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RESEARCH

Overexpression of Wild-Type p53-Induced Phosphatase 1 Confers Poor Prognosis of Patients with Nasopharyngeal Carcinoma

G. G. Sun · J. Zhang · X. B. Ma · Y. D. Wang · Y. J. Cheng · W. N. Hu

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Abstract This study aimed to analyze the expression, clinical significance of proto-oncogene in nasopharyngeal carcinoma and the biological effect in its cell line by siRNA targeting wild-type p53-induced phosphatase 1 (Wip1). Immunohistochemistry and western blot were respectively used to analyze Wip1 protein expression in 85 cases of nasopharyngeal cancer and normal tissues to study the relationship between Wip1 expression and clinical factors. Wip1 siRNA was transiently transfected into papillary nasopharyngeal carcinoma cell by liposome-mediated method and was detected by Quantitative real-time RT-PCR (qRT-PCR) and western blot. MTT assay, cell apoptosis, migration and invasion were also conducted as to the influence of the down-regulated expression of Wip1 that might be found on CNE2 cells biological effect. The level of Wip1 protein expression was found to be significantly higher in nasopharyngeal cancer tissue than normal tissues (P <0.05). There were significant differences between Wip1 expression and T stages, lymph node metastasis, clinical stages, tumor differentiation and radiotherapy response (P<0.05), regardless of age, gender (P>0.05). Meanwhile, Increased expression of Wip1 was significantly with poor overall survival time by Kaplan-Meier analysis (P<0.05). Wip1 expression deletion determines independent risk factors for prognosis of patients with nasopharyngeal carcinoma in addition to tumor T stage, clinical stage, histological grade and lymph node metastasis outside by Cox-2 in the regression analysis (P<0.05). qRT-PCR and Western blot showed that CNE2 cell transfected Wip1 siRNA had a lower relative expressive content than normal cell (P<0.05). MTT assay, cell apoptosis, cell cycles demonstrated that CNE2 cell transfected Wip1 siRNA had a lower survival fraction, higher cell apoptosis, more percentage of the G0/G1 phases, significant decrease in migration and invasion, and higher P53 and P16 protein expression compared with CNE2 cell untransfected Wip1 siRNA (P<0.05). Wip1 protein was increased in nasopharyngeal carcinoma, specifically in T stages, lymph node metastasis, clinical stages and tumor differentiation. Wip1 may involved in the biological processes of nasopharyngeal cancer cell proliferation, apoptosis, and migration and invasion by regulation P53 and P16 protein expression.

Keywords Wip1 · Nasopharyngeal cancer · Cell proliferation · Cell apoptosis · Prognosis

G. G. Sun · W. N. Hu (✉)
Department of Chemoradiotherapy, Tangshan people's hospital, NO.65, Shengli road, Lunan district, Tangshan 063000, Hebei province, China
e-mail: wanning_hu2008@sina.com

J. Zhang · X. B. Ma
Department of Pathology, Tangshan people's hospital, Tangshan 063000, China

Y. D. Wang
Department of Radiotherapy, The Military General Hospital of Beijing PLA, Beijing 100700, China

Y. J. Cheng
Department of Radiotherapy, The Fourth Hospital of Hebei Medical University, Shijiazhuang 050017, China

Introduction

In 2010, there were 52.8 million deaths globally. At the most aggregate level, communicable, maternal, neonatal, and nutritional causeswere 24.9 % of deaths worldwide in 2010, down from 15.9 million (34.1 %) of 46.5 million in 1990[1]. Nasopharyngeal carcinoma (NPC) has a remarkably distinctive ethnic and geographic distribution. In the year 2000, a total of 64,798 new cases were registered worldwide, and more than 80 % of those were reported from China, Southeast Asia, and some Asian countries [2]. The high incidence of nasopharyngeal carcinoma was closely related with the human herpes virus (EBV) and drinking water containing a large number of trace

elements nickel, cadmium and other ingredients. Animal experiments showed that the nickel can promote nitrite-induced nasopharyngeal carcinoma. Southerners like to eat pickled products, while the previous data indicated that salted fish was directly related with poorly differentiated nasopharyngeal cancer[3]. The quantity of EBV DNA detected in blood indicates the stage and prognosis of the disease. Radiotherapy with concomitant chemotherapy has increased survival, and improved techniques (such as intensity-modulated radiotherapy), early detection of recurrence, and application of appropriate surgical salvage procedures have contributed to improved therapeutic results. Screening of high-risk individuals in endemic regions together with developments in gene therapy and immunotherapy might further improve outcome [4]. Thus, further studies will clarify the pathogenesis of nasopharyngeal cancer and provide the molecular targets for effective treatment of nasopharyngeal cancer. Recent studies have found that wild-type p53-induced phosphatase 1 gene demonstrated biological and genetic features similar to proto-oncogenes in some tumors, suggesting that Wip1 plays an important role in the development and progression of nasopharyngeal cancer [5]. We studied Wip1 expression in nasopharyngeal cancer tissue using immunohistochemistry and western blot, and performed a preliminary observation on its effect on the biological behavior of nasopharyngeal cancer using Wip1 knockdown, in an attempt to look for new genetic targets for future clinicalal treatment.

