Biochemical and Biophysical Research Communications xxx (2018) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Downregulation of TXNIP leads to high proliferative activity and estrogen-dependent cell growth in breast cancer

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ARTICLE INFO

Article history:
Received 30 January 2018
Accepted 5 March 2018
Available online xxx

Keywords:
Estrogen receptor
Fulvestrant
GLUT1
p27
VDUP1

ABSTRACT

TXNIP is a potent tumor suppressor with reduced expression in various types of human cancer. The prognostic and predictive power of TXNIP has been recognized in human breast cancer. The aim of this study is to investigate the clinical relevance and functional roles of TXNIP downregulation in breast cancer. We examined TXNIP expression at the protein level in tissue microarray (TMA)-based human breast cancers and its correlation with clinical parameters and molecular markers on immunohistochemistry (IHC). Compared with normal tissues, TXNIP expression was significantly decreased in human breast cancer tissues and animal mammary tumors, along with tumor progression. TXNIP was restored immediately after histone deacetylase inhibitor treatment in breast cancer cells, implying transcriptional regulation of TXNIP by histone modification. Decreased TXNIP protein levels were more common in tumors showing high proliferative activity, such as high Ki-67 labeling indexes and low p27 expression. TXNIP knockdown led to increased *in vitro* and *in vivo* breast cancer cell growth accompanied by p27 reduction and GLUT1 induction. Interestingly, estrogen receptor (ER)-positive breast cancer samples showed higher TXNIP expression compared to ER-negative samples. TXNIP expression decreased when ER signaling was activated by estradiol, while its expression increased under ER blockage by anti-estrogen fulvestrant. In addition, TXNIP knockdown in breast cancer cells caused significant reduction in the cell-growth inhibitory effect of anti-estrogen fulvestrant. In conclusion, our data demonstrated that TXNIP functions to suppress high proliferative activity and estrogen-dependent cell growth in breast cancer.

1. Introduction

Breast cancer is one of the most common cancers in women, with a rising annual incidence in many populations [1]. Measurement of estrogen receptor alpha (ER) in breast cancer cells is used in standard care to identify patients more likely to respond to anti-estrogens such as tamoxifen or fulvestrant. Fulvestrant is a highly selective ER antagonist that is used for the treatment of ER-positive (ER+) locally advanced or metastatic breast cancer. Unlike specific ER modulators such as tamoxifen, which modulates ER function in a tissue-specific manner, fulvestrant directly reduces ER levels and activity [2]. It binds to ER with high affinity, and is a potent competitor against estrogen for ER binding [2]. Generally, ER+ cases, which can be treated with hormone therapy, are generally considered to be less aggressive than ER-negative (ER−) cases. However, since a significant proportion of ER+ breast cancer patients experience disease progression during fulvestrant treatment [3], it is necessary to identify and elucidate the genes responsible for ER signaling and the anti-estrogen response.

Thioredoxin interaction protein (TXNIP) is a 46-kDa multifunctional protein that was isolated as an upregulated protein following vitamin D_3 treatment of HL-60 cells [4]. TXNIP interacts directly with the thioredoxin (Trx) antioxidant gene, regulating the reduction/oxidation status of the cell through inhibition of Trx activity [5]. TXNIP plays an important role in a wide variety of biological functions, including cell growth, differentiation, and energy metabolism [6]. It is also recognized as a tumor-suppressor protein

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https://doi.org/10.1016/j.bbrc.2018.03.020
0006-291X/

due to its inhibitory effect on tumor growth via regulation of tumor cell proliferation and cell-cycle progression [7]. TXNIP deficiency has been shown to promote the development of hepatocellular carcinoma and gastric cancer in mice [8,9]. It has been proposed that TXNIP has potential as a useful biomarker for human breast cancer, as high *TXNIP* mRNA expression is associated with a better prognosis [10].

In this study, we examined TXNIP expression in human breast cancer tissues and its correlation with clinical parameters and molecular markers by IHC. In addition, we investigated whether TXNIP downregulation is observed during animal mammary carcinogenesis. Based on its negative correlation with Ki-67 and positive correlation with ER and p27 in clinical samples, we performed TXNIP-knockdown studies to clarify the roles of TXNIP in cancer cell growth and TXNIP's involvement in ER signaling and the anti-estrogen response.

2. Materials and methods

2.1. Cells

The human breast cancer cell lines MCF7, T47D, and MDA-MB-231 were obtained from the Korean Cell Line Bank (Seoul, Korea). Murine mammary cancer cell lines were primarily cultured from mammary adenocarcinomas arising from *MMTV-Cre;Smad4*$^{F/F}$;*Trp53*$^{F/F}$;*Cdh1*$^{F/+}$ mice [11]. Non-targeting small interfering RNA (siRNA) and siRNAs specific for human *TXNIP* was purchased from Qiagen (SI03648827; FlexiTube). The lentiviral *TXNIP* shRNA constructs (TRCN0000282428) were purchased from Sigma-Aldrich with pLKO.1-puro eGFP control vector (SHC005, Sigma).

2.2. Immunohistochemical staining of TXNIP, Ki-67, and p27

Human breast cancer TMA slides containing tissues and normal breast tissues were purchased from SuperBioChips Laboratories (Seoul, Korea), ISU ABXIS Co., Ltd. (Seoul, Korea), and US Biomax (Rockville, MD, USA). Supplementary data on each patient's clinical and pathological factors, including histological grade, and relevant biomarkers such as ER and progesterone receptor (PR) status, and HER-2/*neu*, were provided by the manufacturers. Canine mammary gland tumors were collected from biopsy specimens submitted for diagnosis at the Veterinary Diagnostic Center of the College of Veterinary Medicine at Seoul National University.

