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Study on the Mechanism of Cell Cycle Checkpoint Kinase 2 (CHEK2) Gene Dysfunction in Chemotherapeutic Drug Resistance of Triple Negative Breast Cancer Cells

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Background: This study aimed to investigate the mechanism of CHEK2 gene dysfunction in drug resistance of triple negative breast cancer (TNBC) cells.


Material/Methods: To perform our study, a stable CHEK2 wild type (CHEK2 WT) or CHEK2 Y390C mutation (CHEK2 Y390C) expressed MDA-MB-231 cell line was established. MTT assay, cell apoptosis assay and cell cycle assay were carried out to analyze the cell viability, apoptosis, and cell cycle respectively. Western blotting and qRT-PCR were applied for related protein and gene expression detection.

Results: We found that the IC₅₀ value of DDP (Cisplatin) to CHEK2 Y390C expressed MDA-MB-231 cells was significantly higher than that of the CHEK2 WT expressed cells and the control cells. After treatment with DDP for 48 h, cells expressing CHEK2 WT showed lower cell viability than that of the CHEK2 Y390C expressed cells and the control cells; compared with the CHEK2 Y390C expressed cells and the control cells, cells expressing CHEK2 WT showed significant G1/S arrest. Meanwhile, we found that compared with the CHEK2 Y390C expressed cells and the control cells, cell apoptosis was significantly increased in CHEK2 WT expressed cells. Moreover, our results suggested that cells expressing CHEK2 WT showed higher level of p-CDC25A, p-p53, p21, Bax, PUMA, and Noxa than that of the CHEK2 Y390C expressed cells and the control cells.

Conclusions: Our findings indicated that CHEK2 Y390C mutation induced the drug resistance of TNBC cells to chemotherapeutic drugs through administrating cell apoptosis and cell cycle arrest via regulating p53 activation and CHEK2-p53 apoptosis pathway.

MeSH Keywords: **Apoptosis • Checkpoint Kinase 2 • Cisplatin • Drug Resistance • Triple Negative Breast Neoplasms**

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Background

Breast cancer is one of the most common diagnosed malignancies in females in the world. Genetic factor is an important risk factor for breast cancer [1]. Up-to-now, a variety of breast cancer susceptibility genes, including BRCA1/2, CHEK2 (cell cycle checkpoint kinase 2), and ATM have been identified and considered to play important roles in DNA damage response [2–4]. BRCA1/2 is the most frequently found breast cancer susceptibility gene. People with BRCA1/2 gene mutations have a significant risk of developing breast cancer and ovarian cancer for a lifetime, with a cumulative risk of breast cancer at the age of 70; and 40% of these patients also have a risk of ovarian cancer. BRCA1/2 is an important gene for DNA damage repair. After DNA damage, BRCA1 protein can be rapidly recruited into the damaged DNA site, and activate its downstream RAD51, CHEK2, and other proteins by phosphorylation of the protein kinase ATM, thus achieving DNA damage repair through homologous recombination (HR), an important pathway for DNA damage repairing.

CHEK2 is another important breast cancer susceptibility gene, found after BRCA1/2. Various studies have reported the critical roles of CHEK2 in the regulation of apoptosis, cell cycle and DNA repair [5]. CHEK2, which is involved in cell cycle G1/S or G2/M phase arrest, is an important signal transduction protein in DNA double-strand breaks. DNA double-strand breaks activate the intracellular ATM kinase, and ATM can activate the nuclear CHEK2 through a series of phosphorylation reactions. CHEK2 can promote the phosphorylation of tumor suppressor gene p53 (Ser20), block the binding of murine double micro-2 (MDM2) protein to p53 and its role in degradation of p53, thus improving the stability of p53 in cells [6]. p53 can induce G1 arrest by activating the transcription of the p21CIP1/WAP1 gene, which inhibits cyclin-dependent CHEK2/cyclin E complex activity. In addition to p53 activation induced G1 arrest, activated CHEK2 can phosphorylate and then degrade CDC25A, function G1/S detection point effect, thus blocking DNA synthesis.

Our previous studies [7–9] have been carried out on multiple related genes of the DNA damage pathway, and we found that CHEK2 Y390C mutation inhibited the efficacy of CHEK2 in response to DNA damage agents, indicating Y390C mutation significantly impaired CHEK2 function during DNA damage response. Based on the previous studies, we propose the following hypothesis: CHEK2 is involved in the regulation of the effect of chemotherapeutic drugs on human breast cancer cells, and CHEK2 mutations may cause drug resistance to chemotherapy agents in breast cancer cells. In this study, we will examine how CHEK2 Y390C mutation can induce the drug resistance of triple-negative breast cancer (TNBC) cells to chemotherapeutic drugs, and explore the underlying molecular

mechanisms through analysis of cell apoptosis, cell cycle arrest, p53 activation, and CHEK2-p53 apoptosis pathway.

Material and Methods

Cell culture

Human TNBC cell line MDA-MB-231 was purchased from American Type Culture Collection (ATCC, USA). MDA-MB-231 cells were grown in DMEM (Gibco, USA) containing 5% (v/v) fetal bovine serum (FBS, Gibco), 1% penicillin-streptomycin, and 2 mM L-glutamine, and incubated at 37°C with 5% CO₂.