Materials and Methods

Main Reagents

Rabbit anti-human Wip1 monoclonal antibody (Epitomics Inc.). immunohistochemistry kit (Zhongshan Goldenbridge Biotechnology Co., Ltd.). Bovine serum albumin (BSA) and β-actin primary antibody were purchased from Sigma Chemical Company (St. Louis, MO, USA). Plenti6/V5-DEST Vector, lentiviral packaging mix, Opti-MEM, Lipofectamine 2000, and the SuperScript III Reverse Transcriptase (RT) kit were obtained from Invitrogen Corporation (Carlsbad, CA, USA). TaqDNA polymerase was purchased from Fermentas, Inc. (Waltham, MA, USA). The DyLight Fluor conjugated to goat anti-mouse IgG was obtained from LI-COR bioscience, Inc. (Lincoln, Nebraska, USA). An immunohistochemistry kit and the Annexin V-FITC/PI apoptosis detection kit were purchased from 4A Biotech Co. Ltd. (Beijing, China). Fetal bovine serum (FBS), cell-culture media, and supplementary materials were obtained from Gibco Co. (Grand Island, NY, USA).

Clinical Data

The protocol for this study was approved by the Ethics Committee of the Tangshan Cancer Hospital, Tangshan, Hebei, China and the fourth Affiliated Hospital of Hebei Medical University College, Shijiazhuang, Hebei, China, and written informed consent was obtained from all study patients. The samples for each patient included nasopharyngeal cancer tissue and normal tissue. There were 55 males and 30 females, aged from 23 to 77 years old, with a median age of 58 years. Of the 85 cases of nasopharyngeal cancer: 31 of them with T1 + T2 stages and 54 with T3 + T4 stages. Meanwhile, 26 patients demonstrated no lymph node metastasis (N0), whereas 59 with identified lymph nodes involvement (N+). As for the clinical stages, 28 cases had I ~ II stages and 57 had a III ~ IV stages. The grades of differentiation were 20 with Grade I (well differentiated) and 65 with Grade II or III (moderately to poorly differentiated). All of the patients did not have any neoadjuvant therapies. The fresh specimens of tumor tissue or adjacent normal epithelium 5 cm apart from the tumor edge were immediately taken after the surgery, one was fixed in 4 % paraformaldehyde solution, then embedded in paraffin for immunohistochemistry, and the other one was stored in liquid nitrogen for western blot assay. The criteria was used to assess the radiotherapy short-term efficacy of 85 cases with nasopharyngeal carcinoma. Then the product of target lesions' maximum diameter and transverse diameter was calculated and divided into complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD) based on the percentage of lesions reduced. Statistical analysis was performed to evaluate the consistency of results between one-dimensional measurement and volume measurement using RECIST 1.0 [6].

Cell Culture and Gene Transfection

In accordance with Wip1 gene sequence in the NCBI database and siRNA design principles, Wip1-specific siRNAs were designed and synthesized by Guangzhou RiboBio Co., Ltd., whose sequences were as follows: sense strand 5′-CCAAUG AAGAUGAGUUAUAdTdT3′, antisense strand 3′-dTdTGGUUACUUCUACUCAAUAU-5′, and target sequence CCAATGAAGATGAGTTATA. In addition, a negative siRNA control that shared no homology to siRNA-Wip1 genome sequence was designed and synthesized. The sense and antisense RNAs were synthesized in vitro and annealed to form double-stranded RNA. CNE2 cells was maintained in RPMI-1640 medium (Gibco BRL, Grand Island, NY) supplemented with 10 % fetal bovine serum (Gibco BRL, Grand Island, NY) in a constant-temperature humidified incubator at 37 °C and in an environment containing 5 % CO_2 and 95 % air. Twenty-four hours before transfection, CNE2 cells in logarithmic growth phase were seeded in 6-well plates at a concentration of 5×10^4 cells/well. When the cells reached a confluence of 30 to 50 %, the cells were transfected with the mixture of siRNA and Lipofectimane 2000 at a concentration of 100 nmol/L. Next, the cells were cultured in serum-free

Opti-MEM culture medium. The liposome group was cultured with Opti-MEM medium containing 5 μL of liposomes/well. The culture medium was discarded 6 h later and the cells were switched to RPMI-1640 medium containing 10 % FBS.

Immunohistochemistry

Immunohistochemistry was performed as previously described [7–9]. A 4 μm section was prepared from paraffin-embedded block and dehydrated, then incubated in 3 % hydrogen peroxide for 10 min to block endogenous peroxidase, followd by using trypsin for repair of 20 min; 10 % goat serum was introduced at room temperature for closure of 20 min, and Wip1 antibody (1: 100) was left in the wet box at 4 °C refrigerator for overnight. Then the secondary and third antibodies were dropped into the wet box at room temperature for incubation of 20 min, respectively; DAB staining was again visualized by the hematoxylin stain, and then came to normal dehydration with the coverslip sealed. Results evaluation: two pathologists without knowing patients' information were responsible for assessing the results. Regarding cell counting under microscope, 5 fields were randomly selected, and 3 slides for each specimen were counted. Wip1 expression was determined based on the percentage of positive cells, combined with the staining intensity. The percentage of positive cells was divided into four levels: 0 point: ≤5 % of positive cells, 1 point: 5~25 %, 2 points: 25~50 %, and 3 points: >50 % of positive cells. The intensity of staining was classified as: 0 point: no staining, 1 point: weak staining (light yellow); 2 points: moderate staining (brown); and 3 points: strong staining (yellowish- brown). The final score of WIP1 expression was the product of the WIP1 expression rate and intensity, graded as 0 for negative, +~+++ for positive (+ for 1–3 points, ++ for 4–6 points, and +++ for 7–9 points). As for the negative control, the primary antibody was replaced with PBS.