IHC was performed to detect TXNIP expression in human and canine mammary tumor tissues using mouse anti-monoclonal TXNIP (K0205-3; Medical and Biological Laboratories Co., Nagoya, Japan). Semi-quantitative assessment of TXNIP levels was conducted for both cell compartments. Nuclear TXNIP expression was categorized into four groups according to the percentage of positively stained nuclei: 0, less than 5%; 1, from 5% to 30%; 2, from 30% to 60%; 3, more than 60%. Cytoplasmic TXNIP expression was scored according to the intensity of staining: 0, negative staining; 1,

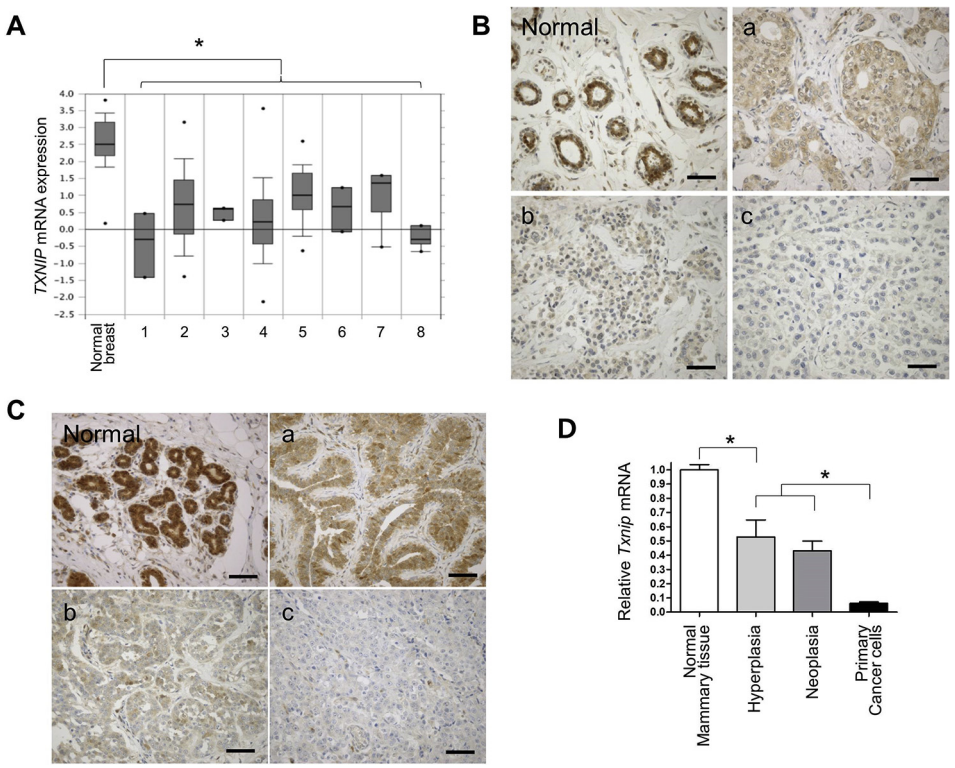

Fig. 1. Downregulation of TXNIP in breast cancer. A. TXNIP mRNA expression in breast cancers and normal breast tissues (n = 61) in the TCGA dataset. Values were presented by the log2 median-centered ratio. 1, intraductal cribriform adenocarcinoma (n = 3); 2, invasive carcinoma (n = 76); 3, invasive ductal and lobular carcinoma (n = 3); 4, invasive ductal carcinoma (n = 389); 5, invasive lobular carcinoma (n = 36); 6, male carcinoma (n = 3); 7, mixed lobular and ductal carcinoma (n = 7); 8, mucinous carcinoma (n = 4). B. Representative IHC staining of TXNIP in human breast cancers. Left top, normal breast epithelial cells showing strong expression of TXNIP (nucleus, grade 3; cytoplasm, grade 3). B-a, cancer cells showing moderate TXNIP expression (nucleus, grade 2; cytoplasm, grade 2). B-b, weak TXNIP expression (nucleus, grade 1; cytoplasm, grade 1). B-c, negative (nucleus, grade 0; cytoplasm, grade 0) immunoreactivity for TXNIP. Bar = 50 μm. C. Representative IHC staining of TXNIP in canine mammary tumors. Left top, normal mammary epithelial cells showing strong expression of TXNIP. C-a, mammary adenoma showing moderate TXNIP expression. C-b, mammary adenocarcinoma showing weak TXNIP expression. C-c, poorly differentiated mammary adenocarcinoma showing negative TXNIP expression. Bar = 50 μm. D. Real-time quantitative RT-PCR (qRT-PCR) analysis for *Txnip* mRNA expression in normal mammary tissues (n = 3) from *MMTV-Cre*-negative mice; hyperplastic (n = 3) and neoplastic (n = 1, adenoma; n = 2, adenocarcinoma) lesions from *MMTV-Cre;Smad4*$^{F/F}$;*Trp53*$^{F/F}$;*Cdh1*$^{F/+}$ mice; and cancer cell lines (n = 2) primarily cultured from mouse adenocarcinoma tissues.

Please cite this article in press as: J.W. Park, et al., Downregulation of TXNIP leads to high proliferative activity and estrogen-dependent cell growth in breast cancer, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.03.020