Cell transfection

To knockdown the CHEK2 gene in MDA-MB-231 cells, cell transfection assay was performed by using Lipofectamine2000 reagent (Invitrogen). In brief, MDA-MB-231 cells (5×10^4 cells/well) were seeded into six-well plates the day before transfection. Then CHEK2-shRNA or control-shRNA (Santa Cruz, CA, USA) was transfected into MDA-MB-231 cells using Lipofectamine2000 reagent (Invitrogen) according to manufacturer's protocol. Then 48 hours after the transfection, the transfection efficiency was detected using qRT-PCR and western blotting.

Subsequently, CHEK2 WT (wild-type CHEK2) or CHEK2 Y390C (CHEK2 Y390C mutation) was re-expressed in the CHEK2 knockdown MDA-MB-231 cells as previously described [7]. Cells transfected with vector control were used as the control group. The transfection efficiency was detected using qRT-PCR and western blotting. The cells were then treated with various concentrations of DDP for 48 hours.

MTT assay

CHEK2 knockdown cells were transfected with retrovirus that encoded CHEK2 WT or CHEK2 Y394C, and then treated with DDP for 48 hours. Cell viability was then measured by performing MTT assay. In brief, 20 mg/mL MTT solution was added into each culture well and incubated at 37°C for four hours. The optical density (OD) at 570 nm was determined using a microplate reader. To determine the drug resistance of MDA-MB-231 cells to DDP, half-maximal inhibitory concentration (IC₅₀) values were calculated.

Apoptosis analysis assay

Forty-eight hours after drug treatment, MDA-MB-231 cells were washed with cold PBS for at least three times, fixed with 70% ethanol for 15 minutes, and then rinsed with PBS. Subsequently, the cells were labeled with Annexin V-FITC and propidium iodide (PI) according to the manufacturer's instructions. After

incubation at room temperature for 30 minutes, flow cytometry (Becton Dickinson, New Jersey, USA) was carried out to analyze cell apoptosis. Tests were repeated at least three times.

Cell cycle assay

Forty-eight hours after drug treatment, MDA-MB-231 cells were harvested, fixed with ethanol, trypsinized and then washed with PBS. After permeabilized with 0.05% Triton X-100 in PBS for 15 minutes, the cells were then stained with propidium iodide solution (10 µg/mL) for 10 minutes without light. Finally, FACS analysis (BD FACSCalibur; BD Biosciences, Franklin Lakes, NJ, USA) was performed to analyze the different phases of the cell cycle.

Western blot analysis

The primary antibodies CHEK2, p-p53, p53, p21, Bax, PUMA, Noxa, CDC25A, and p-CDC25A, and the secondary antibodies, were all purchased from CST (USA). BCA protein assay kit (Beyotime, Shanghai, China) was used to quantify the total protein lysates extracted from MDA-MB-231 cells. Equal amount of protein samples were separated using 12% sodium dodecylsulfate-polyacrylamide gels and then transferred onto a polyvinylidene fluoride membrane (Millipore, Billerica, MA, USA). After blocking with 5% skim milk, the membranes were incubated with primary antibodies overnight at 4°C. Subsequently, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies at room temperature for two hours. The protein bands were visualized by using SuperSignal™ West Pico Chemiluminescent Substrate (Thermo Scientific). The internal control was β-actin.

Quantitative real-time PCR (qRT-PCR)

Total RNA from MDA-MB-231 cells was extracted using TRIzol reagent (Invitrogen) following the manufacturer's instruction. The PrimeScript™ reverse transcription reagent kit (TaKaRa Bio, Otsu, Japan) was used for reverse transcription assay. For cDNA analysis, quantitative real-time PCR (qRT-PCR) was performed by using the SYBR premix Ex TaqII kit (Takara) per as the manufacturer's instructions. GAPDH was used as the internal control. The primer sequences for real-time PCR were as follows: CHEK2 forward, 5'-TCTCGGGAGTCGGATGTTGAG-3', reverse, 5'-CCTGAGTGGACACTGTCTCTAA-3'; p21 forward, 5'-TGCAACTACTACAGAACTGCTG-3', reverse, 5'-CAAAGTGGTCGGTAGCCACA-3'; Bax forward, 5'-CCCAGAGAGTCTTTTCCGAG-3', reverse, 5'-CCAGCCCATGATGTTTCTGAT-3'; PUMA forward, 5'-GCCAGATTTGTGAGACAAGAGG-3', reverse, 5'-CAGGCACCTAATGGGCTC-3'; Noxa forward, 5'-CCAAGCCGTGACCAAGGAC-3', reverse, 5'-CGCCACATTGTGTAGCACCT-3';

GAPDH forward, 5'-GGCATTGCTCTCAATGACAA-3', reverse, 5'-TGTGAGGGAGATGCTCAGTG-3'.

Relative gene expression was analyzed using the 2^{-ΔΔCt} method [10]. Each reaction was performed in triplicate.

Statistical analysis

GraphPad Prism (GraphPad Software, San Diego, CA, USA) was used for data analysis. Data were presented as mean ± standard deviation (SD). Comparison between groups was performed using Student's *t*-test or one-way ANOVA; *p*<0.05 was considered as a significant difference.