Quantitative Real-Time RT-PCR

Total RNA was extracted from the nasopharyngeal cancer CNE2 cell using TRIzol reagent (Invitrogen; Life Technologies, Gaithersburg, Maryland) the manufacturer's protocol. Five hundred nanograms of total RNA was reverse transcribed using TakaRa Reverse Transcriptase Reagents (TakaRa). Quantitative real-time RT-PCR (qRT-PCR) was performed on an ABI Prizm 7,300 according to the standard protocol of SYBR Premix ExTaq perfect real time system (TakaRa). Primers for Wip1 and β-actin as a reference for normalisation were as follows: Wip1 sense 5′- GTTCGTAGCAATGCCT TCTCA, antisense 5′- CACTTTCTTGGGCTTTCATTTG; β-actin sense 5′- ATCGTCCACCGCAAATGCTTCTA, antisense 5′- AGCCATGCCAAT CTCATC TTGTT. Thermal cycling conditions were 95 °C for 1 min, 95 °C for 15 s and 40 cycles at 60°Cfor 1 min. The relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method in SDS 1.3 software.

Western Blot

All operations were completed on the ice. At 4 °C, 12,000 r/min centrifugation lasted for 20 min, and then the supernatant was taken for backup at −20 °C. After the detection of protein concentration with BCA Protein Assay Kit, each hole was given a sample amount of 50ug for SDS-PAGE electrophoresis. Regulator power for ice bath was transferred to nitrocellulose membrane, followed by closure for 2 h with 5 % skim milk. Subsequent to Anti-1 overnight incubation at 4 °C (Wip1 1:1000, p53 1:1000, p16 1:1000, β-actin 1:5000). The latter was from Sigma Chemical Company, St. Louis, MO, USA) in 5 % nonfat dry milk for 1 h at room temperature. After washing, the membrane was incubated with goat anti-rabbit fluorescent secondary antibody (IRDye800, 1:20,000 dilution; the DyLight Fluor conjugated to goat anti-rabbit IgG was obtained from LI-COR Bioscience, Inc., Lincoln, Nebraska, USA) in the dark for 1 h at room temperature. The blots were then scanned and analyzed using the Odyssey Infrared Imaging System (LI-COR Bioscience). Western blot data were quantified by normalizing the signal intensity of each sample to that of β-actin.

MTT Assay

Cell viability was determined by using the tetrazolium salt 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, cells were plated into 96-well culture plates at an optimal density of 5×10^3 cells/ml in 200 μl of culture medium per well. After 24–96 h of culture, 20 μl of 5 mg/ml MTT was added to each well and incubated at 37 °C for 4 h. The medium was then gently aspirated and 150 μl of dimethyl sulfoxide (DMSO) was added to each well to solubilize the formazan crystals. The optical density of each sample was immediately measured using a microplate reader (BioRad) at 570 nm.

Flow Cytometry Assay

An Annexin V-FITC-flow cytometry assay was used as previously described [10, 11] to detect the apoptosis rate. Cell apoptosis: An Annexin V-FITC-flow cytometry assay was used to detect the apoptosis rate in the cells after Wip1 siRNA transfection. Cells were seeded into 60-mm dishes for 48 h and grown to approximately 70–75 % confluence. After quick detachment from the plate, cells were collected, washed with ice-cold PBS, and resuspended at a cell density of 1×10^6/mL in a binding buffer from the Annexin V-FITC apoptosis detection kit (4A Biotech Co. Ltd, Beijing, China) and then stained with 5 μl of Annexin V-FITC and 10 μl of propidium

Table 1 Expressions of Wip1 in nasopharyngeal cancer tissue and in normal nasopharyngeal tissue

Groups	Case	Expression of Wip1 protein					
		–	+	++	+++	χ^2	P
Normal tissue	85	68	7	5	5	42.525	0.000
Cancer tissue	85	26	18	23	18		

iodide (PI, 20 μg/ml). The cells were then incubated in the dark at 25 °C for 15 min before 10,000 cells were analyzed by a FACScan flow cytometer (Becton-Dickinson, Mountain View, CA, USA) and Cellquest software (Becton-Dickinson) for apoptosis rate determination. The data were evaluated using the ModFit software program (Verity Software House, Topsham, ME, USA).

Invasion and Migration Assays

The Costar Transwell 8 μm inserts were coated with 50 μg reduced serum Matrigel (BD Biosciences Franklin Lakes, NJ) for invasion assay according to the manufacturer's instruction. Invasion Chambers (BD China, Shanghai, China) at 10×10^5 cells per chamber. The membrane in the chamber was coated with Matrigel (BD China). Medium supplemented with 10 % FBS was used in the lower chamber. Migration assays were performed in the same manner excluding the Matrigel. After 16 h, non-invading cells, and media were removed with a cotton swab. Cells on the lower surface of the membrane were fixed with polyoxymethylene (Sigma) and stained with 0.1 % crystal violet (Sigma) for 0.5 h. Stained cells were counted under a microscope in 4 randomly selected fields, and the average was used to indicate cell migration and invasion.

