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RESEARCH

TPX2 Overexpression in Medullary Thyroid Carcinoma Mediates TT Cell Proliferation

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Abstract TPX2 (targeting protein for xenopus kinesin-like protein 2), a microtubule-associated protein, plays an important role in the formation of the mitotic spindle. Abnormal expression of TPX2 in various types of malignant tumors has been reported, but less is known for medullary thyroid cancer (MTC). We investigated the expression of TPX2 in human MTC tissues and its potential use as a therapeutic target. Immunohistochemical analysis of TPX2 expression was performed for 32 cases of MTC and 8 cases of normal thyroid. TPX2 expression was found to be significantly higher in MTC compared to normal thyroid tissues ($P<0.05$), and to be associated with tumor size, lymph node metastasis, and advanced disease stage. The cellular effects of TPX2 knockdown, including cell proliferation, apoptosis, cell cycle diffusions, and mitotic gene expression were investigated using small interfering RNA (siRNA). TPX2-siRNA caused G1 and G2-phase cell cycle arrest, inhibited cell proliferation, and induced apoptosis. TPX2-siRNA also downregulated Aurora-A and cyclinB1 protein expression in MTC cells and enhanced the expression of p53 protein ($P<0.05$). These results suggest that TPX2 may be of potential use as a new marker for MTC prognosis and therapy.

Keywords Medullary thyroid cancer · TPX2 · siRNA · Proliferation · Apoptosis

Xiaolin Yang and Geling Liu contributed equally to this work.

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Introduction

Thyroid carcinoma is the most common endocrine malignancy and is following a trend of increasing incidence [1]. Medullary thyroid cancers (MTC) arise from the calcitonin-producing parafollicular C cells of the thyroid and account for about 5–8 % of all thyroid cancers [2]. Over the past decades, surgery has remained the only curative therapy for MTC. Because MTC has the characteristic of early metastasis and fails to respond to radioiodine and TSH suppression, the recurrence and mortality rates of MTC are higher than those of differentiated thyroid carcinomas derived from thyroid follicular epithelial cells [3]. Therefore, further elucidation of the mechanisms of pathogenesis of MTC and the identification of new molecular targets for effective treatment of MTC are urgently needed.

The chromosome instability represents a hallmark of solid cancers (including MTC), for which it has been suggested to underlie the progression to more aggressive phenotypes [4]. Failure in mitosis leading to chromosome instability is known to be involved in tumor development. Over the last decade, our knowledge regarding the molecular processes controlling mitosis has increased considerably. As a result, different mitotic proteins, the expression or functions of which have been found to be altered in human cancer tissues, are now thought to play a role in tumor chromosome instability. These include TPX2, a microtubule-associated protein, implicated in the regulation of multiple aspects of mitotic spindle and chromosome segregation. Abnormally expressed TPX2 has been reported in various malignancies with the expression correlating to tumor invasion and metastasis [5–7]. However, little is known about the role of TPX2 in MTC.

In the current study, we first analyzed the expression of TPX2 in MTC and the correlation between its expression and clinicopathological data from MTC patients. In order to provide a new experimental basis for MTC, we assessed the

biological consequences of siRNA–mediated knockdown of TPX2 expression in cultured MTC cells and identified downstream regulatory molecules that are modulated in response to TPX2 knockdown. These studies provide a molecular basis for the function of TPX2 in MTC, as well as a potentially new target for therapeutic intervention.

