Tropical Journal of Pharmaceutical Research November 2022; 21 (11): 2379-2384
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v21i11.16

Original Research Article

Knockdown of FK506-binding proteins 14 enhances tamoxifen-sensitivity of breast cancer through PI3K/AKT and ERK signaling

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Sent for review: 6 August 2022 *Revised accepted: 28 October 2022*

Abstract

Purpose: *To investigate the effect of FK506-binding proteins 14 (FKBP14) in the development of chemoresistance of breast cancer.*
Methods: *Breast cancer cell lines (MCF-7 and T47D) were exposed to 4-hydroxytamoxifen over a long period to establish tamoxifen-resistant (TamR) cells. Cell proliferation was evaluated by MTT and colony formation assays, while Transwell assay was used to investigate cell migration and invasion.*
Results: *TamR cells showed resistance to 4-hydroxytamoxifen through increase in IC_{50} for 4-hydroxytamoxifen in MCF-7 and T47D. The FKBP14 was significantly up-regulated in TamR cells ($p < 0.05$). Knockdown of FKBP14 reduced the IC_{50} for 4-hydroxytamoxifen in TamR cells. The number of colony formation in TamR cells was also significantly decreased by silencing of FKBP14 ($p < 0.01$). Knockdown of FKBP14 inhibited the migration and invasion of TamR cells. Protein expression of p-AKT, p-PI3K and p-ERK in TamR cells were down-regulated by silencing of FKBP14.*
Conclusion: *Loss of FKBP14 enhances sensitivity to tamoxifen in TamR MCF-7 and T47D cells through inactivation of PI3K/AKT and ERK signaling. The role of FKBP14 in tamoxifen-resistant animal models needs further investigation.*

Keywords: *FKBP14, Tamoxifen-resistant, Breast cancer, Phosphatidylinositol-3-kinase (PI3K)/AKT, extracellular signal-regulated kinase (ERK)*

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INTRODUCTION

Breast cancer (BC) is a common cancer worldwide, and its incidence has been increasing in recent years [1]. Endocrine therapy contains aromatase inhibitors and antagonists of the estrogen receptors, and is the major therapy for estrogen-receptor positive patients [2]. Tamoxifen is the first-line drug of endocrine therapy which shows benefits for BC patients [3]. However, the acquired resistance to tamoxifen promotes BC metastasis and reduces the therapeutic efficiency of endocrine therapy [3]. Nearly 40 % of breast cancer patients show tamoxifen-resistance [4]. Therefore, research

about genes involved in tamoxifen-sensitivity is helpful for the amelioration of drug resistance.

Emerging evidence has shown that FK506 binding proteins (FKBPs) bind to the immunosuppressive drug, FK506, suppresses phosphatase activity of calcineurin, and exerts immunosuppressive effect in endocrine- and cardiovascular-related diseases [5]. Moreover, FKBPs were associated with radioresistance and chemoresistance of tumors [6].

The FKBP14, a member of FKBPs family, functions as an oncogene in tumors, such as colon cancer, gastric cancer, cervical cancer, ovarian cancer, and osteosarcoma [7]. The FKBP14 was used as a tool in prognostic model that predicted prognosis in patients with BC [7] or patients after chemotherapy [8]. The FKBP14 was upregulated in tamoxifen-resistant MCF-7 [9]. However, the effect of FKBP14 in tamoxifen-resistant BC remains unclear.

The effects of FKBP14 on cell proliferation and metastasis of tamoxifen-resistant BC cells were investigated in this study. The underlying mechanism might provide potential strategy for the management of BC patients with tamoxifen-resistance.

EXPERIMENTAL

Cell culture and treatment

Breast cancer cell lines (MCF-7 and T47D) were purchased from Lonza Japan (Tokyo, Japan). The cells were grown in RPMI 1640 (Thermo Fisher Scientific, Waltham, MA, USA) with 10 % fetal bovine serum (Sigma-Aldrich, St. Louis, MO, USA). To induce tamoxifen-resistance (TamR), MCF-7 and T47D were cultured in RPMI 1640 medium containing 0.05 μM 4-hydroxytamoxifen for 30 days, and then incubated with graded concentrations of 4-hydroxytamoxifen at 0.1, 0.2, 0.4, 0.8, 1.6 or 3 μM for another 24 weeks.

