RESEARCH ARTICLE

Expression of Fragile Histidine Triad (FHIT) and WW-Domain Oxidoreductase Gene (WWOX) in Nasopharyngeal Carcinoma

Xu Chen[&], Ping Li[&], Zheng Yang, Wu-Ning Mo*

Abstract

The aim of the present study was to analyze the expression of FHIT and WWOX in nasopharyngeal carcinoma (NPC) and correlations with clinical pathologic features. mRNA expression of the FHIT and WWOX was assessed by real-time fluorescent relatively quantitative PCR in 61 NPC tissues and 45 non-cancerous nasopharyngeal tissues. As a result, mRNA expression levels of both FHIT and WWOX were significantly lower in NPC patients than in control samples (P=0.049 and 0.045, respectively). Moreover, the mRNA expression of both had an inverse relation with larger invasive range (P=0.035 and 0.048, respectively), poor histologic differentiation (P=0.012 and 0.016) and advanced clinical stage (P=0.026 and 0.038). Consistency was found between expression of FHIT and WWOX in the same NPC tissues (r=0.681, P=0.00). In conclusion, synergy between FHIT and WWOX may exist in the development of NPC so that the two factors may be considered as important genetic markers. Detecting the expression of FHIT and WWOX should provide clinically significant information relevatn to tumor diagnosis, progression and treatment modalities for NPC.

Keywords: WWOX - FHIT - nasopharynx carcinoma - prognosis

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Introduction

Nasopharyngeal carcinoma (NPC) is one of the most frequent malignant diseases in the Southern China and other south-east Asians. The annual incidence rate is over 20 cases per 100,000 populations. Men are twice as likely to develop NPC as women. The incidence generally increases from ages 20 to 50 (Cho, 2007). Unfortunately, because of the deep primary anatomical site of tumor growth and vague symptoms at early stage, most NPC patients tend to present a more advanced stage when first diagnosed. Therefore, it is of great interest and importance to find valuable biomarkers for early diagnosis for this malignancy.

The latest research proved that more than 80 common fragile sites had been found in the human genome database (Tuner et al., 2002). Common fragile sites are chromosome regions which observed in metaphase chromosomes. Those genes in these regions are more susceptible to breakage, rearrangements and deletions than other genes (Ilsley et al., 2002; Nunez et al., 2005). Substantial studies demonstrated that those genes related to common fragile sites played an important role in the carcinogenesis.

Carcinogenesis is a complicated and multiple procedure with the involvement of genetic alterations, including inactivation of tumor suppressor genes (Solomon et al., 1991). The fragile histidine triad (FHIT) and the WW-domain oxidoreductase gene (WWOX) are tumor suppressor genes that encompass the FRA3B and FRA16D fragile sites at chromosomes 3p14.2 and 16q23.3, respectively.

The FHIT gene is a tumor suppressor gene and is located in FRA3B which is the most active common fragile site, where DNA damage leading to aberrant transcripts and translocations frequently occur (Barnes et al., 1996; Huebner et al., 1997; Huebner et al., 1998). Abnormal transcripts of FHIT have been detected in various types of cancer (Baffa et al., 1998; Noguchi et al., 1999). Alteration of the FHIT gene through damage to the associated fragile region by environmental carcinogens, contributes substantially to the human cancer burden (Sozzi et al., 1998; Pylkkanen et al., 2002). The FHIT expression could be detected in almost all the tissues such as kidney, brain, prostate as well as liver. Overexpression of FHIT protein was found to inhibit tumorigenic activity and cause cell apoptosis in cancer cells (Dumon et al., 2001). FHIT protein is involved in apoptotic signal pathways, although the mechanisms of apoptosis induction have not been defined in detail. The FHIT gene can be inactivated by several mechanisms, including deletions, point mutations, methylation, loss of a whole chromosome and genetic recombination. It was reported that the loss or decreased RNA and protein products of FHIT was found in stomach, liver, cervix, esophagus, breast, renal, pulmonary, gall bladder, colon, and oral cavity tumors, as well as leukemia (Huebner et al., 1998; Campiglio et al.,

Department of Clinical Laboratory, First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi, China
*[&]Equal contributors *For correspondence: mown163@163.com*

1999).