Results

CHEK2 Y390C mutation induces drug resistance of MDA-MB-231 cells to DDP

To investigate the mechanism of CHEK2 gene dysfunction in drug resistance of TNBC cells to DDP, a stable CHEK2 WT or CHEK2 Y390C expressed MDA-MB-231 cell line was established. Firstly, CHEK2 gene was silenced in MDA-MB-231 cells using CHEK2-shRNA. Then 48 hours after transfection, the transfection efficiency was detected using qRT-PCR and western blotting respectively. As shown in Figure 1A and 1B, compared with the control groups, the protein and mRNA level of CHEK2 was significantly decreased in CHEK2 knockdown MDA-MB-231 cells. The IC₅₀ value of DDP was significantly increased in CHEK2 knockdown cells (Figure 1C).

Then, CHEK2 WT or CHEK2 Y390C was re-expressed in the CHEK2 knockdown MDA-MB-231 cells. As shown in Figure 2A and 2B, no significant difference of CHEK2 expression was found in CHEK2 WT and CHEK2 Y390C re-expressed MDA-MB-231 cells. And the IC₅₀ value of DDP to CHEK2 Y390C expressed MDA-MB-231 cells was significantly higher than that of the CHEK2 WT expressed MDA-MB-231 cells, and no significant difference was found between CHEK2 Y390C expressed cells and the control cells (Figure 2C).

CHEK2 Y390C mutation impairs cell viability inhibition upon DNA damage induced by DDP

After treatment with 3 µM DDP for 48 hours, cell viability was detected using MTT assay. We found that compared with the control group, the cell viability was significantly decreased in CHEK2 WT expressed MDA-MB-231 cells. CHEK2 Y390C expressed MDA-MB-231 cells showed higher cell viability compared to the CHEK2 WT expressed MDA-MB-231 cells. No significant difference was found between CHEK2 Y390C expressed cells and the control cells (Figure 3).

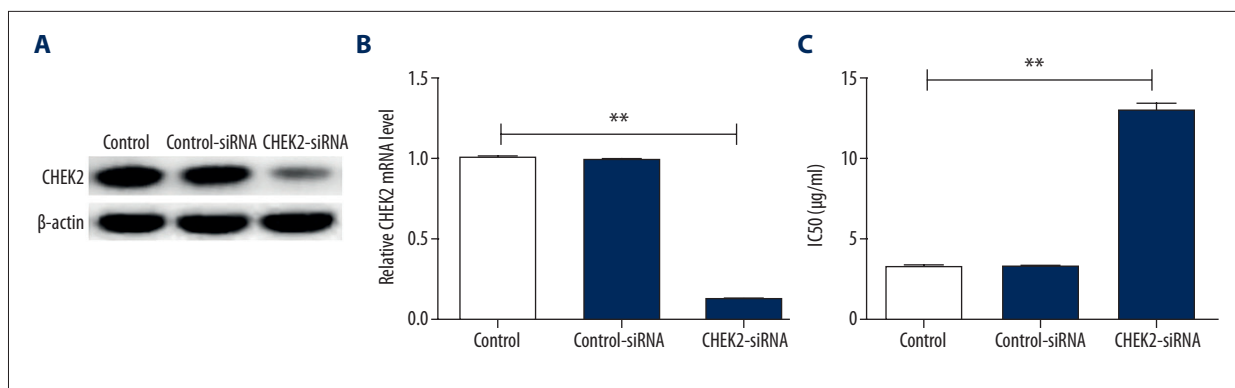


Figure 1. Suppression of CHEK2 in MDA-MB-231 cells. CHEK2 gene was silenced in MDA-MB-231 cells by transfection with CHEK2-shRNA. Forty-eight hours after transfection, the transfection efficiency was detected using western blotting (A) and qRT-PCR (B), respectively. And IC₅₀ value of DDP to MDA-MB-231 cells was detected (C). Data were presented as mean ±SD; ** *p*<0.01 versus control.

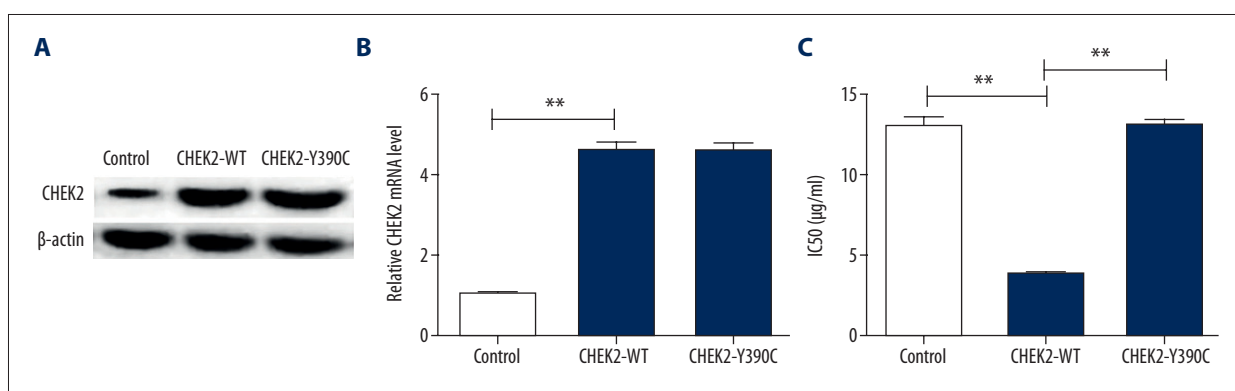


Figure 2. Re-expression of CHEK2 WT and CHEK2 Y394C in CHEK2-knockdown MDA-MB-231 cells. CHEK2 WT (wild-type CHEK2) or CHEK2 Y390C (CHEK2 Y390C mutation) was re-expressed in CHEK2 knockdown MDA-MB-231 cells. CHEK2 knockdown MDA-MB-231 cells transfected with vector control were used as the control group. CHEK2 expression was detected using western blotting (A) and qRT-PCR (B), respectively. The IC₅₀ value of DDP to MDA-MB-231 cells was detected (C). Data were presented as mean ±SD; ** *p*<0.01 versus control.

