Effects of *TPX2* gene on radiotherapy sensitization in breast cancer stem cells

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Abstract. The present study explored the link between the targeting protein for Xenopus kinesin-like protein 2 (*TPX2*) gene and breast tumor stem cells in order to screen novel radiosensitizers. Expression of TPX2 protein and gene in breast cancer cells was analyzed by western blot analysis and RT-PCR. Three kinds of broad-spectrum sensitizers were selected and their effects on radiotherapy were analyzed by immunohistochemistry in breast tumor stem cells. *TPX2* gene and protein were expressed in breast tumor cells and increased gradually along with the expression of cancer cell differentiation; 25 mg/l lovastatin showed best radio-sensitizing effects on breast cancer cells. Furthermore, immunohistochemical results showed that the positive rate of breast cancer cells processed by 25 mg/l lovastatin were significantly decreased. In conclusion, *TPX2* gene is closely related to the development of breast cancer stem cells. Moreover, the sensitizing effects of lovastatin on breast tumor stem cells are the result of its influence on the *TPX2* gene.

Introduction

Breast cancer is one of the most common malignant tumors of females with high incidence and high mortality (1,2). It was traditionally believed that tumor was originated from cell mutation and could grow unlimitedly. However, theories on tumor stem cells have extended knowledge on tumor cells, indicating that tumor develops from tumor stem cells in tissues (3,4). The above recent theory, not only explained biological behavior of breast cancer cells, but also provided new research directions for tumor treatment.

Radiotherapy involves X- or gamma-ray treatment onto tumor region and is one of the main methods of treating tumor. The rays interact with molecules (mainly water molecules) in tumor cells to produce cytotoxic OH-free radical resulting in cell death or apoptosis (5). However, during conventional radiotherapy, resistance of cancer cells towards rays is commonly observed (6,7). Therefore, seeking strategies to improve breast cancer radiosensitivity is a recent hot spot in radiotherapy research against tumor.

Targeting protein for Xenopus kinesin-like protein 2 (*TPX2*) gene, also called XKIP2-targeted protein, is necessary for the microtubule structuring process of cell kinetochore (8-10). Abundant research in recent years has shown that *TPX2* gene is closely related with the development of cancer cells such as lung, colon and cervical cancer cells (11). However, there are few studies on the association between *TPX2* gene and breast cancer cells.

To the best of our knowledge, the present study examined for the first time *TPX2* expression in breast tumor stem cells and investigated the association between *TPX2* gene and breast tumor stem cells. The present study also involved exploration of radiotherapy with various sensitizers on breast stem cells by targeting the *TPX2* gene.

Materials and methods

Experimental materials. Bcap37, MCF7, SKBR3 and MDAMB231 breast cancer cells were purchased from the American Type Culture Collection (ATCC) cell bank (Manassas, VA, USA). Tissue samples were collected from 55 cases of breast cancer cells removed during surgeries from March 2014 to June 2016 in the Hexian Memorial Hospital of Panyu (Guangzhou, China). The samples were confirmed by biopsy. According to clinical staging by International Federation of Gynecology and Obstetrics (FIGO). There were 12, 13, 9 and 21 cases of I-IV stages, respectively. The patients were aged 29-55 years with an average of 38.4±3.9 years. Normal breast cells were collected for comparison. Compounds with spectral sensitization such as docetaxel, lovastatin and β-santalene were obtained.

Extraction of cell total proteins. Specific experimental methods were previously described (12). Extracted proteins were saved at -80˚C.

Western blot analysis. Specific experimental methods were carried out as previously described (13).

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Table I. TPX2 protein relative expression in four types of breast cancer cells (mean ± SD).

Variables	Normal cell	Bcap37	MCF7	SKBR3	MDAMB231
TPX2 (OD)	0.028±0.012	0.318±0.023	0.377±0.019	0.373±0.018	0.374±0.022
β-actin (OD)	0.93±0.13	0.89±0.11	0.93±0.22	0.95±0.13	0.97±0.21
Relative expression	0.003±0.001[a]	0.357±0.043[b]	0.406±0.093[b]	0.393±0.025[b]	0.386±0.036[b]

[a,b]In one-way ANOVA among groups, the same letter indicated (P>0.05) no statistical significance; different letters indicated (P<0.05) statistical significance. Same below. *TPX2*, targeting protein for Xenopus kinesin-like protein 2.

Extraction of total RNA, detection and determination of purity. Experimental methods were conducted as in the literature (14), with some modifications. Extracted proteins were saved at -80˚C.