Statistical Analyses

All statistical analyses were performed using SPSS16.0 software (IBM, Armonk, NY, USA). For the clinicalopathologic features, P values were calculated using the χ^2 test. Survival distributions were estimated with the Kaplan-Meier method and compared with the log-rank test. Multiple Cox regression analysis was platfomed to analyse relationship beetween multiple variables and overall survival. Wip1 mRNA expression was shown in mean ± SD. Student *t*-test was used to analyze the difference between groups. A 5 % or lower *P-value* was considered to be statistically significant.

Results

Wip1 Protein Expression in Nasopharyngeal Cancer and Normal Tissue

In normal nasopharyngeal tissues, Wip1 staining was negative or weak. In nasopharyngeal cancer tissues, Wip1 staining ranged from light yellow to brown. Statistically, Wip1 was expressed in 69.4 % (59/85) of nasopharyngeal cancer tissues, which was higher than the 20.0 % (17/85) in normal tissues. The difference was statistically significant (P <0.05, Table 1, Fig. 1). While Western blot showed that the relative expression of Wip1 protein presented volume between cancer lesion and adjacent normal tissue were 0.859±0.089 and 0.437± 0.051, showing the difference with statistical significance (P <0.05, Fig. 2). The expression of Wip1 was correlated with T stages, clinical stages, lymph node metastasis, tumor differentiation and radiotherapy response (P <0.05, Table 2), regardless of age, gender (P>0.05, Table 2). There was obvious relationship beetween clinical stage and therapeutic response (P <0.05).

Wip1 Expression and Prognosis

Survival analysis was performed in all the patients and follow-up data were collected. All patient follow-ups ended in 2012 after a revisit time of 60 months. Among all cases, 35 were still alive at this time and 50 were dead. Patients were divided into two groups according to Wip1 expression level. There were 59 individuals with positive levels of Wip1 expression, among whom 20 were still alive and 39 were dead. The survival rate was 33.9 %. There were 26 individuals with negative levels of Wip1 expression, among whom 15 were

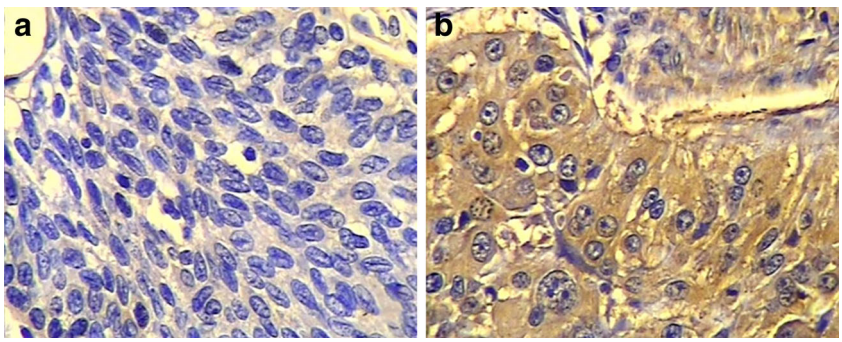

Fig. 1 Expressions of Wip1 protein in nasopharyngeal carcinoma and normal tissue. A normal tissue (SP×400); B nasopharyngeal carcinoma (SP×400)

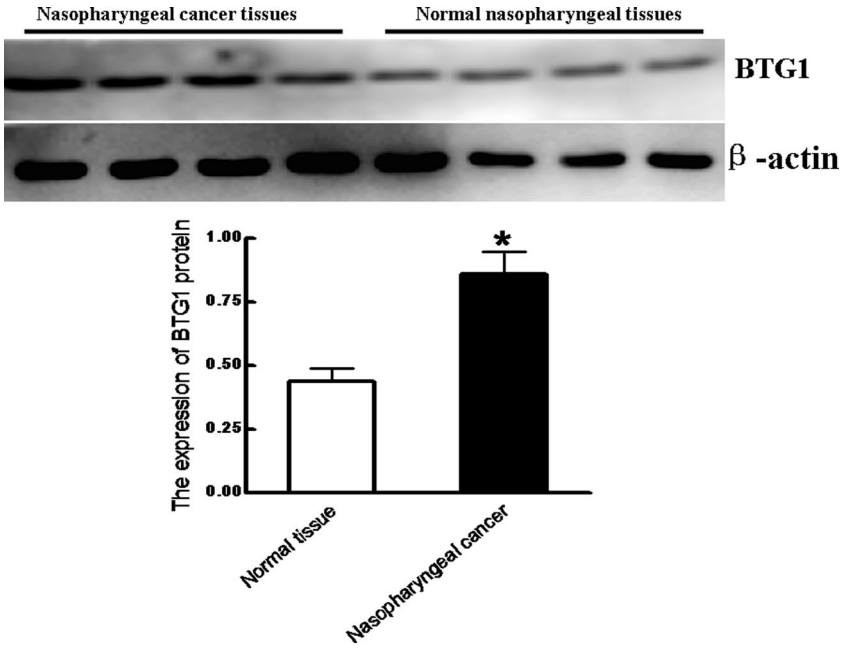

Fig. 2 Expressions of Wip1 protein in nasopharyngeal carcinoma and normal tissue. *$p<0.05$ compared to the normal tissue