CHEK2 Y390C mutation impairs cell apoptosis upon DNA damage induced by DDP

CHEK2 knockdown cells were transfected with retrovirus that encoded CHEK2 WT or CHEK2 Y394C and then treated with 3 µM DDP for 48 hours, and then cell apoptosis was analyzed using FCM. As shown in Figure 4A and 4B, cell apoptosis was significantly induced in CHEK2 WT expressed MDA-MB-231 cells compared to the control group. However, compared with CHEK2 WT group, cell apoptosis markedly reduced in the CHEK2 Y390C group. No significant difference was found between CHEK2 Y390C expressed cells and the control cells.

CHEK2-p53 apoptosis pathway was determined in the present study, and the level of p21, Bax, PUMA, and Noxa was detected. The findings suggested that cells expressing CHEK2 WT showed higher protein/mRNA level of p21, Bax, PUMA, and Noxa than that of the CHEK2 Y390C expressed cells and

the control cells. No significant difference was found between CHEK2 Y390C expressed cells and the control cells (Figure 5).

CHEK2 Y390C mutation impairs cell cycle arrest upon DNA damage induced by DDP

The cell cycle analysis suggested that CHEK2 WT expressed MDA-MB-231 cells arrested in G1/S phase after treatment with DDP. However, this arrest was eliminated when CHEK2 Y390C mutation was expressed in MDA-MB-231 cells, and no significant difference was found between CHEK2 Y390C expressed cells and the control cells (Figure 6A).

In addition, the phosphorylation of CDC25A was measured in the present study, and we found that compared with the control cells, p-CDC25A significantly enhanced in CHEK2 WT expressed MDA-MB-231 cells. And this elevation was notably eliminated by CHEK2 Y390C mutation (Figure 6B, 6C).

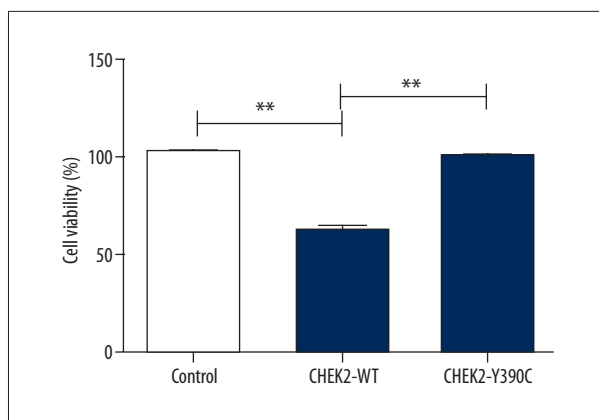


Figure 3. Effect of CHEK2 Y390C mutation on MDA-MB-231 cell viability. CHEK2 WT (wild-type CHEK2) or CHEK2 Y390C (CHEK2 Y390C mutation) was re-expressed in CHEK2 knockdown MDA-MB-231 cells. CHEK2 knockdown MDA-MB-231 cells transfected with vector control were used as the control group. After treatment with 3 μ M DDP for 48 hours, the cell viability was detected using MTT assay. Data are presented as mean \pm SD; ** $p < 0.01$ versus control.