Radiotherapy experiments on the sensitizing compounds. Docetaxel, lovastatin and β-santalene of the same concentration (20 mg/l) were prepared to function in Bcap37 breast cancer cells. Sensitizing compounds of the same concentration (10, 15, 20, 25, 30, 40 and 50 mg/l) were prepared to investigate effects of concentration on breast cancer cells (15).

Grouped processing of sensitization. Two microliters of culture and buffer solutions were added to group A for comparison. Different sensitizers at a rate of 20 mg/l were added to group B. In group C no sensitizer was given and underwent radiotherapy only. After the experiment, the cell apoptotic rate was recorded by flow cytometry (16).

Radiotherapy and flow cytometry. Specific experimental process was carried out as published (15).

Immunohistochemistry experiment. For detection of TPX2 expression in breast tissues, we performed inmmunohistochemical staining according to a previous study (17).

Statistical analysis. Experimental data were analyzed by SPSS software (Chicago, IL, USA). Differences among groups underwent homogeneity test for variance and t-test; testing level was α=0.05. (P<0.05 was considered to indicate a statistically significant difference).

Results

Detection of TPX2 protein expression in different breast cancer cells by western blot analysis. TPX2 protein expression in four types of breast cancer cells, Bcap37, MCF7, SKBR3 and MDAMB231 were detected by western blot analysis. Normal breast cells were used for comparison, and the OD value ratio of TPX2 and β-actin referred to TPX2 protein relative expression. By combining markers, it was confirmed that the bands between a molecular weight of 100 and 43 kDa were TPX2 protein and β-actin (Fig. 1). It was observed that TPX2 was expressed in the four types of breast cancer cells but not expressed in the normal cells. This experiment compared the detected OD values with image scanning and analyzing software and conducted statistical processing. Table I shows

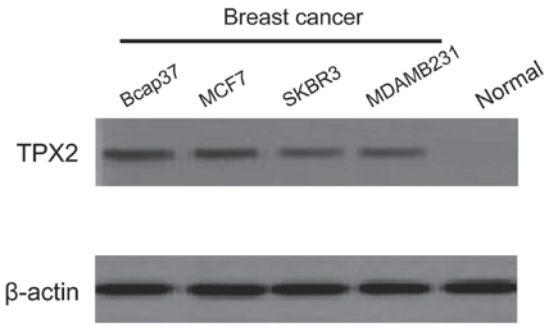

Figure 1. Targeting protein for Xenopus kinesin-like protein 2 (*TPX2*) expression in different breast cancer cells.

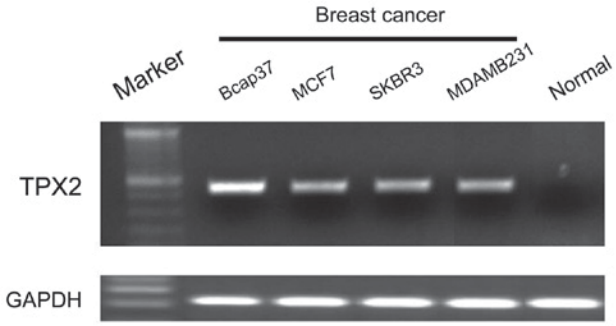

Figure 2. Targeting protein for Xenopus kinesin-like protein 2 (*TPX2*) mRNA expression in different breast cancer cells.

that TPX2 protein expression in four types of breast cancer cells was significantly higher than that in normal cells (0.003±0.001), but TPX2 protein expression between each pair of breast cancer cells had no statistical significance (P>0.05).

Detection of TPX2 mRNA expression in different breast cancer cells by RT-PCR. TPX2 mRNA expression in Bcap37, MCF7, SKBR3 and MDAMB231 was detected by RT-PCR. Normal breast cells were used for comparison, and OD value ratio of TPX2 and GAPDH referred to TPX2 gene expression. It is clear from Fig. 2 that TPX2 mRNA expression was similar to its protein expression in that it was expressed in the four types of breast cancer cells but not expressed in the normal cells. This experiment compared the detected OD values with image scanning and analyzing software and statistical processing was conducted. The TPX2 mRNA expression (Table II) in the four types of breast cancer cells (0.536±0.039 on average) was

Table II. TPX mRNA expression in different breast cancer cells (mean ± SD).

Variables	Normal cells	Bcap37	MCF7	SKBR3	MDAMB231
TPX2 (OD)	0.004±0.012	0.464±0.034	0.520±0.028	0.478±0.017	0.499±0.052
GAPDH (OD)	0.86±0.11	0.84±0.18	0.90±0.28	0.93±0.15	0.89±0.17
Relative expression	0.005±0.002[a]	0.553±0.023[b]	0.578±0.091[b]	0.514±0.033[b]	0.561±0.062[b]

[a,b]In one-way ANOVA among groups, the same letter indicated (P>0.05) no statistical significance; different letters indicated (P<0.05) statistical significance. *TPX2*, targeting protein for Xenopus kinesin-like protein 2.