still alive and 11 were dead. The survival rate was 57.7 %. Patients with positive levels of Wip1 expression had significantly lower 5-year survival rates than those with low levels of Wip1 expression group (P <0.05, Fig. 3). Wip1 expression deletion determines independent risk factors for prognosis of patients with nasopharyngeal carcinoma in addition to tumor T stage, clinical stage, histological grade and lymph node metastasis outside by Cox-2 in the regression analysis (P <0.05, Table 3).

Expression and Identification of Wip1 siRNA in CNE2 Cell

Wip1 siRNA was transiently transfected into papillary nasopharyngeal carcinoma cell by liposome-mediated method and was detected by qRT-PCR and western blot. qRT-PCR data showed that the Wip1 mRNA expression level was 0.905±0.064 in empty vector-transfected cells. In contrast, the amount of Wip1 mRNA in the Wip1 siRNA -transfected cell lines was 0.323±0.028. The difference was statistically significant (P <0.05, Fig. 4a). Furthermore, western blot analysis showed that the control cells had approximately equal amounts of immunoreactive protein (0.875±0.082). In contrast, the amount of Wip1 protein in the Wip1 siRNA-transfected cell lines was 0.351±0.059. The difference was statistically significant (P <0.05, Fig. 4b).

Effects of Wip1 siRNA on Nasopharyngeal Cancer CNE2 Cell

Next, we assessed the effect of Wip1 siRNA on the regulation of nasopharyngeal cancer cell viability. MTT assay showed that relative proliferative capacity of the Wip1 siRNA cell relative grew slower at 24, 48, 72 h and 96 h compared with the parental cell. The difference was statistically significant

Table 2 Relation between Wip1 expression and clinical characteristics in nasopharyngeal cancer tissue

Groups	Case	Expression of Wip1 Protein			
		–	+~+++	χ^2	P
Sex					
Male	54	16	38	0.064	0.800
Female	31	10	21		
Age					
≤60	68	20	48	0.222	0.638
>60	17	6	11		
Tumor invasion					
T1+ T2	31	14	17	4.881	0.027
T3+ T4	54	12	42		
Lymph node metastasis					
N0	26	13	13	6.648	0.010
N+	59	13	46		
Clinical stages					
I ~ II	28	13	15	4.935	0.026
III ~ IV	57	13	44		
Histological grade					
I	20	11	9	7.341	0.007
II ~ III	65	15	50		
Radiotherapy Response					
CR + PR	51	20	31	4.470	0.034
SD + PD	34	6	28		

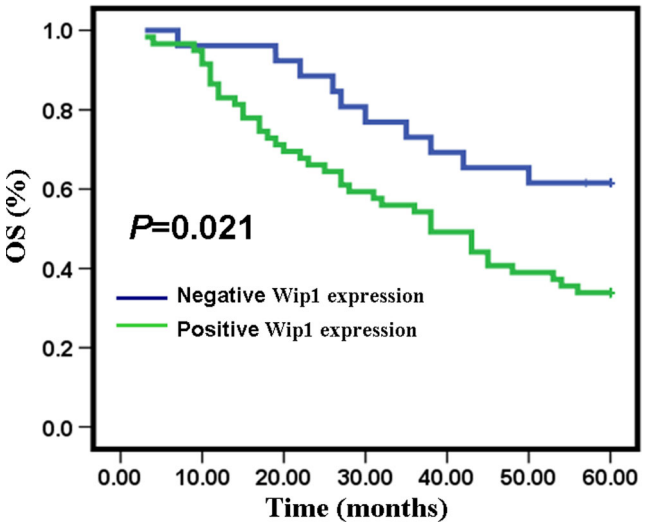

Fig. 3 Relation between Wip1 expression and overall survival in nasopharyngeal carcinoma by Kaplan-Meier analysis

