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Low expression of Cyfip1 may be a potential biomarker in nasopharyngeal carcinoma

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Cytoplasmic FMR1 interacting protein 1 (Cyfip1) is a new candidate tumor suppressor gene, which may play an important role in the occurrence and development of cancers. However, the role of Cyfip1 in nasopharyngeal carcinoma (NPC) remains poorly known. The aim of this study was to investigate the Cyfip1 mRNA expression in NPC and its association with clinicopathological features. The study population comprised 114 Chinese individuals, including 69 NPC tissues and 45 non-cancerous nasopharyngeal tissues. We used real-time fluorescent relatively quantitative PCR to evaluate the Cyfip1 mRNA expression in NPC tissues and non-cancerous nasopharyngeal tissues. The expression level of Cyfip1 mRNA was significantly lower in patients with NPC than in the control samples (p=0.001). Furthermore, low expression level of Cyfip1 mRNA was significantly associated with invasive range (T3-T4 vs T1-T2, p=0.001), lymph node metastasis (N1-N3 vs N0, p=0.010), distant metastases (M1 vs M0, p=0.040) and clinical stage (III-IV vs I-II, p<0.001). Our results suggest the association between Cyfip1 mRNA expression and NPC. Detecting the expression of Cyfip1 may provide clinically useful information for diagnosis, progression and treatment methods in NPC.

Key words: nasopharyngeal carcinoma, Cyfip1, diagnosis, prognosis

Nasopharyngeal carcinoma (NPC) is a malignant tumor that originates in the upper epithelial lining of the nasopharynx. Previous epidemiologic studies suggest that NPC has a distinctive geographical distribution and is highly epidemic in South-Eastern China, especially in Hong Kong, Guangdong and Guangxi areas [1–3]. According to GLOBOCAN estimates, there were approximately 87,000 new cases of NPC and 51,000 deaths occurred around the world in 2012 [4]. Among them, the NPC estimated 33,198 new cases and 20,404 deaths in China [5]. Furthermore, between 1998–2002 the incidence rates were 19.76 in males and 7.33 in females in Cangwu county, Guangxi Province per 100,000 and 30.94 in males as well as 13.00 in females in Sihui City, Guangdong Province per 100,000 [6]. Although NPC is highly sensitive to chemoradiotherapy, most NPC patients tend to display an advanced stage when first diagnosed [7]. Therefore, identifying a potential biomarker for early diagnosis and prognosis of NPC is indispensable.

The Cyfip1 gene, encoding a cytoplasmic protein named cytoplasmic FMR1-interacting protein 1(CYFIP1) as a component of WAVE, is located on chromosome 15q. In recent years, Silva and colleagues found that Cyfip1 was involved in normal epithelial morphogenesis after testing 29 new genes [8]. Furthermore, the loss of expression of Cyfip1 has been found in a number of human cancers, including breast cancer, colon cancer, lung cancer, bladder cancer, and acute lymphoblastic leukemia [8, 9]. They concluded that Cyfip1 was a potential tumor suppressor that regulates tumor invasion [8]. However, the role of Cyfip1 in NPC has not been explored. Therefore, the aim of this study was to investigate the mRNA expression of Cyfip1 in NPC and its correlation with clinicopathological features.

Materials and methods

Patients and samples. Sixty nine NPC tissues and 45 non-cancerous nasopharyngeal tissues were collected from the First Affiliated Hospital of Guangxi Medical University (Nanning City, Guangxi Zhuang Autonomous Region, and China) during the period between February 1st, 2012 and

September 20th, 2015. NPC cases included 45 males and 24 females with the median age of 49 years (range 28–83). A control group consisted of 27 males and 18 females with the median age of 44 years (range 26–77). All fresh tissues from patients who have not previously received any treatment were immediately conserved in liquid nitrogen and confirmed by pathologic diagnosis. Cancer stage was defined according to the 2008 NPC staging criterion [10]. Pathologists assessed pathologic features, including histological type, clinical stage, invading range and metastasis (lymph nodes metastasis and distant metastasis). This study obtained the patient's consent and was approved by the ethics committee of the First Affiliated Hospital of Guangxi Medical University.