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

The BC cells (MCF-7 and T47D) and the TamR cells were lysed in TRIzol (Life Technologies, Carlsbad, CA, USA). The isolated RNAs (2 μg) were then reverse-transcribed into cDNAs using Multiscribe™ Reverse transcription Kit (Applied Biosystems, Foster City, CA, USA). The mRNA expression of FKBP14 was determined by PreTaq II kit (Takara, Dalian, Liaoning, China) using the primers in Table 1.

Table 1: Primers used in PCR

Genes	Forward (5′-3′)	Reverse (5′-3′)
FKBP14	TGAAGGACAGCA CCAATAG	GCACATTTACCA CCAACTC
GAPDH	GGCATGGACTGT GGTCATGAG	TGCACCCCAACT GCTTAGC

Cell transfection and viability assays

The siRNA targeting FKBP14 (siFKBP14) and the negative control (siNC) were synthesized by Genepharma (Suzhou, China). TamR cells were seeded into 96-well plates, and then transfected with the siRNAs via Lipofectamine 2000 (Invitrogen). The BC cells (MCF-7 and T47D) and the TamR cells were seeded into 96-well plates, and incubated with different concentrations of 4-hydroxytamoxifen (10^{-3}, 10^{-2}, 10^{-1}, 1, 10, and 100 μM) for 48 h. Cells were then incubated with MTT solution (Beyotime, Beijing, China) for another 4 h, and DMSO (Sigma-Aldrich) was added to dissolve the formazan crystal formed. Absorbance at 570 nm was measured using microplate reader (Thermo Fisher Scientific). The IC_{50} for 4-hydroxytamoxifen in the cells was then calculated.

Cell proliferation assay

The TamR cells were seeded into 6-well plates, and then transfected with the siRNAs via Lipofectamine 2000 for 24 h. Cells were then grown for ten days, and the cell colonies were fixed in methanol, stained with 0.1 % crystal violet (Sigma-Aldrich, St. Louis, MO, USA), and photographed under light microscope (Olympus, Tokyo, Japan).

Transwell assays

The TamR cells with siRNAs transfection in serum-free RPMI 1640 medium were plated into upper Transwell insert chamber (Corning Incorporated, Corning, NY, USA). RPMI 1640 medium with 15 % fetal bovine serum was placed into the lower chamber. The invasive cells in the lower chamber were stained with crystal violet, and observed under microscope (Olympus) 24 h later to detect the cell migration. To assess cell invasion, TamR cells in serum-free medium were also plated into the Matrigel-coated upper chambers, and then subjected to the same protocol to investigate the cell invasion.

Western blot

The BC cells (MCF-7 and T47D) and the TamR cells were lysed in RIPA buffer (Beyotime), and the isolated proteins were then separated using

10 % SDS-PAGE. Proteins were transferred onto nitrocellulose membranes, and the membranes were blocked in 5 % bovine serum albumin. Then, the membranes were probed with specific antibodies: anti-FKBP14, and anti-β-actin (1:2000), anti-p-ERK and anti-ERK (1:3000), anti-p-AKT and anti-AKT (1:4000), anti-p-PI3K and anti-PI3K (1:5000) at 4 ^{o}C overnight. The membranes were then washed and incubated with horseradish peroxidase-conjugated secondary antibody (1:5000) at 37 ^{o}C for 1 h. Immunoreactivities were visualized using enhanced chemiluminescence (Sigma-Aldrich). All the antibodies were acquired from Abcam (Cambridge, MA, USA).

Statistical analysis

All the data obtained at least triplicate are expressed as mean ± SEM, and analyzed using Student's t test or one-way analysis of variance (ANOVA) and SPSS software. A *p*-value of < 0.05 was considered indicative of statistically significant differences.

RESULTS

FKBP14 was elevated in TamR BC cells

To induce tamoxifen-resistance, MCF-7 and T47D were exposed to 4-hydroxytamoxifen for a long time. The IC_{50} of TamR cells for 4-hydroxytamoxifen was higher than that in MCF-7 and T47D (13.48 μM for TamR MCF-7 vs. 0.92 μM for MCF-7 and 11.72 μM for TamR T47D vs. 1.32 μM for MCF-7) (Figure 1 A), suggesting that tamoxifen-resistance of TamR cells to 4-hydroxytamoxifen. The mRNA expression of FKBP14 was up-regulated in the TamR cells when compared to MCF-7 and T47D (Figure 1 B). The TamR also expressed higher protein of FKBP14 than MCF-7 and T47D (Figure 1 C), demonstrating the possible relationship between FKBP14 and tamoxifen-resistance in BC.

