attention is now being focused on targeting miRNAs as a novel strategy for therapeutic intervention in the clinical significance and feasibility [9, 10]. MiRNA-452 was a widely studied human miRNA; for example, it was useful as tumor stratification, metastasis, and diagnostic biomarker [11, 12], in regulating several cancer stem/progenitor cell populations, and in accelerating tumor progression through regulation of their target genes dihydropyrimidinase-like 2 (*DPYSL2*) and kirsten rat sarcoma viral oncogene homolog (*KRAS*) [13, 14]. In addition, miRNA-452 regulated reciprocal epithelial–mesenchymal signaling in the pharyngeal arch [15] and was overexpressed in the Wnt signaling to regulate tumor/metastasis suppressive activity [16]. Despite the well-established role of miRNA-452 in cancer, to the best of our knowledge, it has been not reported whether miRNA-452 is involved in drug resistance. In the present study, we confirmed

that the expression of miRNA-452 in MCF-7/DOC cells was markedly higher than that in MCF-7 cells by miRNA micro-array and RT-qPCR. To further demonstrate whether miRNA-452 expression might affect the sensitivity of breast cancer cells to DOC, MTT assay and apoptosis assay were performed. The results showed that overexpression of miRNA-452 could significantly decrease the sensitivity of MCF-7 cells to DOC, and downregulation of miRNA-452 could partially restore the sensitivity of MCF-7/DOC cells to DOC.

MCF-7 cells, transfected with miRNA-452, expressed miRNA-452 at 212.2 times compared to its NC, and MCF-7/DOC cells expressed miRNA-452 at 78.9 times compared to its MCF-7 cells. However, the IC50 value of MCF-7 cells transfected with miRNA-452 was significantly lower than blank control of MCF-7/DOC cells. The following can be seen as possible reasons: First, the MCF-7/DOC cells were established by a stepwise increase of docetaxel concentrations in the culture over 12 months; however, the transfection experiment was transient, resulting in the role of miRNA-452 mimic in transfected MCF-7 cells as maybe transitory. Second, MCF-7/DOC cells contained a group of endogenous miRNAs, except miRNA-452, many other miRNAs, such as miRNA-34a [17], miRNA-222, and miRNA-29a [5], could contribute to docetaxel resistance. We had upregulated significantly the expression level of miRNA-452; however, the role of one single miRNA in modulating chemosensitivity may be limited. Importantly, one miRNA can target multiple mRNAs, and one target can be repressed by multiple miRNAs. Therefore, the complex net or inherent changes in molecular biology and physiopathology in different kinds of cells may affect the IC50 value.

The MCF-7/DOC cells were established and achieved statistically significant degrees of resistance relative to parental MCF-7 cell line. Thus, the MCF-7/DOC cells had a higher degree of docetaxel resistance than the parental MCF-7 cells in nature. In apoptotic analysis, both MCF-7/DOC cells and MCF-7 cells have an appropriate docetaxel concentration, respectively. The high or low docetaxel concentration will affect the accuracy of apoptotic analysis. According to the groping experimental conditions, 0.5 μM of DOC was applied to MCF-7 cells and 30 μM of DOC was used for MCF-7/DOC cells.