Real-time fluorescence relatively quantitative PCR. The total RNA was extracted from NPC tissues and non-cancerous nasopharyngeal tissues using TRIzol (TiangenInc, Beijing, China), followed by reverse transcription into cDNA with oligo-dT primers (Tiangen Bio Inc, China) according to the manufacturer' instructions. Reverse transcription was performed on the basis of instructions in the TIANscript RT kit. The 20 μl system included 2 μg RNA, 2 μl Olig(dT), 2 μl dNTP Mix, and RNase-Free ddH_2O to a volume of 14.5 μl, 4 μl 5xFirst-Strand Buffer, 0.5 μl RNasin, 1 μl (200U) TIANScriptM-MLV. Reaction conditions were as follows: 70 °C for 5 minutes, 42 °C for 50 minutes, 95 °C for 5 minutes.

qRT-PCR taking cDNA as a template was performed in the machine of ABI7500 (according to ABI7500 instructions). The sequences of the forward and reverse primers for CYFIP1 were 5'-AGGCCAACCACAACGTGTC-3'and 5'-CTCAGACTTTGGGGAGTGG-3', respectively. 18S gene was used as an internal reference using the forward primer 5'-CAGCCACCCGAGATTGAGCA-3', reverse primer 5'-TAGTAGCGACGGGCGGTGTG-3'. The 20 μl reaction mix consisted of 10 μl SYBR Green real-time PCR Master Mix, 0.6 μl forward primer, 0.6 μl reverse primer, 2 μl ROX Reference Dye, 2 μl cDNA and 4.8 μl double-distilled water. The amplification of PCR was performed under the following conditions: denaturation at 95 °C for 10 min, 40 cycles with denaturation at 95 °C for 15 s, annealing at 60 °C for 20 s and extension at 72 °C for 15 s. Cyfip1 was normalized to 18S using the adjusted cycle threshold (ΔCT) method: the relative expression of Cyfip1=$2^{-\Delta CT}$, ΔCT=CT value of Cyfip1 – CT value of 18S.

Statistical analysis. All statistical analyses were performed using the software SPSS17.0. Data are showed as mean±SD and analyzed by employing Student's t-test as well as One-way ANOVA. Difference with a $p<0.05$ was considered statistically significant.

Results

Cyfip1 mRNA expression in cancerous and non-cancerous nasopharyngeal tissues. Significant difference was found in Cyfip1 mRNA expression level between NPC tissues and non-cancerous nasopharyngeal tissues ($p<0.01$, Figure 1). Furthermore, the expression level of Cyfip1 mRNA in NPC tissues was just 0.23% of non-cancerous nasopharyngeal tissues (Table 1).

Association between Cyfip1 mRNA expression and pathological characteristics of NPC patients. Cyfip1 mRNA expression and pathological characteristics of NPC are shown in Table 2. We did not observe any significant association between Cyfip1 mRNA expression and patient gender, age or histological type. However, we found that low expression level of Cyfip1 mRNA was significantly associated with invasive range (T3–T4 vs T1–T2, p=0.001), lymph node metastasis (N1–N3 vs N0, p=0.010), distant metastases (M1 vs M0, p=0.040) and clinical stage (III–IV vs I–II, $p<0.001$).

Discussion

NPC is one of the most common malignancies in Southern China, South-Eastern Asia, Micronesia/Polynesia and Northern Africa [4, 5]. In order to control and prevent relatively high mobility and mortality of NPC in these areas, more and more researchers pay attention to the genetic mechanisms for both activation of oncogenes and inactivation of tumor suppressor genes. Among the two mechanisms, tumor suppressor genes play an important role in tumor growth and progression. However, Cyfip1 as a new tumor suppressor gene has been found to have decreased expression in several human cancers. Hence, we believe it is necessary to investigate whether similar phenomenon exists in NPC. To the best of our knowledge, this is the first study to investigate the expression of Cyfip1 in NPC.

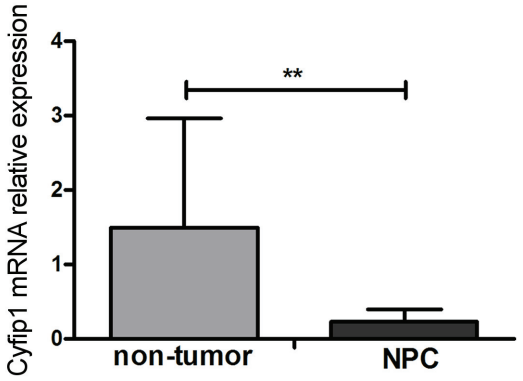

Figure 1. qRT-PCR analysis of Cyfip mRNA expression. ** $p<0.01$.

Table 1. Comparison of Cyfip1 mRNA expression in NPC tissues and non-cancerous nasopharyngeal tissues.