FKBP14 contributed to cell proliferation

The TamR cells were transfected with siFKBP14 to down-regulate FKBP14 (Figure 2 A). Transfection with siFKBP14 reduced the IC_{50} for 4-hydroxytamoxifen in TamR cells compared to that with siNC (Figure 2 B). Moreover, knockdown of FKBP14 reduced the number of colonies in the TamR cells (Figure 2 C), indicating that the anti-proliferative effect of FKBP14 loss on TamR BC cells.

FKBP14 contributed to cell metastasis

The number of cell that migrated (Figure 3 A) and invaded (Figure 3 B) cells in TamR cells were decreased by transfection with siFKBP14 when compared to transfection with siNC, indicating the anti-metastatic effect of FKBP14 loss on TamR BC cells.

FKBP14 activated PI3K/AKT and ERK signalings

Knockdown of FKBP14 reduced expression of p-ERK in TamR cells (Figure 4). The levels of p-AKT and p-PI3K were also reduced in TamR cells transfected with siFKBP14 (Figure 4), suggesting that loss of FKBP14 reduced the activation of PI3K/AKT and ERK signalings in TamR BC cells.

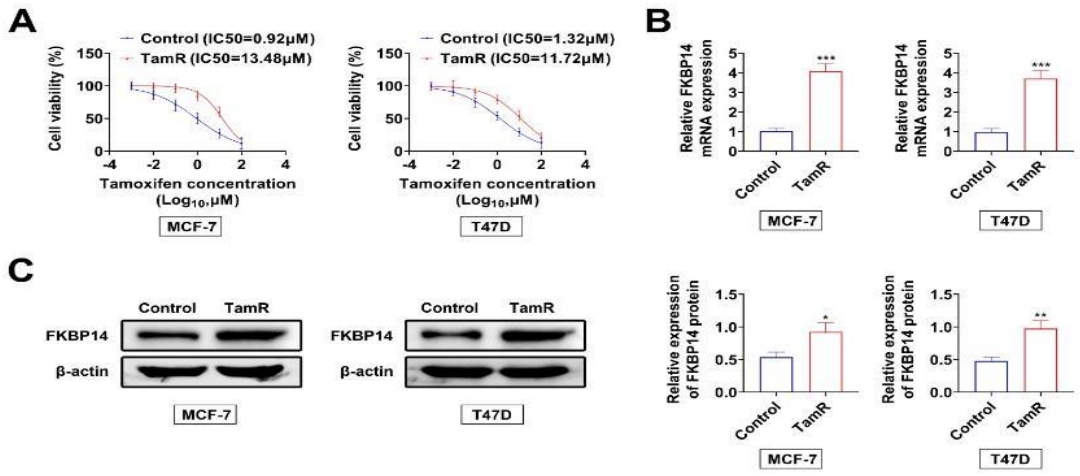

Figure 1: FKBP14 was elevated in TamR BC cells. (A) The IC_{50} values for 4-hydroxytamoxifen in the TamR cells. (B) The mRNA expression of FKBP14 was up-regulated in the TamR cells when compared to the control (MCF-7 and T47D). (C) The protein expression of FKBP14 was up-regulated in the TamR cells compared to MCF-7 and T47D vs. control. **P* < 0.05, ***p* < 0.01, ****p* < 0.001