Anaphase-promoting complex or cyclosome (APC) is an unusually large E3 ubiquitin ligase. It is responsible for regulating defined cell cycle transitions and for modulating Wnt signaling cascade through downregulation of β-catenin. Freshly, Shen et al. [18] reported that inhibition of Wnt/β-catenin signaling downregulated P-glycoprotein and reversed multidrug resistance of cells to chemotherapy. Loss of APC expression not only led to the nuclear accumulation of β-catenin and the inappropriate activation of its target genes, such as c-myc and cyclin-D1 (growth-promoting genes) and survivin (anti-apoptotic gene), but also reduced paclitaxel-induced apoptosis by the serine–threonine kinase gene AURORA-A [19]. So knockdown APC might increase the drug resistance. In addition, APC is a regulator of Wnt signaling as well as cytoskeletal function. APC localized to kinetochores and formed a complex with kinetochore-bound protein. Microtubule-plus end proteins at kinetochores were thought to attach microtubules to chromosomes and to regulate the local polymerization and depolymerization of microtubules. This was similar to what happened in response to docetaxel, which regulated microtubule dynamics to trigger cell death. In the polymerization of microtubules, APC was more efficient than docetaxel, which indicated that docetaxel resistance cells might benefit from the upregulated APC expression [20]. However, whether there was a functional link between Wnt signaling and regulation of microtubules through APC was still unknown. In addition, APC regulated the mitotic spindle checkpoint, and dysfunctions of mitotic checkpoint had been associated with reduced paclitaxel-induced apoptosis [21]. All the findings indicated that APC was in close contact with microtubule function and drug resistance. APC4, as a scaffolding subunit, is the structural basis for the subunit assembly of the APC [22]. It seems that the loss of APC4 will influence the architecture of the APC and may resultantly change the drug resistance.

To explore the potential mechanisms of miRNA-452 in the drug resistance of breast cancer cells to DOC, APC4, a predicted target gene of miRNA-452, was chosen as our object. First, according to the predicted results of bioinformatics analysis, APC4 was a target gene of KEGG pathway which is a bioinformatics resource consisting of genomic, chemical, network information, and possible mechanisms important in the development of resistance to drugs [23]. It indicated that the miRNA-452 might target APC4 to modulate the drug resistance. Second, in MCF-7/DOC cells with high miRNA-452 expression, both APC4 mRNA and protein expression were downregulated compared with parental MCF-7 cells. More importantly, the enforced decrease of miRNA-452 level in the inhibitor-transfected MCF-7/DOC cells not only upregulated APC4 expression but also relaxed the resistance of cells to DOC. Contrarily, the enforced increase of miRNA-452 level in the mimic transfected MCF-7 cells downregulated APC4 expression and reduced the sensitivity of cells to DOC. These data suggested that miRNA-452-regulated APC4 expression, and this interaction might have an important functional consequence in the formation of breast cancer cells resistance to DOC. According to the overall results of bioinformatics analysis, mRNA, and protein expression, it showed dysregulation of miRNA-452 involved in the DOC resistance formation of breast cancer cells in vitro may be, in part, via targeting APC4. However, the role of APC4 and its related signaling pathway in miRNA-452-mediated drug resistance needs further exploration in vivo including a large amount of breast cancer tissues and animal model.

Aberrant expression of miRNA-452 is an important feature of breast cancer cells with an acquired drug-resistant phenotype and may be a significant factor contributing to its development. Evidence of miRNA-mediated drug resistance is accumulating, and much attention is now being focused on targeting miRNAs as a novel strategy for therapeutic intervention. To address the clinical significance and feasibility of implementing miRNA-based approaches to modulate sensitivity to chemotherapeutic agents, further investigation of preestablished drug-resistant animal models or clinical tumor samples is crucial for exploring the miRNA involvement in clinical resistance, and using this information for therapeutic benefit. Despite the difficulties to overcome at present, the value of miRNAs in clinical applications is projected to be monumental. Identifying the miRNAs that are able to affect the sensitivity to microtubule-poisoning drugs would be a good start and would aid in improving the targeting and development of new drugs that target the microtubule, helping clinicians to select more effective drug treatment strategies.

Conclusions

In conclusion, we demonstrate that miRNA-452 is involved in docetaxel resistance in breast cancer in vitro and miRNA-452-mediated docetaxel resistance maybe, at least in part, via targeting *APC4*. The result provides a supportive rationale for the development of miRNA-based therapeutic strategies aiming to overcome cancer cells drug resistance. Further studies, including the function analyses of its other target pathways, its expression changes in more clinical breast cancer tissues, and the association of its alteration with clinical drug resistance *in vivo*, would be warranted.

Conflicts of interest None.

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