Tissue	N	Cyfip1 mRNA(ΔCT)	$2^{-\Delta\Delta CT}$	p-value
Non-cancerous nasopharyngeal tissues	45	3.24±1.65	0.23	0.001
NPC tissues	69	5.37±2.54		

Abbreviations: NPC, nasopharyngeal carcinoma; CT, cycle threshold

Table 2. Association between Cyfip1 mRNA expression and clinicopathological features in NPC patients.

Clinical features	Number of patients	Cyfip1 mRNA	
		mean±SD	p-value
Gender			
Male	45	5.24±2.65	0.859
Female	24	5.36±2.68	
Age			
0 ~50	19	5.35±2.16	0.710
>51	50	5.12±2.33	
Histological Type			
LDSC	17	5.54±2.78	
HDSC	9	5.47±2.62	0.945
NKC	43	5.73±2.55	
Clinical Stage			
I ~ II	20	6.54±2.12	<0.001
III ~ IV	49	4.10±2.07	
Invasive Range			
T1 ~T2	32	5.73±2.31	0.001
T3 ~T4	37	4.02±1.84	
Lymph node Metastasis			
N0	14	6.64±2.47	0.010
N1 ~N3	55	4.86±2.18	
Distant Metastases			
M0	64	6.58±2.32	0.040
M1	5	4.35±1.83	

Abbreviations: SD, standard deviation; LDSC, low differentiated squamous carcinoma; HDSC, high differentiated squamous carcinoma; NKC, non-keratinizing carcinoma

In our study, we found that the expression level of Cyfip1 mRNA in NPC tissues was lower than the level in non-cancerous nasopharyngeal tissues. More interestingly, we also found that low expression level of Cyfip1 mRNA was significantly associated with invasive range, lymph node metastasis, distant metastases and clinical stage. In a certain degree, the present study conforms to the former view that Cyfip1 is a tumor suppressor gene and it may play an important role in the occurrence and progression of NPC. By an examination of 249 tumor samples, Silva et al. found that loss of expression of Cyfip1 was among 63% of lung cancer, 59% of colon cancer, 24% of bladder cancer, and 31% of breast cancer [8]. Furthermore, they also showed that the loss of expression of Cyfip1 results in the disruption of epithelial cell architecture and increased tumor invasion [8]. Interestingly, Cyfip1 expression associates with tumor progression and a poor clinical outcome, suggesting that cyfip1 is a tumor suppressor gene [8, 11]. Then, Cowell et al. further demonstrated that knockdown of CYFIP1 and NCKAP1 proteins *in vitro* leads to suppression of invasion [12]. Afterwards, Sreenivasan et al. reported that the tumor suppressor gene Cyfip1 was upregulated upon curcumin treatment in retinoblastoma cells [13]. More recent studies by Dziunycz et al. showed that Cyfip1 was downregulated in the cutaneous squamous cell carcinoma [14]. Our study is consistent with those views.

Tumor suppressors play a critical role in preventing tumor growth and progression by opposing the phenomenon of increased proliferation, reduced cell death and adhesion-independent cell survival. Cyfip1 as an integral member of the Scar/WAVE complex regulates actin cytoskeleton architecture. The Scar/WAVE complex uses its VCA domain to stimulate the actin nucleating activity of the Arp2/3 complex in response to upstream signals from the small GTPase Rac1 [15]. Silva *et al.* found that interference with the WAVE complex and its function in regulating actin dynamics results in the phenotypes similar to those generated by suppression of Cyfip1 [8]. It was hence hypothesized that Cyfip1 might conduce to tumor suppression through its role in the WAVE complex. Interestingly, Teng *et al.* showed that knockdown of CYFIP1 in cancer cells caused destabilization of WASF3 complex and suppressed invasion [16]. In addition, other researchers investigated the cellular role of fragile X mental retardation protein (FMRP) and CYFIP1, demonstrating that FMRP and CYFIP1 modulate mTor signaling in an antagonistic manner [17]. However, the mechanisms of low expression of Cyfip1 in tumors are still poorly understood.

We are aware that this study has several limitations. First, our study method only used real-time fluorescent relatively quantitative PCR. Second, although we have detected decreased expression of Cyfip1 mRNA in NPC, the specific roles of Cyfip1 should be confirmed by an *in vitro* model of NPC cell lines. Third, the mechanisms of low expression of Cyfip1 mRNA in NPC are unclear. However, our results suggest that reduced expression of Cyfip1 is associated with the occurrence and development in patients with NPC, and therefore, Cyfip1 may be considered as a potential biomarker for diagnosis, progression and treatment modalities in NPC.

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