Materials and Methods

Tissue Microarray and Immunohistochemical Staining

A paraffin-embedded thyroid tissue microarray (TH806) containing 32 cases of MTC and 8 cases of normal thyroid was purchased from US Biomax, Inc. Detailed description of the tissues used for the microarray can be found on the company website (http://www.biomax.us/tissue-arraysthyroid/TH806). The microarray was processed for immunohistochemical staining. Experimental procedures were performed according to the SP kit manual (Zhong shan Jin qiao Biotech, Beijing, China). The microarray was conventionally dewaxed, then repaired in a pressure cooker at 130 °C for 10 min, with 3 % H_2O_2 added for 15 min to eliminate endogenous peroxidase. Next, 20 % normal goat serum was applied at 37 °C for 30 min, followed by rabbit anti-TPX2 antibody (1:200, Bioworld Technology, St. Louis Park, MN, USA) at 4 °C overnight. The next day, the microarray was washed with PBS twice and then incubated with a horseradish peroxidase-conjugated secondary antibody (Zhongshan Jinqiao Biotech, Beijing, China) at room temperature for 30 min. The microarray was washed three times for 5 min, the third antibody was added, and it was incubated at 37 °C for 30 min. After three additional washes with PBS, the microarray was incubated with diaminobenzidine solution as the chromogen (Zhongshan Jinqiao Biotech) to visualize the positive signal and then counterstained with Mayer's haematoxylin for 20 s. The microarray was dehydrated in upgraded ethanol solution, clarified with xylene and sealed with cover slips. The negative control microarray was processed as above, using PBS instead of the primary antibody. Cells with brown or yellow staining in the nucleus were considered to be TPX2 positive. Cells within five randomly selected fields of each core were counted under a microscope (10×20). The percentages of positive tumor cells were scored. A threshold of >10 % positively stained tumor cells was used to define TPX2 positivity [5].

Cell Lines and Culture

Human MTC-derived TT cells were obtained from ATCC. These cells are hypodiploid with a modal chromosome number of 40 (range=32 to 86) and a wild-type p53 gene (wt-p53) [8]. The cells were cultured in RPMI 1640 containing 10 % FBS (Gibco Co., Grand Island, NY, USA), 2 mM/L-glutamine at 37 °C in 5 % CO_2 humidified atmosphere.

SiRNA and Transfection

Human TPX2-siRNA (sense strand, 5′-GCACAGAUGAGC GAAUCAATT-3; antisense strand, 5′-UUGAUUCGCUCA UCUGUGCTT-3′) and negative control siRNA (nonsilencing siRNA) were purchased from GenePharma RNAi Company (Shanghai, China). Non-transfected cells were also tested as the blank control group. To introduce the siRNA, TT cells were seeded in 6-well culture plates at a density of 3×10^5 cells. After incubation overnight, cells were transiently transfected with TPX2-siRNA (2.7 μg/well) or the negative control siRNA using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

Real-Time PCR Analysis

Total RNA was isolated from cells of each group 24 h after transfection using Trizol reagent (Invitrogen) according to the manufacturers' instructions, and the purity and concentration of RNA were determined. One microgram of RNA was used to prepare cyclic DNA (cDNA), using a cDNA Synthesis kit (Invitrogen). The cDNA was subjected to real-time PCR using a SYBR Green PCR Supermix kit (Invitrogen) with a Rotor gene-3000 instrument (Corbett). Reactions were carried out in 20 μL total volume with 1 μL cDNA. Primer sequences used for TPX2 were 5′-AGAGGACGAACCGGTAGTGA-3′ and 5′-GTGCCTTCAG- AGCACCTCTT-3′. Primer sequences used for GAPDH were 5′-GAACGGTGAAGGTGACA-3′ and 5′-AAGGGACTTCCTGTAACAACGCA-3′. The PCR program was as follows: 95 °C for 10 s; and then 40 cycles of 94 °C for 15 s and 60 °C for 20 s. Relative expression was calculated using the $2^{-\Delta Ct}$ method with GAPDH serving as a reference for gene for normalization [9].

Cell Proliferation Assay

The effect of TPX2-siRNA on the viability of TT cells was determined by the MTT assay. Briefly, 1×10^4 TT cells were seeded in each well of 96-well plates. At 0, 24, 48, 72, and 96 h post siRNA transfection, cell viability was determined. Ten microliters of MTT solution (5 mg/ml; from Sigma, St Louis, MO, USA) was added, and the cells were incubated for 4 h. After that, the cell culture medium was removed and 150 μl of DMSO (Sigma) was added to dissolve the MTT. Absorbance at 490 nm was measured using a microplate reader (Bio-Rad, Richmond, CA). The cell culture medium without siRNA treatment served as the negative control.