Several loss of heterozygosity (LOH) studies has suggested that the chromosome 16q23.3-16q24.1 region is frequently changed in various cancer types (Bednarek et al., 2001; Paige et al., 2001; Driouch et al., 2002; Kuroki et al., 2002; Macias et al., 2002; Yendamuri et al., 2003). These observations indicate that this chromosome region harbors a tumor suppressor gene involved in different kinds of cancer. In the year 2000, the WWOX gene was mapped to this chromosomal region (Macias et al., 2002). The WWOX gene is located again in the common fragile site FRA16D, a chromosome area frequently affected by allelic losses in breast and other cancers (Ramos et al., 2006). The WWOX gene consists of nine exons and eight introns. Its main product is a 414 amino acid long, 46 KDa proteins which contains two WW domains, a short-chain dehydrogenase/reductasedomain (SRD) domain, and a nuclear localization sequence between these domains (Macias et al., 2002). These different domains of WWOX gene each has their own different functions. The two WW domains regulate complexes associated with signaling pathways implicated in a variety of cellular functions, such as transcriptional regulation and protein stability, degradation and transcription (Ludes-Meyers et al., 2003; Ramos et al., 2006). The SRD domain is involved in the process where WWOX protein plays a role in suppressing tumor (Bednarek et al., 2001; Paige et al., 2001). In addition, there is evidence that this gene product behaves as a suppressor of tumor growth. WWOX overexpression in prostate cancer cells suppressed colony growth and induced apoptosis (Qin et al., 2007). The evidence for its tumor suppressor activity was demonstrated for the first time in several cancer cell lines (Bednarek et al., 2001). The normal WWOX protein inhibits tumor cell growth. Mouse knock-out experiments support the tumor suppressor function of the WWOX gene (Aqeilan et al., 2007; Aqeilan et al., 2007). By these knock-out models, it has been shown that in juvenile WWOX-null mice, osteosarcoma develop spontaneously or loss of one allele increases the incidence of spontaneous (Kurek et al., 2010) and chemically induced lung and forestomach tumors in these animals (Aqeilan et al., 2007; Aqeilan et al., 2007). All these above findings support the view that WWOX is an important tumor suppressor gene and takes highly responsibility for inhibiting tumors. It was reported that WWOX expression was detected to be higher in organs such as testis, ovary and prostate where its activity is regulated hormonally (Nunez et al., 2006). Therefore, WWOX was conjectured to be a regulator of the steroids signaling pathways. Studies showed that its function in cellular metabolism is likely to modulate gene expression by interactions with other proteins. Some proteins such as p73, LITAF/SIMPLE, SCOTIN, AP-2k, YAP, EZRIN, truncated c-met, and RUNX2 had been proven to be interacted with WWOX by the WW domain in the regulation of cell growth and apoptosis (Aqeilan et al., 2004; Aqeilan et al., 2004; Ludes-Meyers et al., 2004; Aqeilan et al., 2005; Aqeilan et al., 2008). However, there is little evidence on the function of the SDR domain, although the functions of the WW domain and the interacting proteins with this domain have been identified. Regardless of its function in cell metabolism, WWOX which was considered as a tumor suppressor gene has been associated with various kinds of tumors, including breast (Bednarek et al., 2000; Iliopoulos et al., 2005), ovarian and lung cancer (Tuner et al., 2002; Aqeilan et al., 2005), hematopoietic malignancies (Ishii et al., 2003), pancreatic carcinoma (Kuroki et al., 2004; Nakayama et al., 2008), bladder cancer (Tuner et al., 2002; Iliopoulos et al., 2005). Furthermore, so far numerous studies have shown either loss or reduction of the WWOX expression in a variety of human tumors of breast, ovary, liver, stomach, pancreas, esophagus, lung and haematopoietic malignancies (Aqeilan et al., 2007). Since the FHIT and WWOX were first found by Ohta et al. (1996) in 1996 and Bednarek et al. (2000) in 2002, lost or reduced FHIT and WWOX expression has been shown to be an important step in the initiation of tumorigenesis in a variety of tumors, including breast, lung, esophagus, kidney, cervix and other organs (Sozzi et al., 1997; Tseng et al., 1999; Mori et al., 2000; Connolly et al., 2000; Guler et al., 2005; Aqeilan et al., 2007). We purposed in this study to determine if there is a similar association in NPC and we are also interested in possible associations between FHIT and WWOX in the development of NPC. In the current study, we explore the mRNA expression of FHIT and WWOX in NPC and its correlation with clinical pathologic features.

Materials and Methods

Sample collection

Sixty-one undifferentiated NPC tissues and 45 non-cancerous nasopharyngeal tissues were obtained from the First Affiliated Hospital of Guangxi Medical University, Nanning City, China. All fresh tissues were obtained after diagnosed before any therapy and were immediately preserved in liquid nitrogen as soon as possible. Two pathologists reassessed pathologic features, including histologic type, clinical stage, invasive range and metastasis (Lymph node Metastasis and Distant Metastases). For the use of these clinical materials for research purposes, prior consents from the patients and approval from the Ethics Committees of the hospital were obtained. All tissues had confirmed pathological diagnosis and were staged according to the 2008 NPC staging criterion.

Real-time fluorescent relatively quantitative PCR analysis

The following primers were designed according to published mRNA sequences of WWOX gene and FHIT gene. Conservative domain of the sequences were analyzed and chosen. Forward primer: 5'-TCGCAGCTGGTGGGTGTAC - 3_, reverse primer: 5'-AGCTCCCTGTTGCATGGACTT -3' for WWOX gene, forward primer: 5'- CAACATCTCATCAAGCCCTCT -3', reverse primer: 5'- TCCACCACTGTCCCGACT -3' for FHIT gene and 18S gene was used as an internal control using the forward primer 5'-GCACCGTCAAGGCTGAGAAC-3' and reverse primer 5'-TGGTGAAGACGCCAGTGGA-3'. All the primers were synthesized (ShengGong Inc, China) and