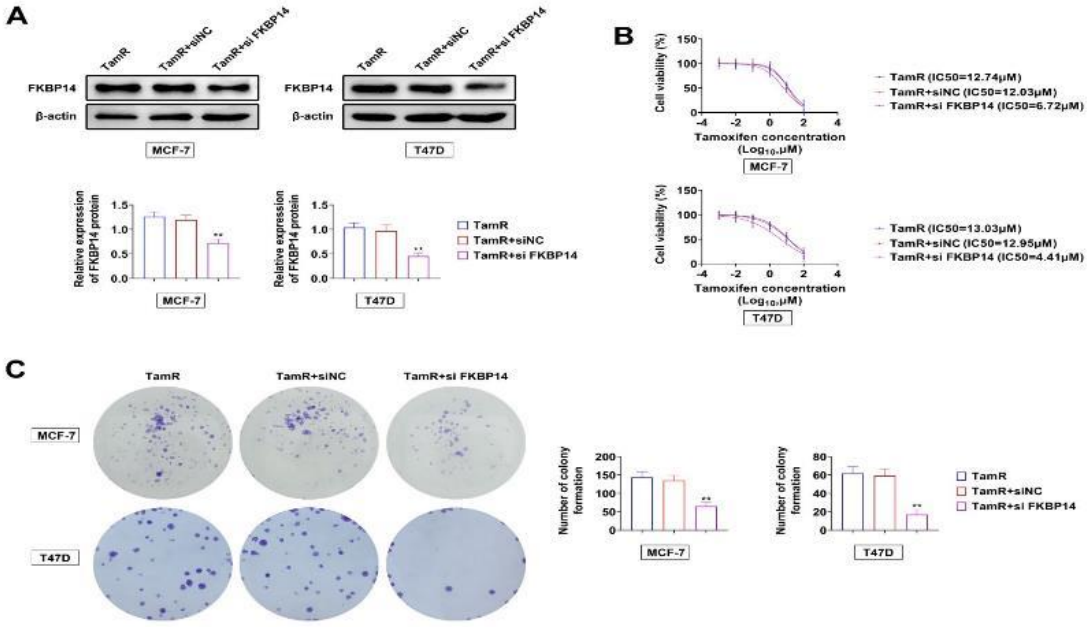

Figure 2: FKBP14 promoted proliferation of TamR BC cells. (A) Transfection with siFKBP14 down-regulated protein expression of FKBP14 in the TamR cells. (B) Transfection with siFKBP14 reduced the IC_{50} for 4-hydroxytamoxifen in the TamR cells when compared to that with siNC. (C) Transfection with siFKBP14 reduced the number of colonies in the TamR cells. ***P*<.0.01 vs siNC

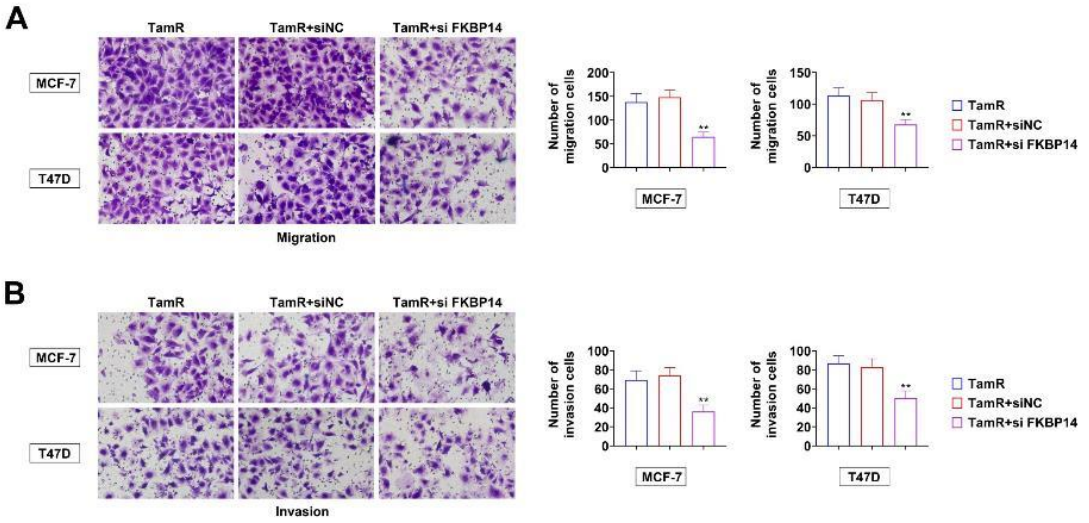

Figure 3: FKBP14 contributed to cell metastasis TamR BC cells (A) Transfection with siFKBP14 reduced the number of migrated cells in the TamR cells. (B) Transfection with siFKBP14 reduced the number of invaded cells in the TamR cells. ***p* < 0.01 vs. siNC

DISCUSSION

The FKBPs, with the ability to regulate cell cycle, survival, apoptosis and metastasis of tumors, have been shown to be promising prognostic or diagnostic biomarkers in various cancers [10]. Moreover, FKBPs are involved in chemoresistance of cancers through regulation of apoptotic and survival pathways [11,12]. This study identified a novel oncogene for BC, FKBP14, and silence of FKBP14 enhanced the tamoxifen sensitivity of TamR BC. Genome-wide expression analysis based on cold atmospheric plasma treatment and tamoxifen resistance showed that FKBP14 was up-regulated in TamR MCF-7 cells [9]. This study also confirmed the elevation of FKBP14 in TamR MCF-7 and T47D cells. Moreover, heterotrimeric transcription factor, NFYB, promoted the up-regulation of FKBP14 in patients who received chemotherapy, and high FKBP14 expression predicted poor prognosis in the patients [8].