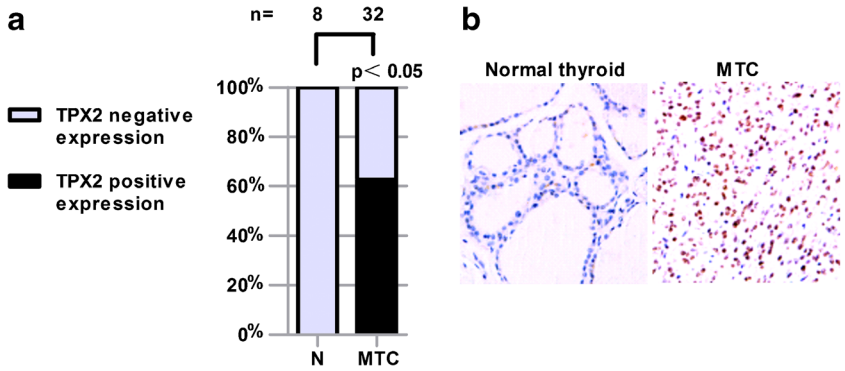

Fig. 1 TPX2 expression in MTC. **a** TPX2 is overexpressed in MTC as compared with normal thyroid tissue as assessed by microarray of 32 MTC and 8 normal thyroid tissue samples. *N* normal thyroid. **b** Representative staining from the microarray is shown as follows: normal thyroid tissue (*left*) and MTC (*right*). Brown staining represents TPX2 positivity, whereas blue staining represents a haematoxylin background stain

Flow Cytometric Analysis

For cell apoptosis analyses, cells of each group at 72 h post transfection were trypsinized and collected by centrifugation at 800 r/min for 5 min, and then incubated with 0.5 ml of the binding buffer and 1 μl Annexin V-FITC from an Annexin V-FITC apoptosis detection kit (Merck, Darmstadt, Germany) at room temperature for 15 min. After that, the cells were resuspended in a fresh 0.5 ml of the binding buffer containing 5 μl propidium iodide (PI). TT cells apoptosis was measured using a FACScalibur flow cytometer (BD Biosciences, San Jose, CA, USA).

For cell cycle analyses, cells were treated with siRNA oligonucleotides as described above for 72 h and harvested by trypsinization. The cells were washed twice with PBS, fixed with cold 70 % ethanol at 4 °C overnight, and stained with 400 μl PI (100 μg/ml, Sigma) at room temperature for 30 min. Cell cycle was analyzed by flow cytometry.

Western Blot Analysis

After transfection for 72 h, total protein of each group was extracted in RIPA buffer (Beyotime Biotech Institute, Shanghai, China) with protease inhibitors (PMSF). Concentrations of total cellular protein were measured using a BCA kit (Suolaibao Biotech, Beijing, China). Aliquots of 50 μg of cell protein extracts were electrophoresed on 10 % polyacrylamide gels and transferred onto PVDF membranes. The membranes were incubated overnight at 4 °C with primary antibodies against TPX2 (1:250; Bioworld), β-actin (1:300; Santa Cruz Biotechnologies, Santa Cruz, CA, USA), Aurora-A (1:300; Santa Cruz), cyclin B1 (1:300; Epitomics Biotechnology, Burlingame, CA, USA), and wt-p53 (1:300; Epitomics Biotechnology). The membranes were washed and probed with anti-mouse or anti-rabbit horseradish peroxidase–linked secondary antibodies (Beyotime Biotech Institute) and visualized with DAB detection reagent (Beyotime Biotech Institute). The protein bands were scanned and quantified using the Quantity One Software (Bio-Rad). Expression of β-actin protein served as a loading control.

Statistical Analysis

All statistical analyses were performed using SPSS16.0 software (SPSS, Chicago, IL, USA). The $\chi 2$ test was used to analyze the difference in TPX2 immunohistochemical staining between medullary thyroid tumor and normal thyroid tissues. Other statistical data are presented as mean ± standard deviation. Single-factor analysis of variance (One-way ANOVA) was used to compare multiple samples. *P*-values of less than 0.05 were considered statistically significant.