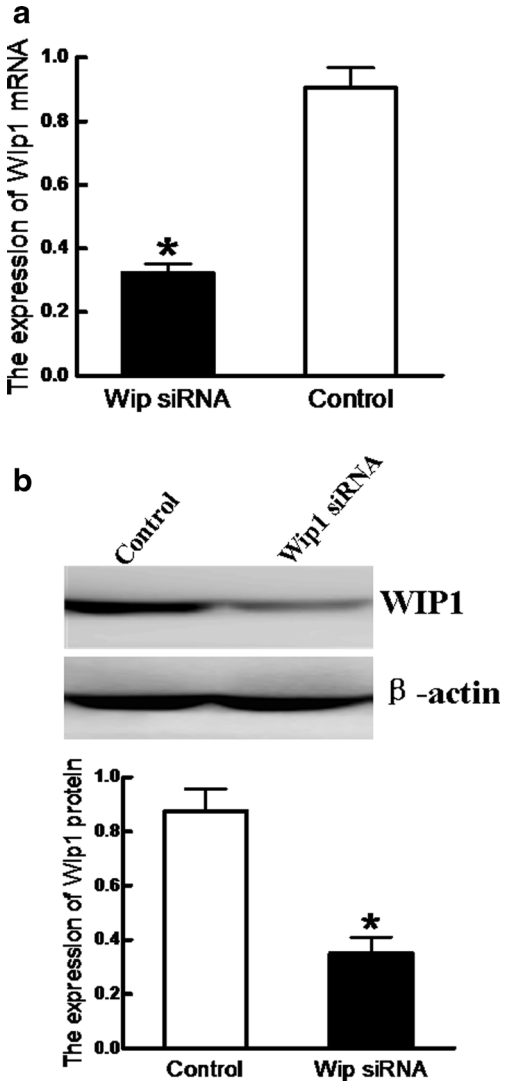

Fig. 4 Expression and identification of the Wip1 gene. **a** qRT-PCT, **b** Western Blot. *$p<0.05$ compared to the control cell

(Fig. 5, P <0.05). Meanwhile, there was a relative large increase in the early and late apoptosis rate was 15.6±1.5 % in Wip1 siRNA cell compared to control cells (5.5±0.6 %). The difference was statistically significant (Fig. 6, P <0.05). Furthermore, the CNE2 cells transfected with Wip1 siRNA or empty vector were transferred to transwell chambers or Matrigel-coated transwell chambers to evaluate the effect of Wip1 siRNA on cell invasion potential. Introduction of Wip1 siRNA clearly led to a significant decrease in CNE2 cell migration and invasion (186.0±17.0 and 151.0±17.0, respectively) compared with the cells transfected with empty vector (268.0±22.0 and 223.0±21.0, respectively). The difference was statistically significant (P <0.05, Fig. 7). To further identify the mechanisms by which Wip1 siRNA inhibited nasopharyngeal cancer cell proliferation, promoted cell apoptosis and decreased migration and invasion, we analyzed the expression level of P53 and P16 protein due to their critical roles in cell proliferation, cell apoptosis, and migration and invasion. Western blot analysis revealed that Wip1 siRNA significantly upregulated P53 and P16 protein expression in CNE2 cell transfected with Wip1 siRNA (0.785±0.083, 0.468±0.048, respectively) compared to control cell (0.336±0.031, 0.217±0.021, respectively). The difference was respectively statistically significant (P <0.05, Fig. 8).

Table 3 Multivariate analyses of affecting prognosis of nasopharyngeal cancer

Group	Wip1 (+vs-)	Sex M vs F	Age ≤60 vs >60	Tumor invasion T1+T2 vs T3+T4	Clinic stages I~II vs III~IV	Histological grade Ivs II~III	Lymph node metastasis N0 vs N+
Beta	1.733	0.275	−0.018	2.195	1.519	1.112	1.487
SE	0.656	0.435	0.017	0.953	0.706	0.503	0.625
Wald	7.244	0.483	1.233	5.333	4.685	4.636	5.932
Exp(B)	5.735	1.356	0.985	8.855	4.525	2.893	1.335
95%CI	1.635–17.018	0.598–2.933	0.946–1.014	1.385–26.633	1.158–17.855	1.103–7.635	0.463–3.956
P alue	0.006	0.496	0.276	0.022	0.031	0.029	0.017

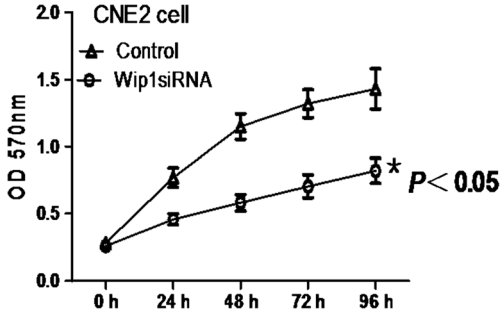

Fig. 5 The effects of Wip1 siRNA on the cell proliferation. *$p<0.05$ compared to the control cell

Discussion

Wip1 is a serine/threonine protein phosphatase whose encoding sequence is located in human chromosome 17q22/q24 region. Human Wip1 protein has a molecular weight of about 61kD and consists of 605 amino acids. In recent years, studies have shown that Wip1 is highly expressed in breast cancer [12], neuroblastoma [13], and medulloblastoma [14] and exercises a negative feedback regulation on p53 through the p38MAPK/p53 signaling pathway, leading to decreased expression of p53 and p53 mutation, suggesting a close association between Wip1 and prognosis [15].