Table III. Comparison of radiotherapy effects of three types of broad-spectrum sensitizers on breast cancer stem cells (cell apoptosis rate, %; mean ± SD).

Groups	Docetaxel	Lovastatin	β-santalene
A (control)	0.5±0.02	0.7±0.05	0.4±0.03
B (adding medicine)	0.6±0.12	0.3±0.11	3.3±0.08
C (radiotherapy)	15.2±0.22[a,b]	21.3±1.21[a,b]	6.9±0.52[a,b]
D (radiotherapy and adding medicine)	18.5±1.11[a-c]	33.2±2.10[a-c]	11.3±1.15[a-c]

[a]Compared with group A, P<0.05; [b]compared with group B, P<0.05; [c]compared with group C, P<0.05.

Table IV. Comparison of radiotherapy effects of lovastatin at different concentrations on breast cancer stem cells (concentration, mg/l; cell apoptosis rate, %; mean ± SD).

	Concentrations (mg/l)						
Groups	10	15	20	25	30	40	50
C (radiotherapy)	21.5±1.21	19.6±1.42	22.8±1.11	18.1±1.32	19.4±1.47	20.8±2.01	20.2±1.96
D (radiotherapy and adding medicine)	25.4±2.12[a]	30.4±2.31[a]	35.6±2.51[a]	38.7±2.12[a]	38.9±1.58[a]	37.8±2.45[a]	38.2±2.66[a]

[a]Compared with group C, P<0.05.

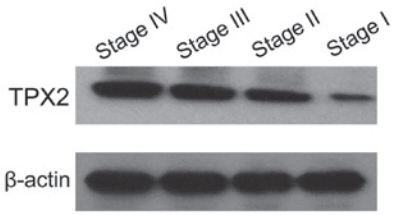

Figure 3. Targeting protein for Xenopus kinesin-like protein 2 (*TPX2*) mRNA expression in breast cancer cells at different clinical stages.

significantly higher than that in normal cells (0.005±0.002), but TPX2 mRNA expression between each pair of breast cancer cells had no statistical significance (P>0.05).

Detection of TPX2 expression in breast cancer cells at various differentiation stages by western blot analysis. *TPX2* expression in breast cancer cells at the various differentiation stages was detected by western blot analysis to investigate TPX2 protein changes in the development of breast cancer cells. Experimental results are shown in Fig. 3. The results showed that TPX2 protein expression at stages I-IV was significantly increased along with the increased of differentiation stages.

Investigation on radiotherapy effects on three types of broad-spectrum sensitizers. In the present study, in comparison to the control group, docetaxel and lovastatin produced less effect on breast cancer cells confirming that cytotoxicity of the two compounds was not strong. However, β-santalene between two groups had no significant differences, reflecting its strong cytotoxicity. Furthermore, lovastatin had the strongest effects. By comparing three kinds of sensitizers, it was observed that lovastatin had little cytotoxicity but obvious radiotherapy improving effects; thus, it is a relatively reasonable radiotherapy sensitizer on breast cancer cells. As a result, lovastatin was chosen to investigate the effects of different concentration on breast cancer cells (Tables III and IV).

Immunohistochemical results of different groups. Cells undergoing various processing were used as research subjects,