Table 1 Association of TPX2 protein expression with clinicopathological features from medullary thyroid cancer patients

Characteristics	No.	TPX2			*P*
		+	−	%	
Gender					
Male	9	5	4	55.6	0.696
Female	23	15	8	65.2	
Age (yrs)					
>40	24	16	8	66.7	0.443
≤40	8	4	4	50.0	
Tumor size(cm)					
≤4	15	4	11	26.7	<0.001
>4	17	16	1	94.1	
Lymphnode metastasis					
+	7	7	0	100	0.029
−	25	13	12	52.0	
Disease stage					
I, II	14	4	10	28.6	0.001
III, IV	18	16	2	88.9	
Thyroiditis					
+	1	1	0	100	1.000
−	31	19	12	61.3	

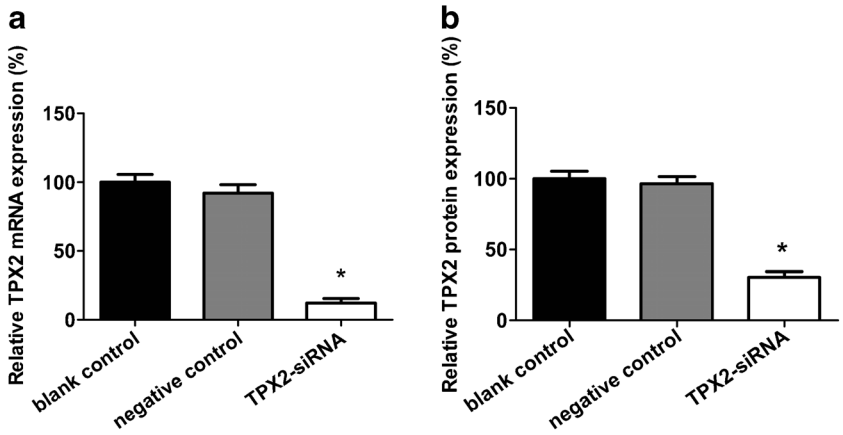

Fig. 2 Verification of gene silencing activity of TPX2-siRNA at the mRNA and protein level in TT cells. **a** Reduced TPX2 mRNA expression was demonstrated by real-time PCR following transfection with TPX2-siRNA. Expression was standardized to the expression of GAPDH mRNA. **b** Reduced TPX2 protein levels in TPX2-siRNA-transfected TT cells were verified by western blotting. β-actin is shown as a loading control. The TPX2 mRNA and protein levels were normalized to an average of 100 % in control cells. * $P<0.05$ versus blank control and negative control. Results represent the averages ± SD of three independent experiments

Results

Expression of TPX2 Protein in Thyroid Tissues as Detected by Immunohistochemistry

To determine whether TPX2 expression is differentially expressed in thyroid tissues during MTC, we assessed levels of TPX2 expression by immunohistochemisty using a microarray containing 32 MTC and 8 normal thyroid tissue samples. Of the 32 evaluable MTC tissue samples, 20 (62.5 %) tested positive for TPX2 expression (as defined by >10 % positive staining). In contrast, no expression in normal thyroid tissue was observed (Fig. 1). These results verify that, similar to other types of malignancy [5–7], TPX2 may be abnormally expressed in MTC.

Association of TPX2 Protein Expression with Clinicopathological Features of MTC

To determine whether TPX2 protein expression is associated with the clinicopathological stage of MTC, we assessed the correlation between the TPX2 expression level and an array of disease features among the 32 MTC patient samples in the microarray from Fig. 1. Our results showed a clear correlation between TPX2 expression and tumor size, lymph node metastasis and advanced disease stage ($P<0.05$, Table 1). In contrast, no association was found with gender, age, or existence of thyroiditis ($P>0.05$, Table 1). These results suggest that TPX2 overexpression is associated with MTC disease progression.

TPX2-siRNA Reduces TPX2 mRNA and Protein Levels in TT Cells

To determine the function of TPX2 in MTC, we used siRNA-mediated silencing to specifically reduce the expression of TPX2 in TT cells, a human MTC cell line. The ability of TPX2-siRNA to reduce TPX2 mRNA and protein expression was analyzed using real-time PCR and western blotting. Transfection of TPX2-siRNA clearly reduced the expression of TPX2 mRNA in TT cells as compared with non-transfected cells or cells transfected with a non-targeting siRNA (Fig. 2a, 87.9±6.46 % reduction, $P<0.05$). TPX2 protein levels were also reduced in TT cells transfected with TPX2-siRNA as assessed by densitometric analysis of western blots (Fig. 2b, 69.5±3.96 % reduction, $P<0.05$).