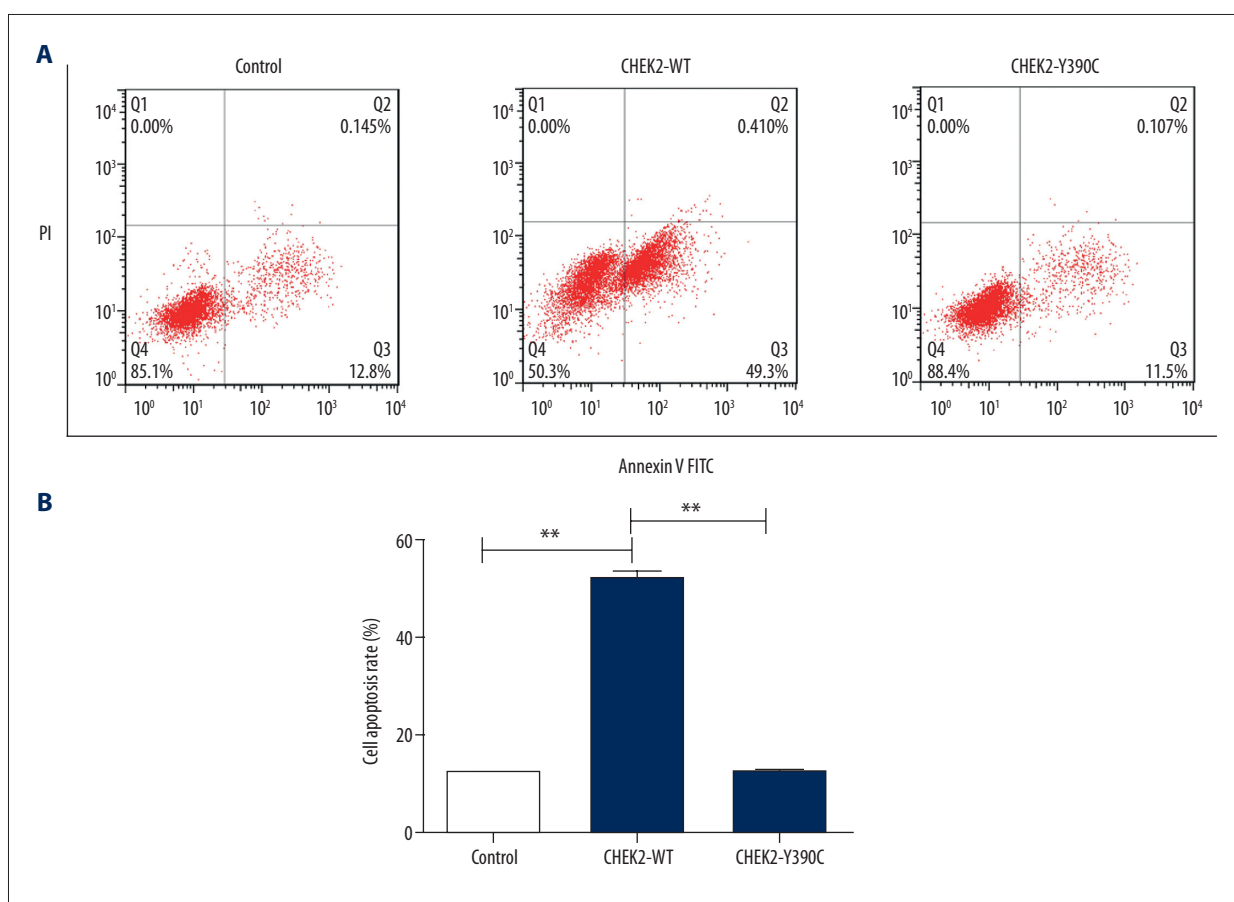


Figure 4. Effect of CHEK2 Y390C mutation on MDA-MB-231 cell apoptosis. CHEK2 WT (wild-type CHEK2) or CHEK2 Y390C (CHEK2 Y390C mutation) was re-expressed in CHEK2 knockdown MDA-MB-231 cells. CHEK2 knockdown MDA-MB-231 cells transfected with vector control were used as the control group. After treatment with 3 μ M DDP for 48 hours, the cell apoptosis was analyzed using FCM. Data are presented as mean \pm SD; ** $p < 0.01$ versus control.

CHEK2 Y390C mutation impairs p53 activation upon DNA damage induced by DDP

The activation of p53 plays an important role in the regulation of DNA repair, cell death, and cell cycle. The present study found that after treatment with 3 μ M DDP for 48 hours, the

phosphorylation of p53 was significantly enhanced in CHEK2 WT expressed MDA-MB-231 cells compared to the control cells. Compared with CHEK2 WT expressed MDA-MB-231 cells, p-p53 markedly reduced in CHEK2 Y390C expressed MDA-MB-231 cells. No significant difference was found between CHEK2 Y390C expressed cells and the control cells (Figure 7).