This study quantified for the first time Wip1 expression in nasopharyngeal tissue and normal nasopharyngeal tissue using immunohistochemistry and western blot. Our results showed that Wip1 protein levels were significantly higher in nasopharyngeal cancer tissue than in normal tissue. Our results are consistent with a previous research finding that Wip1 expression is significantly higher in glioblastoma multiforme [16], ependymoma [17], thyroid papillary carcinoma [18] than in normal tissue. The univariate analysis in the present study also showed that Wip1 protein levels were correlated with T stages, lymph node metastasis, clinical stage and pathological differentiation. Recent studies indicate that when DNA damage occurs, ATM causes Chk2 phosphorylation to prevent tumorigenesis. Wip1 combines with Chk2 to dephosphorylate and inactivate Chk2, leading to tumorigenesis. In vivo and in vitro experiments confirmed that Wip1 could also contribute to tumorigenesis by inhibiting Chk1 dephosphorylation and inactivation [19]. Further studies demonstrated that Wip1 high expression disrupted the homeostasis maintained by the

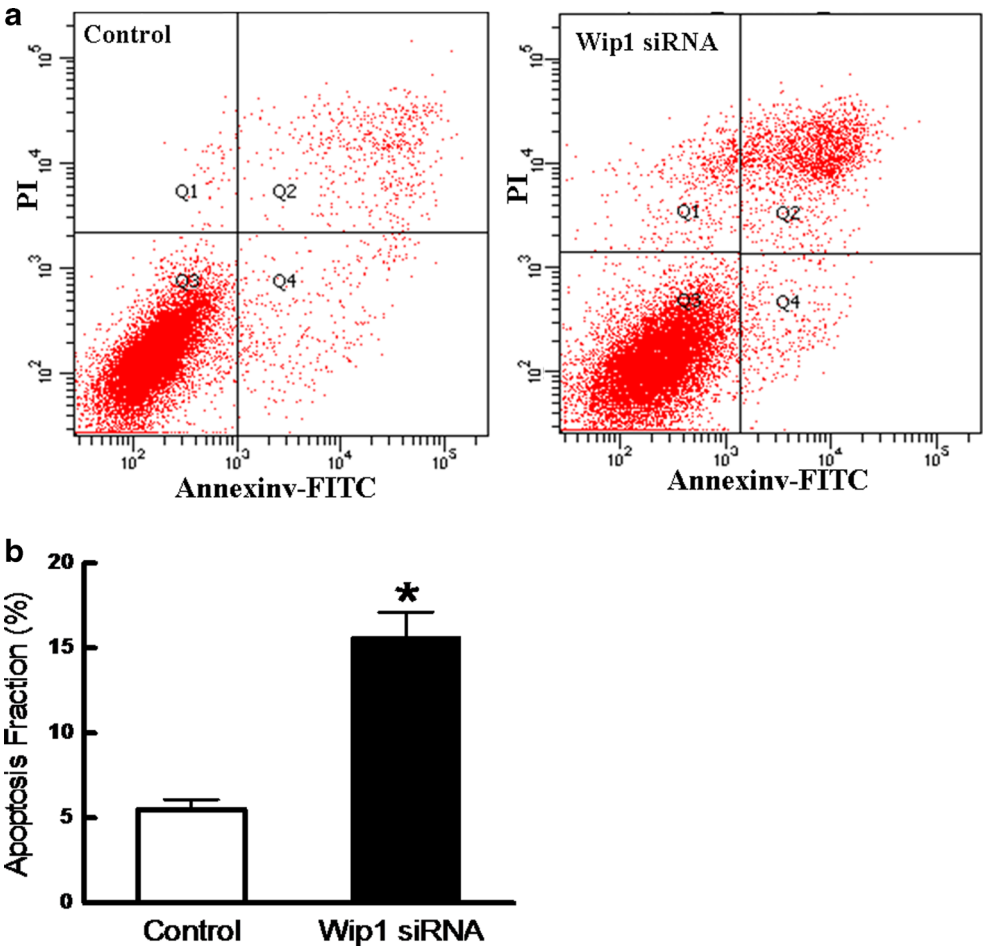

Fig. 6 The effects of Wip1 siRNA on the cell apoptosis. *$p<0.05$ compared to the control cell