Effect of TPX2-siRNA on TT Cell Proliferation

We assessed the effects of TPX2-siRNA on TT cell proliferation by MTT assay. Our data showed that the proliferation rate of TT cells was significantly lower in the TPX2-siRNA group than in the blank control groups after 48 h transfection ($P<0.05$, Fig. 3). There was no statistical difference between

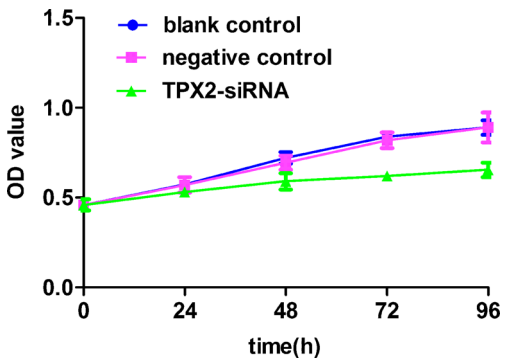

Fig. 3 Effects of TPX2-siRNA on the regulation of cell proliferation. Average OD values from three independent MTT assays are shown over a timecourse following transfection with blank control, negative control, or TPX2 siRNA. The reduced OD values for TPX2-siRNA suggest an inhibitory effect of TPX2-siRNA on the proliferation of TT cells

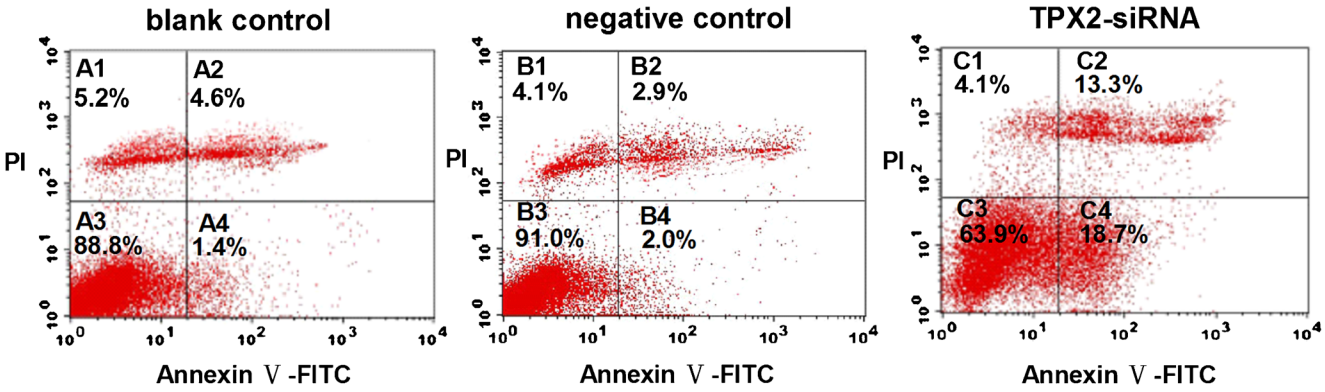

Fig. 4 Effects of TPX2-siRNA on MTC cell apoptosis. Representative flow cytometric analysis (Annexin V/propidium iodide staining) revealed induction of apoptosis in TT cells transfected with TPX2-siRNA compared to the blank control and negative control. Mean ± SD of apoptotic cells as determined by positive Annexin V/propidium iodide staining for three independent experiments were 1.91±0.90 % (blank control), 2.24± 0.95 % (negative control) and 14.96±3.44 % (TPX2-siRNA)

the blank control and negative control groups ($P>0.05$). These results suggest that downregulation of TPX2 significantly inhibits the proliferation of TT cells.

TPX2 Silencing Promotes Apoptosis in TT Cells

We performed Annexin V/PI staining and flow cytometry to determine whether TPX2-siRNA induces apoptosis. Significantly enhanced apoptosis was observed 72 h following transfection of TT cells with TPX2-siRNA as compared to the blank control and negative control ($P<0.05$, Fig. 4). There was no statistical difference between the blank control and negative control groups ($P>0.05$). These results indicate that TPX2-siRNA induces cell apoptosis in TT cells.