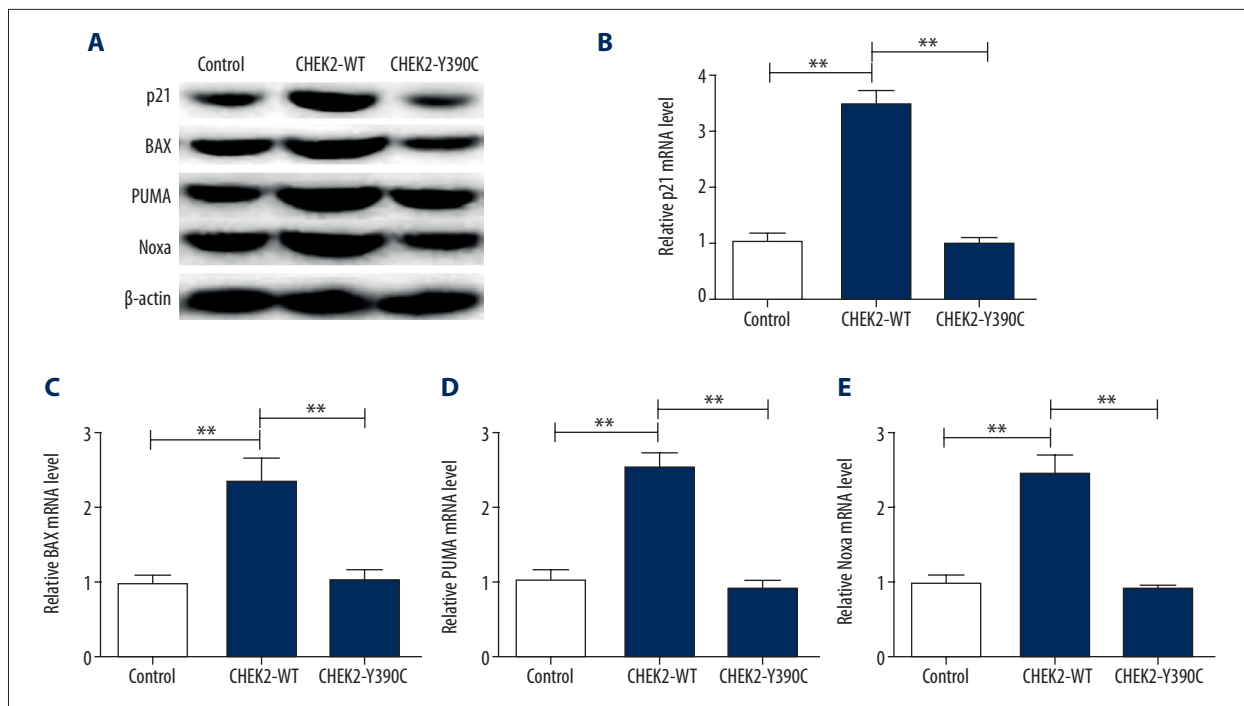


Figure 5. Effect of CHEK2 Y390C mutation on CHEK2-p53 apoptosis pathway in MDA-MB-231 cell apoptosis. CHEK2 WT (wild-type CHEK2) or CHEK2 Y390C (CHEK2 Y390C mutation) was re-expressed in CHEK2 knockdown MDA-MB-231 cells. CHEK2 knockdown MDA-MB-231 cells transfected with vector control were used as the control group. After treatment with 3 μ M DDP for 48 hours, the protein (A) and mRNA (B–E) level of p21, Bax, PUMA, and Noxa were determined using western blot and qRT-PCR, respectively. Data are presented as mean \pm SD; ** $p < 0.01$ versus control.

Discussion

Drug resistance is a major factor that affects the efficacy of chemotherapy in patients with breast cancer. There are many factors that affect TNBC drug resistance, including the evaluation of multi-drug resistance associated protein (MRP) genes, resistant multi-drug (MDR) gene, and their products, as well as reduced Topoisomerase II (Topo II) and glutathione (GSH), and increased glutathione S transferase (GST) system. Furthermore, studies have shown that the abnormal expression of related factors in cell signal transduction, DNA repair abnormality in tumor cells, and the aberrant expression of other related genes are closely related to TNBC drug resistance [11–16].

As a tumor suppressor gene, CHEK2 plays a critical role in DNA repair and maintenance of chromosome stability, and it is prone to mutate and cause cancer [15–17]. Studies have shown that CHEK2 mutations are involved in cancer occurrence and chemotherapeutic drug resistance [18–21]. Our previous study [7] have shown that CHEK2 Y390C mutation could inhibit the efficacy of CHEK2 in response to DNA damage agents, thus indicating Y390C mutation significantly impaired CHEK2 function during DNA damage response. In the present study, we investigated the mechanism of CHEK2 gene dysfunction in drug resistance of TNBC cells. Firstly, we confirmed that CHEK2 Y390C

mutation induced drug resistance of MDA-MB-231 cells to DDP; DDP is a recognized chemotherapeutic agent which can cause DNA damage. Further analysis found that CHEK2 Y390C mutation could impair cell viability inhibition, cell apoptosis, and cell cycle arrest induced by DDP.

CHEK2 is phosphorylated and activated by ATM kinases in response to DNA damage. Then the activated CHEK2 activates or phosphorylates lots of substrates, including proteins of serine 988 BRCA1 genes, tumor suppressor upstream gene p53, cell division cycle (CDC25) family, and cell cycle control proteins [15]. Thus, to improve DNA repair and triggers cell cycle checkpoints as well as p53-mediated apoptosis [22,23]. To explore the underlying molecular mechanism of CHEK2 Y390C mutation induced drug resistance in TNBC cells, we analyzed p53 activation, CDC25 phosphorylation, and CHEK2-p53 apoptosis pathway by detecting the level of p-p53, p-CDC25, p21, Bax, PUMA, and Noxa. Findings indicated that cells expressing CHEK2 WT showed higher level of p-p53, p-CDC25A, p21, Bax, PUMA, and Noxa than that of the CHEK2 Y390C expressed cells and the control cells. No significant difference was found between CHEK2 Y390C expressed cells and the control cells. These results indicated that CHEK2 Y390C manages cell apoptosis and cell cycle through regulating p53 activation and CHEK2-p53 apoptosis pathway.