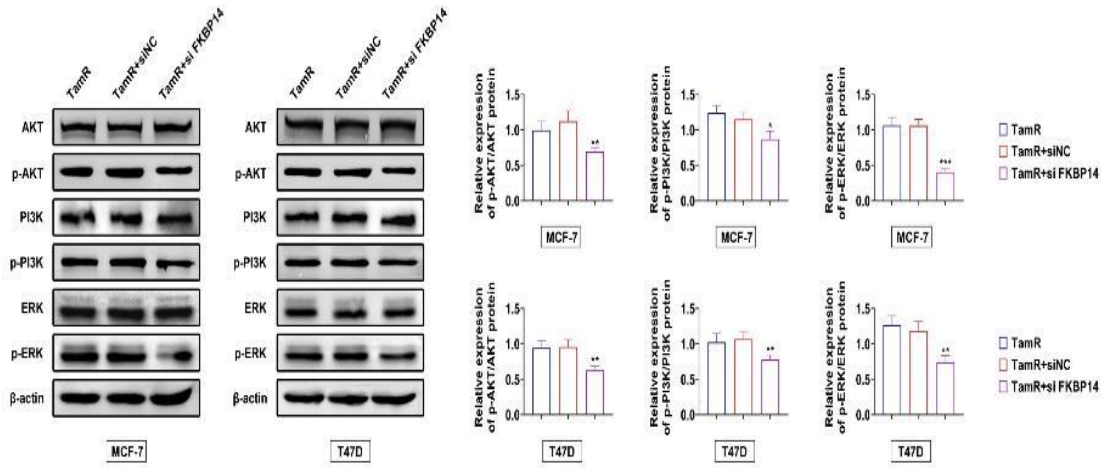

Figure 4: FKBP14 activated PI3K/AKT and ERK signalings in TamR BC cells. Transfection with siFKBP14 reduced the protein expression of p-ERK, p-AKT and p-PI3K in TamR cells. *P < 0.05, **p < 0.01, ***p < 0.001 vs. siNC

Therefore, the correlation of FKBP14 expression and clinical characteristics of tamoxifen-resistant patients should be investigated to indicate the prognostic or diagnostic roles of FKBP14 in the tamoxifen-resistance.

Functional assays showed that silencing of FKBP14 reduced the IC_{50} for 4-hydroxytamoxifen in the TamR cells, thereby enhancing the tamoxifen sensitivity of TamR cells. Moreover, the cell proliferation and metastasis of the TamR cells were inhibited by knockdown of FKBP14. Resistance to tamoxifen showed decrease in cell apoptosis in BC [13]. Therefore, silencing of FKBP14 might exert pro-apoptotic role in TamR cells to promote the tamoxifen sensitivity.

Alteration in the expression of microRNAs, tamoxifen metabolism, and modification in the PI3K cell survival pathway contribute to resistance of BC to tamoxifen [14,15]. Moreover, ERK signaling is associated with tamoxifen-resistance of BC through regulation of cell apoptotic pathway [16]. Suppression of PI3K/AKT [17] and ERK [18] pathways ameliorated the tamoxifen-resistance.

CONCLUSION

The findings of this study show that knockdown of FKBP14 reduces the expressions of p-ERK, p-AKT and p-PI3K in TamR cells, thus inhibiting the activation of PI3K/AKT and ERK signaling. The loss of FKBP14 suppresses cell proliferation and metastasis of TamR cells through inactivation of PI3K/AKT and ERK signaling. However, the role of FKBP14 in tamoxifen-resistant animal model should be further investigated.

DECLARATIONS

Acknowledgements

This work was supported by Beijing Medical Award Foundation (Grant no. YXJL-2020-0941-0741).

Funding

None provided.

Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Yongqiang Ma and Mingchuan Zhao designed the experiments and carried them out. Chengcheng Gong and Haitao Miu analyzed and interpreted the data, prepared the manuscript with contributions from all co-authors.

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