Downregulation of TPX2 Alters the Cell Cycle Distribution in TT Cells

Previously, TPX2-siRNA has been shown to induce arrest at the G2/M transition in types of malignant cells other than MTC cells [12, 17]. To examine the effects of TPX2-siRNA in MTC cells, we evaluated the cell cycle distribution by flow cytometry assay following transfection. Control and TPX2-siRNA cells were collected 72 h after transfection. Similar to previous studies [12, 17], the percentage of G2-phase cells in the TPX2-siRNA transfected group was higher, indicating G2 arrest. Additionally, the percentage of S-phase cells was lower than that in the blank control and negative control groups ($P<0.05$, Fig. 5, Table 2). This indicates that G1 arrest also occurs for MTC cells. These results suggest that TPX2 silencing arrests the cell cycle at both the G2 phase and G1 phase in TT cells.

TPX2-siRNA Modulates the Expression of Cell Cycle Regulatory Proteins in TT Cells

To determine the effects of TPX2 at the molecular level, we assessed whether TPX2-siRNA affects the regulation of mitosis-related genes. Our data showed that TPX2-siRNA significantly reduced the expression of Aurora-A and cyclin B1 proteins in TT cell compared to the blank control and negative control (60.4±2.02 % and 49.3 ±7.84 % reduction, respectively; $P<0.05$, Fig. 6), while wt-p53 protein was significantly induced in TT cell (81.5±23.3 % induction;

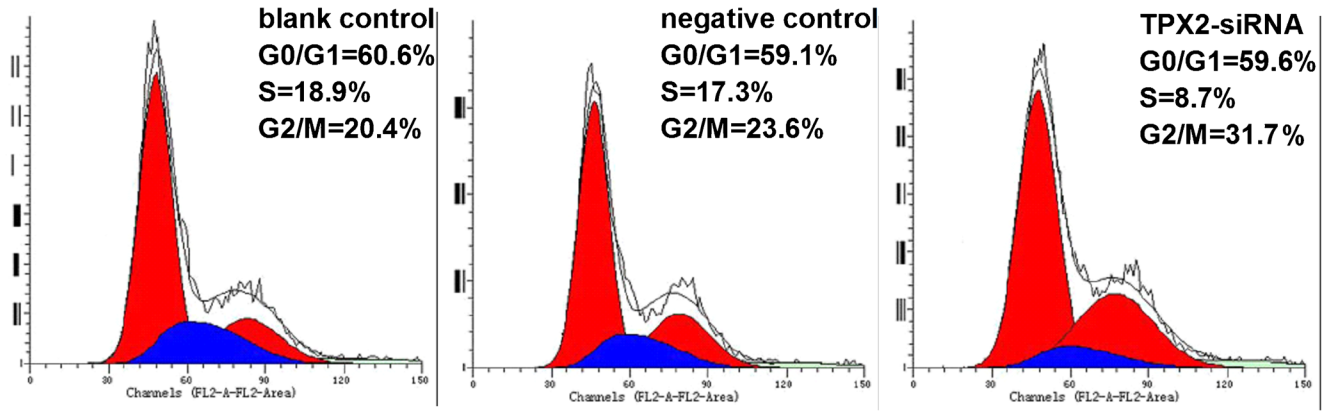

Fig. 5 Cell cycle distributions of TT cells following transfection with control or TPX2-targeting siRNAs. Flow cytometry analysis images (propidium iodide staining) of the cell cycle distributions are representative of three independent experiments

Table 2 Effect of TPX2-siRNA on cell cycle distributions of TT cells (%, mean ± standard deviation)

Group	G0/G1	S	G2/M
Blank control	61.15±1.98	19.76±0.86	19.09±1.86
Negative control	61.26±2.01	20.33±2.95	18.41±4.49
TPX2-siRNA	58.84±1.64	8.69±1.23*	32.46±2.82*

*$P<0.05$ compared with blank control and negative control

$P<0.05$). There were no statistical differences between the blank control and negative control groups ($P>0.05$). These results indicate that TPX2 may function to promote cell cycle progression through the modulation of each of these three cell cycle regulators.