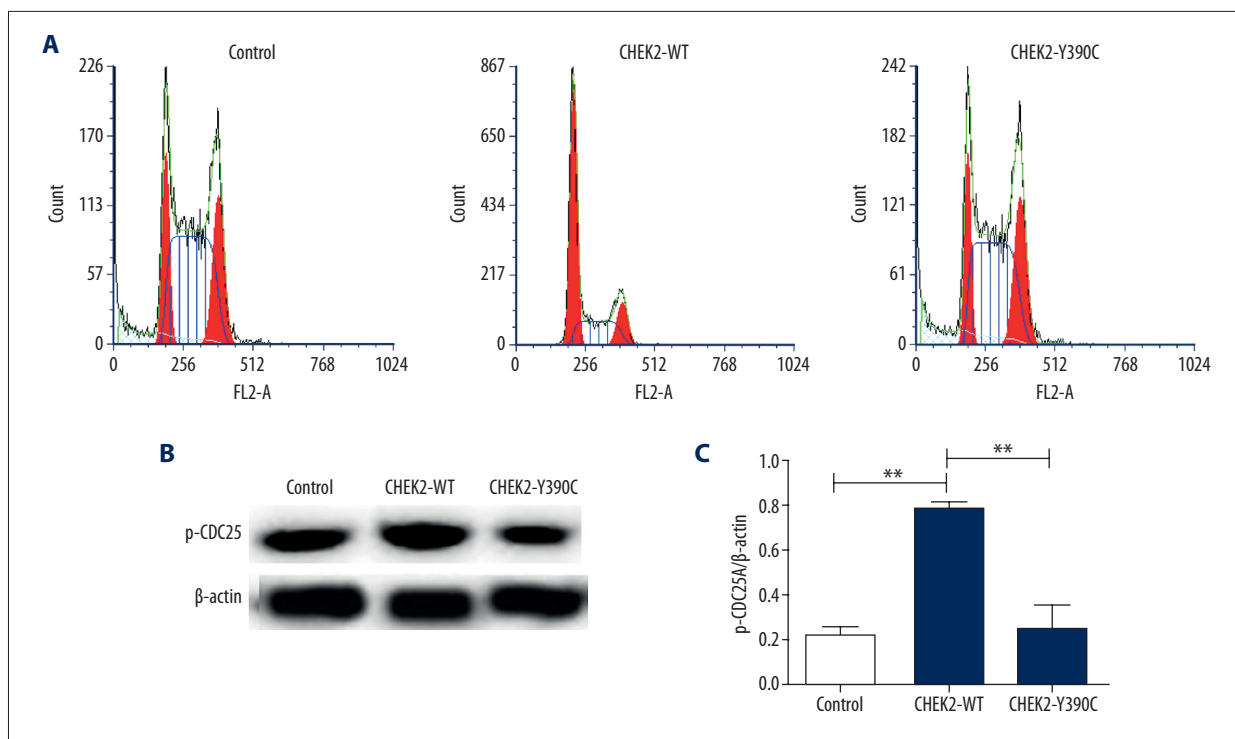


Figure 6. Effect of CHEK2 Y390C mutation on MDA-MB-231 cell cycle. CHEK2 WT (wild-type CHEK2) or CHEK2 Y390C (CHEK2 Y390C mutation) was re-expressed in CHEK2 knockdown MDA-MB-231 cells. CHEK2 knockdown MDA-MB-231 cells transfected with vector control were used as the control group. After treatment with 3 μM DDP for 48 hours, cell cycle was analyzed by FCM. Meanwhile, the protein level of p-CDC25 was determined using western blot. Data are presented as mean ±SD; ** $p < 0.01$ versus control.

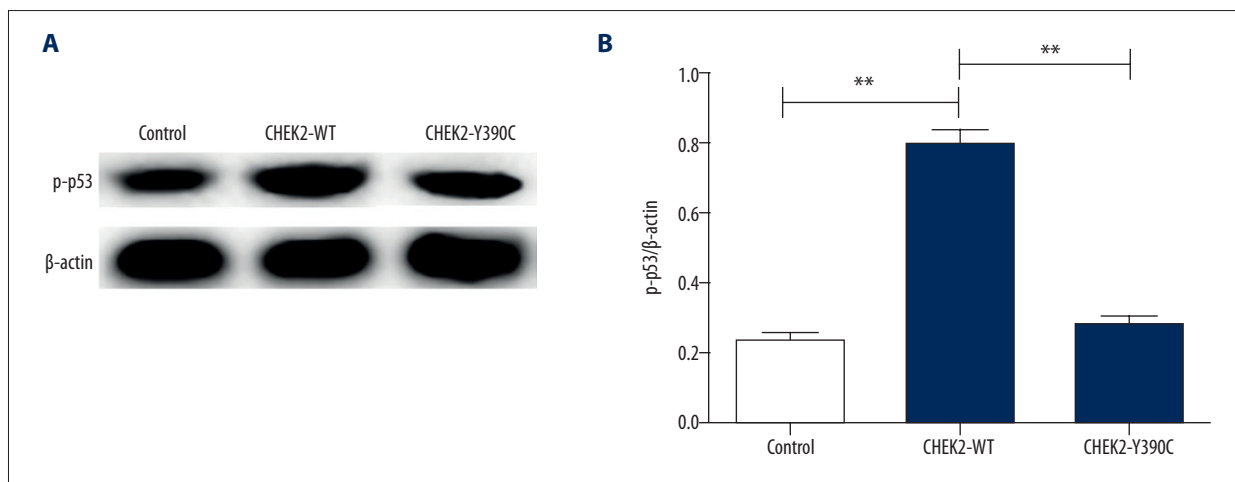


Figure 7. Effect of CHEK2 Y390C mutation on p53 activation in MDA-MB-231 cells. CHEK2 WT (wild-type CHEK2) or CHEK2 Y390C (CHEK2 Y390C mutation) was re-expressed in the CHEK2 knockdown MDA-MB-231 cells. CHEK2 knockdown MDA-MB-231 cells transfected with vector control were used as the control group. After treatment with 3 μM DDP for 48 hours, the protein level of p-p53 was determined using western blot. Data are presented as mean ±SD; ** $p < 0.01$ versus control.

In summary, our present research suggested that CHEK2 Y390C mutation induced the drug resistance of TNBC cells to chemotherapeutic agents through administration of cell apoptosis and

cell cycle arrest via regulating p53 activation and CHEK2-p53 apoptosis pathway.

Conclusions

Our data indicate that CHEK2 Y390C mutation induces resistance of TNBC cells to chemotherapeutic drugs via regulating p53 activation and CHEK2-p53 apoptosis pathway. Therefore, CHEK2 Y390C may serve as a novel therapeutic target in TNBC treatment in the future. In addition, it may be an effective

molecular marker to predict the sensitivity of patients to chemotherapy drugs, and thus better guide individual treatment decisions and improve the efficacy of chemotherapy.