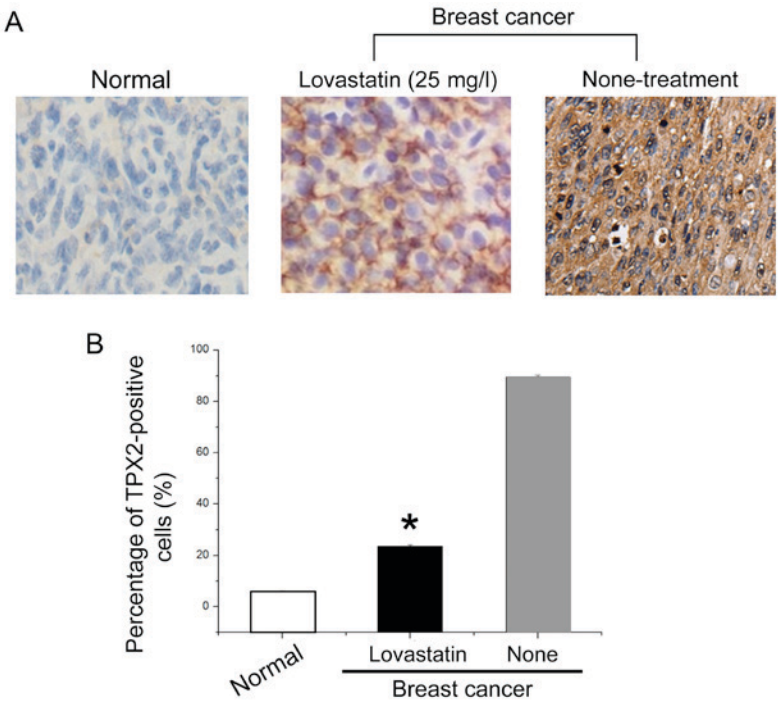

Figure 4. Immunohistochemical staining of targeting protein for Xenopus kinesin-like protein 2 (*TPX2*) in different groups. (A) Immunohistochemical results. *TPX2*-negative cells are blue; *TPX2*-positive cells are brown. (B) Counting results of *TPX2*-positive cells undergoing various processing. *P<0.05, compared with 'none' group.

and TPX2 protein expression in the cells of different groups were detected by immunohistochemistry. Fig. 4A shows that TPX2 protein-positive rate of breast cancer cells processed by 25 mg/l lovastatin were significantly lower than that of normal cancer cells. Counting results of positive cells (Fig. 4B) indicated that TPX2 protein positive rate of breast cancer cells processed by 25 mg/l lovastatin was 23.6%, while TPX2 protein-positive rate of breast cancer cells in the control group was 89.5%. Thus, processing with 25 mg/l lovastatin improved radiotherapy sensitization significantly.

Discussion

As living standards improve and dietary habits change, a significant rise in the incidence of breast cancer has been recorded. Although theories on tumor stem cells have improved knowledge on tumor cells, findings suggest new medical directions (18). Breast cancer is a malignant tumor and there is a possibility that incidence and cell migration may occur following surgery. Therefore, treatment methods for breast cancer have no obvious improvement yet, and it is imperative that new treatment initiatives are identified. Advances in life sciences with regard to treating cancer based on genetics has gradually become a research focus (19).

TPX2 gene is a microtubule-associated protein, and research in recent years has shown that it is closely related with the development of multiple cancer cells (20) including breast cancer. Further, radiotherapy has the ability to inhibit cancer cell proliferation but is very cytotoxic. Therefore, seeking a *TPX2* gene-targeted compound sensitizer for radiotherapy on breast cancer may be an effective curative method that could alleviate associated side effects. In the present study, we first explored the relationship between *TPX2* gene and breast cancer cells, then used three broad-spectrum sensitizers to conduct radiotherapy *in vitro* to identify the interactive relations between sensitizer and *TPX2* gene (21).

By analyzing the relationship between *TPX2* gene and breast cancer cells, using western blot analysis and RT-PCR, the present study revealed that *TPX2* gene and protein were hardly expressed in normal breast cells but were expressed significantly more in all four types of breast cancer cells. However, expressions in various breast cancer cells were not significantly different. It showed that if *TPX2* gene and protein were detected in breast cells, malignant pathological changes could probably exist. This experiment also investigated TPX2 protein expression in breast cancer cells at various differentiation stages and found that TPX2 protein expression increased significantly along with the increase in differentiation stages, which further indicated *TPX2* gene is closely related to the development and deteriorating severity of breast tumor cells. Moreover, monitoring *TPX2* gene expression in tumor tissues could evaluate tumor severity and prove useful in the prediction and prognostic treatment of the disease.

After confirming the relationship between *TPX2* gene and breast cancer stem cells, the present study selected three kinds of broad-spectrum sensitizers, docetaxel, lovastatin and β-santalene in threatment with breast cancer cells by conducting radiotherapy *in vitro*. This experiment compared three kinds of sensitizers and found that docetaxel and lovastatin had little cytotoxicity when no radiotherapy was conducted, while lovastatin had the strongest sensitizing effects and the highest cell apoptotic rate. Therefore, lovastatin was chosen as the sensitizer of radiotherapy for breast cancer. Further effects of lovastatin at different concentrations

on radiotherapy were investigated and it was found that the cell apoptotic rate was the highest at 25 mg/l concentration. Since association between *TPX2* gene and breast tumor stem cells have been confirmed and lovastatin has significant sensitizing radiotherapy effects on breast tumor stem cells, our research group suspected that lovastatin affects *TPX2* gene expression to increase the death rate of cancer cells. However, further investigation is required.

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