Discussion

The proper establishment and maintenance of the bipolar mitotic spindle is essential for the equal segregation of genetic material into daughter cells. Any defect in this process can result in chromosome instability, which is often associated with tumorigenesis [10]. TPX2 is a microtubule-associated protein that plays a central role in mitotic spindle formation and, therefore, proper segregation of chromosomes during cell division. TPX2 expression appears in the cell cycle at the G1-S phase and disappears after the completion of cytokinesis. Overexpression of TPX2 results in abnormal spindle formation, chromosome instability, and malignant transformation [11]. Recently, TPX2 was identified as a candidate oncogene from an amplicon on 20q11.2 [12–14]. Additionally, high levels of TPX2 mRNA and protein were detected in various malignancies [12, 15, 16]. However, little work has been done to explore TPX2 protein levels in MTC cell lines and tumor samples. In the present study, immunohistochemical staining of a MTC tissue microarray shows that TPX2 is highly expressed in MTC tissues taken directly from patients, with 62.5 % of the tumors expressing TPX2 compared with 0 % of the normal thyroid tissue samples. These data verify that TPX2 is overexpressed in the majority of MTC cases.

TPX2 expression in MTC was further found to correlate with disease stage, tumor size and nodal status. Therefore, TPX2 may function to enhance the proliferation capability and induce cell survival in MTC cells. Consistent with this possibility, using siRNA targeting TPX2, we demonstrated that inhibition of TPX2 expression results in the inhibition of cell proliferation and induction of apoptosis in the MTC cell line TT, which is in agreement with other reports in which pancreatic and cervical cancer cells are suppressed through targeted inhibition of TPX2 mRNA [12, 15]. However, the G1 and G2-phase arrest that we observe in TT cells following transfection of TPX2-siRNA differs from the sole G2/M arrest observed other types of malignant cells [12, 17]. TT cells possess a wild-type p53 gene, and we have shown that TPX2-siRNA leads to an increase in p53 protein levels. P53 is a well known tumor suppressor able to drive cell cycle arrest at the G1/S checkpoint and induce apoptosis or senescence when DNA is damaged or cell integrity is threatened. Its potent role in cell proliferation is highlighted by the fact that it is mutated in 50 % of human tumors [18]. Therefore, it is likely that the differential effects of TPX2 gene silencing on cell cycle arrest are related to the difference in p53 gene status.

Our results also show that in addition to the increase in p53, the effects of TPX2-siRNA on cell cycle diffusion, proliferation, and apoptosis are associated with a reduction in Aurora-A and cyclinB1 protein levels. In support of these findings, Gaetan and coworkers showed that in *Xenopus* newly synthesized TPX2 is required for full Aurora-A activation and p53 synthesis and phosphorylation during oocyte maturation [19]. Aurora-A is a serine/threonine kinase that regulates centrosome maturation and mitotic spindle formation [20]. Its abundance is cell cycle regulated, with peaks in G2 and M phases, followed by regulated proteolysis at the end of mitosis. Overexpression of Aurora-A results in centrosome amplification and genetic instability preceding mammary tumor formation,

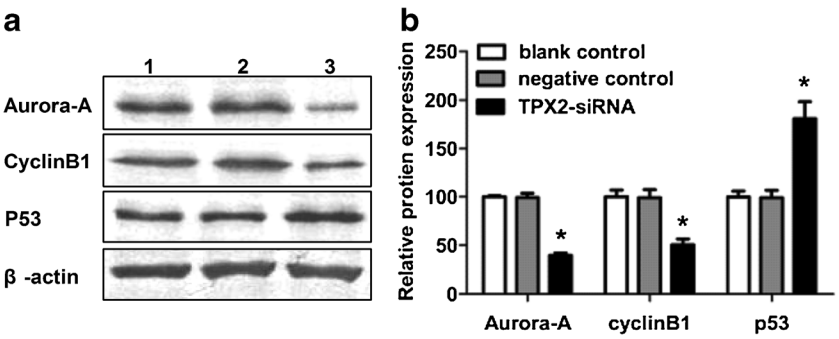

Fig. 6 Effects of TPX2-siRNA on the regulation of mitosis-related gene expression in MTC cells. **a** Representative image shows downregulation of TPX2, Aurora-A, and cyclin B1, but upregulation of p53 protein after transfection with TPX2-siRNA. *Lane 1*: blank control; *Lane 2*, negative control; *Lane 3*, TPX2-siRNA. **b** Relative protein levels were quantified by densitometry of western blots (three replicates). Values were normalized to an average of 100 % in control cells. (*$P<0.05$ vs. blank control and negative control)