Conflicts of interests

None.

References:

1. Lacroix M, Leclercq G: The "portrait" of hereditary breast cancer. *Breast Cancer Res Treat*, 2005; 89: 297–304
2. Gutiérrez-Enríquez S, Bonache S, de Garibay GR et al: About 1% of the breast and ovarian Spanish families testing negative for *BRCA1* and *BRCA2* are carriers of RAD51D pathogenic variants. *Int J Cancer*, 2014; 134: 2088–97
3. Walsh T, King MC: Ten genes for inherited breast cancer. *Cancer Cell*, 2007; 11: 103–5
4. Meindl A, Hellebrand H, Wiek C et al: Germline mutations in breast and ovarian cancer pedigrees establish RAD51C as a human cancer susceptibility gene. *Nat Genet*, 2010; 42: 410–14
5. Bartek J, Falck J, Lukas J: CHK2 kinase—a busy messenger. *Nat Rev Mol Cell Biol*, 2001; 2: 877–86
6. Dumaz N, Milne DM, Jardine LJ, Meek DW: Critical roles for the serine 20, but not the serine 15, phosphorylation site and for the polyproline domain in regulating p53 turnover. *Biochem J*, 2001; 359: 459–64
7. Wang N, Ding H, Liu C et al: A novel recurrent CHEK2 Y390C mutation identified in high-risk Chinese breast cancer patients impairs its activity and is associated with increased breast cancer risk. *Oncogene*, 2015; 34: 5198–205
8. Liu C, Ding H, Li X et al: A DNA/HDAC dual-targeting drug CY190602 with significantly enhanced anticancer potency. *EMBO Mol Med*, 2015; 7: 438–49
9. Hu J, Wang N, Wang YJ: XRCC3 and RAD51 expression are associated with clinical factors in breast cancer. *PLoS One*, 2013; 8: e72104
10. Livak KJ, Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods*, 2001; 25: 402–8
11. Chen X, Ma N, Zhou Z et al: Estrogen receptor mediates the radiosensitivity of triple-negative breast cancer cells. *Med Sci Monit*, 2018; 24: 2674–83
12. Deng X, Apple S, Zhao H et al: CD24 Expression and differential resistance to chemotherapy in triple-negative breast cancer. *Oncotarget*, 2017; 8: 38294–308
13. Blake A, Dragan M, Tirona RG et al: G protein-coupled KISS1 receptor is overexpressed in triple negative breast cancer and promotes drug resistance. *Sci Rep*, 2017; 7: 46525
14. Zheng H, Shao F, Martin S et al: WEE1 inhibition targets cell cycle checkpoints for triple negative breast cancers to overcome cisplatin resistance. *Sci Rep*, 2017; 7: 43517
15. Das S: Identification and targeting of microRNAs modulating acquired chemotherapy resistance in Triple negative breast cancer (TNBC): A better strategy to combat chemoresistance. *Med Hypotheses*, 2016; 96: 5–8
16. Chakravarthy R, Mnich K, Gorman AM: Nerve growth factor (NGF)-mediated regulation of p75(NTR) expression contributes to chemotherapeutic resistance in triple negative breast cancer cells. *Biochem Biophys Res Commun*, 2016; 478: 1541–47
17. Bayram S, Topaktaş M, Akkız H et al: CHEK2 1100delC, IVS2+ 1G> A and I157T mutations are not present in colorectal cancer cases from Turkish population. *Cancer Epidemiol*, 2012; 36: 453–57
18. Mohelnikova-Duchonova B, Havranek O et al: CHEK2 gene alterations in the forkhead associated domain, 1100delC and del5395 do not modify the risk of sporadic pancreatic cancer. *Cancer Epidemiol*, 2010; 34: 656–58
19. Palmero EI, Alemar B, Schüler-Faccini L et al: Screening for germline BRCA1, BRCA2, TP53 and CHEK2 mutations in families at-risk for hereditary breast cancer identified in a population-based study from Southern Brazil. *Genet Mol Biol*, 2016; 39: 210–22
20. Näslund-Koch C, Nordestgaard BG, Bojesen SE: Increased risk for other cancers in addition to breast cancer for CHEK2*1100delC heterozygotes estimated from the Copenhagen general population study. *J Clin Oncol*, 2016; 34: 1208–16
21. Jalilvand M, Oloomi M, Najafipour R et al: An association study between CHEK2 gene mutations and susceptibility to breast cancer. *Comp Clin Path*, 2017; 26: 837–45
22. Knappskog S, Chrisanthar R, Løkkevik E et al: Low expression levels of ATM may substitute for CHEK2/TP53 mutations predicting resistance towards anthracycline and mitomycin chemotherapy in breast cancer. *Breast Cancer Res*, 2012; 14: R47
23. Chrisanthar R, Knappskog S, Løkkevik E et al: CHEK2 mutations affecting kinase activity together with mutations in TP53 indicate a functional pathway associated with resistance to epirubicin in primary breast cancer. *PLoS One*, 2008; 3: e3062
24. Baloch AH, Daud S, Raheem N et al: Missense mutations (p.H371Y, p.D438Y) in gene CHEK2 are associated with breast cancer risk in women of Balochistan origin. *Mol Biol Rep*, 2014; 41: 1103–7
25. O'Driscoll M, Jeggo PA: The role of double-strand break repair insights from human genetics. *Nat Rev Genet*, 2006; 7: 45–54