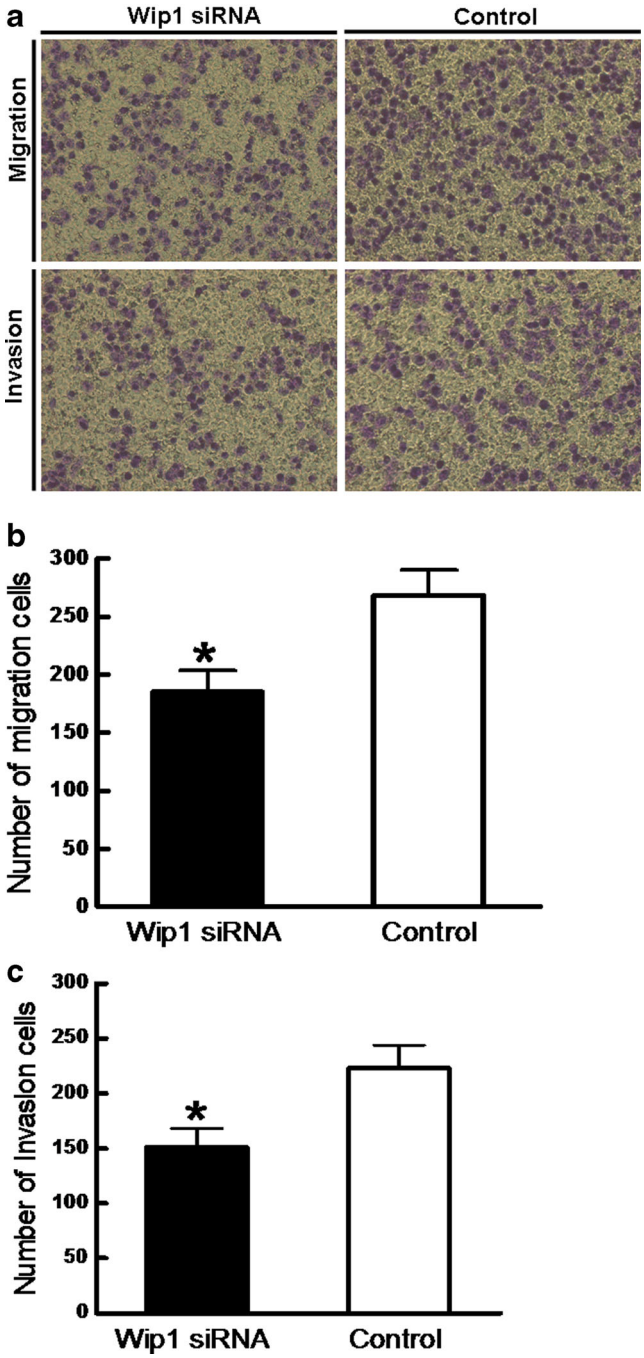

Fig. 7 The effects of Wip1 siRNA on the cell migration and invasion. *$p<0.05$ compared to the control cell

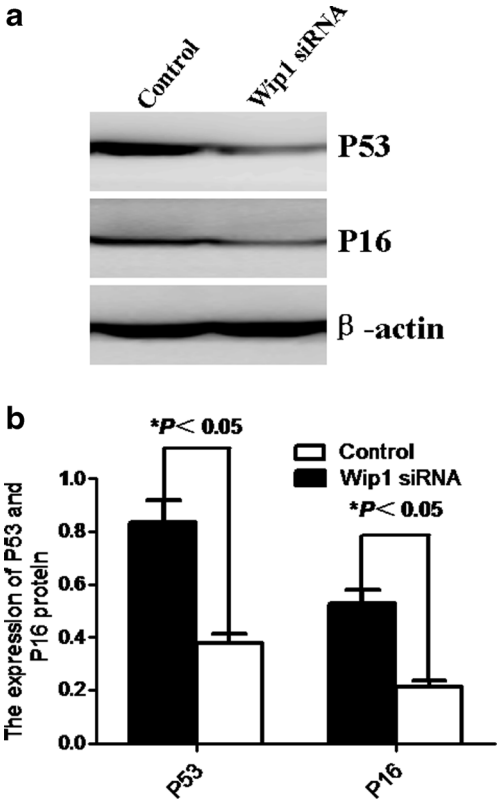

Fig. 8 The effects of Wip1 siRNA on P53 and P16 protein expression. *$p<0.05$ compared to the control cell

p38MAPK-p53-Wip1 pathway, caused downstream Wnt-p53 inactivation through p38 MAPK dephosphorylation, and promoted the development of malignant breast cancer in humans by reducing p16 protein levels [13].

The determination of the prognosis of patients with nasopharyngeal cancer is an extremely important part of clinicalal work. Current studies have shown tumor staging as the preferred prognostic indicator. However, for cancer patients in the same stage and with a similar state, their prognosis still varies considerably [20]. Therefore, it is of particular significance to find an ideal molecular marker in the clinical practice. Moreover, traditional treatment of nasopharyngeal cancer and prognosis rely mainly on TNM classification [21]. This system is subjective and not informative for early nasopharyngeal cancer, and offers limited information about disease severity, prognosis, and response to treatment, while early detection of nasopharyngeal cancer is the most effective way to improve survival [22]. This study showed in survival analysis that Wip1 expression-positive patients had a significantly lower 5-year overall survival rate than patients without Wip1 expression. Therefore, the combination of TNM classification system and Wip1 expression scores may provide some valuable information for clinicalians in the choice of treatment options, prognosis judgment and prediction of disease severity.

Further in vitro experiments demonstrated for the first time that nasopharyngeal cancer cells with high Wip1 expression had significantly weakened proliferation and migration and invasion, significantly increased apoptosis, and markedly increased P53 and P16 protein levels. A Chinese team reported the use of siRNA silencing of high Wip1 expression in U251 glioblastoma cell line to inhibit tumor cell proliferation [23]. Baxter et al. [24] knocked down highly expressed Wip1 in medulloblastoma D283 cells with RNA interference technique, which increased p53 expression and induced tumor cell

apoptosis. A Chinese study also found that sRNAi knockdown of highly expressed Wip1 in medulloblastoma D283 cells increased P53 expression and induced tumor cell apoptosis [15]. A study suggests that this phenomenon may be related to the extensive roles of Wip1 in NF-κB, Notch, and Wnt signaling pathways, which play an important role in proliferation, apoptosis, cell cycle, angiogenesis and epithelial-mesenchymal transition [25].

The results of this study showed that Wip1 protein and mRNA were both increased in nasopharyngeal cancer and that the increase was correlated with lymph node metastasis, clinical stage and poor prognosis of patients with nasopharyngeal carcinoma. Wip1 is involved in a number of biological processes including cell proliferation, apoptosis, and invasion in nasopharyngeal cancer cell. However, carcinogenesis is a complex and integrated process [25, 26], and further research is warranted to gain more insight into the signaling mechanism whereby Wip1 is involved in the process.

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