which is associated with breast cancer [21], pancreatic cancer [22], and thyroid cancer [23]. Molecular and structural studies indicate that the full activation and correct mitotic localization of Aurora-A require its interaction with TPX2 [24, 25]. Furthermore, in many tumor types, TPX2 and Aurora-A frequently are co-ordinately overexpressed [14, 26]. Recently, Maria et al. reported a novel regulatory role of TPX2 on Aurora-A that protects Aurora-A from proteasome-dependent degradation both in interphase and in mitosis in human cells. In turn, Aurora-A might regulate the function of TPX2 by phosphorylation during spindle assembly [24]. Previously, human MTC tissues have been shown to express the Aurora kinases and their functional inhibition has been shown to prevent proliferation and in vitro tumorigenicity of MTC derived cells [8]. Given the downregulation of Aurora-A after silencing of TPX2 and the concomitant inhibition of TT cell proliferation, we propose that TPX2 and Aurora-A interplay with each other to jointly give rise to a functional unit that results in an abnormal spindle, chromosome instability, and malignant transformation of parafollicular C cells of the thyroid.

Chromosome abnomalities provoke a number of different mitotic checkpoints that prevent the progression of mitosis in response to gene dysfunction. Therefore, we propose that the roles of TPX2 and Aurora-A in cell transformation and tumorigenesis are conventionally attributed to the abrogation of mitotic checkpoints. The major checkpoints that control mitotic progression are the G1/S and G2/M checkpoints. The interactions between Aurora-A, p53 and cyclin B1 are characterized to be associated with both of these checkpoints. P53 is degraded via ubiquitin-mediated proteolysis. Katayama et al. reported that Aurora-A phosphorylates p53 at Ser315, facilitating ubiquitination mediated by the E3 ligase Mdm2 and destabilization of p53 [27]. Furthermore, it has been documented that p53 is phosphorylated by Aurora-A on Ser215, which abrogates p53 DNA binding and transactivation activity [28]. Thus, it is likely that the release of p53 from inhibition by Aurora-A explains its increased expression in TPX2-siRNA TT cells and the associated enhancement of the G1/S checkpoint arrest.

The G2/M transition is regulated by the complex of cyclin-dependent kinase 1 (CDK1) and cyclin B1 [29]. Upregulation of cyclin B1 leads to runaway cell proliferation and malignant transformation [30]. The CDK1-cyclin B complex phosphorylates Aurora-A, resulting in the activation of Aurora-A kinase. In turn, the activation of Aurora-A, leads to the nuclear translocation and full activation of CDK1-cyclin B by forming a positive feedback loop for triggering mitotic entry [31]. Consequently, premature activation and nuclear translocation of CDK1-cyclin B results in premature entry into mitosis [32]. Sasayama and coworkers [30] demonstrated that overexpressed Aurora-A can also stimulate polyadenylation of mRNA tails of cyclin B1 and CDK1 in human somatic cultured cells by phosphorylating and interacting with a human ortholog of cytoplasmic polyadenylation element binding protein, h-CPEB, to further extend its mRNA stability and cause upregulation of cyclin B1. Therefore, the inhibited upregulation of cyclin B1 by Aurora-A might explain the G2/M arrest observed in TPX2 knockdown cells.

In summary, we have demonstrated that TPX2 is overexpressed in MTC and that its expression correlates with disease state. Furthermore, silencing of TPX2 affects the expression of Aurora-A, cyclin B1, and p53 to cause growth arrest at the G2-M and G1/S phases, leading to an inhibition of proliferation and increased apoptosis. Given the coordinate roles of Aurora-A, cyclin B1, and p53 in cell cycle progression, the overexpression of TPX2 in MTC may explain the development and progression of malignant tumours. Additionally, TPX2 may serve as a valuable target in MTC due to its direct link to the Aurora A activation pathway.

Conflict of Interest All authors have no conflicts of interest.

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