Table 1. Comparison of FHIT mRNA Expression in NPC Tissues and Normal Nasopharyngeal Tissues

Tissue	n	FHIT mRNA (△CT)	$2^{-\triangle CT}$	*P*
non-cancerous nasopharyngeal tissues	45	11.24±5.27		
NPC tissues	61	13.36±5.51	0.23	0.049

NPC, nasopharynx carcinoma; CT, cycle threshold

Table 2. Comparison of WWOX mRNA Expression in NPC and Normal Nasopharyngeal Tissues

Tissue	n	WWOX mRNA (△CT)	$2^{-\triangle CT}$	*P*
non-cancerous nasopharyngeal tissues	45	11.24±5.27		
NPC tissues	61	13.36±5.51	0.15	0.045

NPC, nasopharynx carcinoma; CT, cycle threshold

used. Expected PCR product will be 73 base pairs for WWOX gene, 191 base pairs for FHIT gene and 253 base pairs for 18S. No similar sequences were found in Genebank.

The mRNA from 100 mg tissues was prepared using the Tiangen Kit (Beijing, China), followed by reverse transcription into cDNA with oligo-dT primers (Tiangen Bio Inc, China). GeneAmp PCR system 2400 (GeneAmp, USA) was used to perform PCR. A reaction mix of 40μl was prepared, incubated at 42°C for 60 min, reacted at 70°C for 6 min and then denaturation of reverse transcriptase. PCR was performed, in a total mix of 50μl system including 5μl 10×PCR Buffer, 1.5μl Mgcl2, 1μl dNTP Mix, 1μl forward primer, 1μl reverse primer, 0.4μl Taq polymerase and 38.1μl distilled water. The PCR program was as follows: 30 s at 94°C, followed by 30 cycles of 10 s at 94°C, 15 s of 62°C and 20 s at 72°C. A final extension at 72°C for 5 min will then be carried. PCR products were purified and extracted as recommended by manufacturer. Agarose gels were used to visualize and confirm the PCR products under UV light.

PCR reactions containing SYBR-green were amplified on a Corbett Real Time PCR machine (Roche Diagonostics, USA). The 20μl Tag reaction mix included 10μl SYBR Green real-time PCR Master Mix, 0.6μl forward primer, 0.6μl reverse primer, 2μl cDNA, 2μl ROX Reference Dye, and 4.8μl deionizer water. Put the 20μl reaction system on centrifuge for 3500 turns of 30s and then perform in the machine of ABI7500. The PCR program was as follows: 10 min at 95°C, followed by 45 cycles of 5 s at 95°C, 15 s of 60°C and 20 s of 72°C. Collected fluorescence in 72°C. We regarded 18S gene as an internal control and calculated to get the adjusted cycle threshold (△CT) value for expression level of WWOX gene and FHIT gene. Calculation formula for calculating the adjusted CT value: relative expression of target gene (WWOX or FHIT) $=2^{-\triangle CT}$. △CT=CT value of target gene (WWOX or FHIT)-CT value of 18S.

Statistical analysis

The software SPSS13.0 was applied to analyze all the statistical analyses. Data were presented as mean ± SD. The correction-T test was also used for comparison of two groups. A P-value of less than 0.05 was considered statistically significant.

Table 3. Comparison of FHIT and WWOX mRNA Expression in NPC Tissues by Classification Based on Clinical Feature

Clinical feature	FHIT mRNA		WWOX mRNA	
	mean ± SD	*P*	mean ± SD	*P*
Age(year)				
0-50	13.16±5.96		12.54±3.88	
>51	13.77±4.89	0.971	12.64±3.97	0.883
Gender				
Male	13.06±5.67		12.77±4.07	
Female	13.82±5.81	0.883	12.08±3.26	0.691
Histologic Type				
LDSC	15.24±6.12		14.18±5.07	
HDSC	11.36±4.89		10.91±3.90	
NKC	13.15±5.88	0.012	12.75±8.01	0.016
Invasive Range				
T1~ T2	12.18±5.93		11.36±4.51	
T3 ~T4	15.45±5.02	0.035	12.99±3.99	0.048
Lymph node Metastasis				
N0	11.77±6.11		12.15±5.18	
N1~N3	14.96±5.98	0.092	13.64±3.98	0.084
Distant Metastases				
M0	13.87±5.09		12.01±3.17	
M1	14.17±5.22	0.641	12.98±4.42	0.842
Clinical Stage				
I~II	12.92±5.13		12.07±4.84	
III~IV	15.46±6.79	0.026	4.31±5.22	0.038

All NPC tissues had confirmed pathological diagnosis and were staged according to the 2008 NPC staging criterion; NPC, nasopharynx carcinoma; SD, standard deviation

Results

Clinicopathologic Data

In the 61 NPC cases, there were 45 male and 16 female with age ranging from 23 to 80 years (median, 45.7 years). 46 cases were low differentiated squamous carcinoma (LDSC), 12 cases were high differentiated squamous cell carcinoma (HDSC), 3 cases were non keratin carcinoma (NKC). In the 45 non-cancerous nasopharyngeal specimens, there were 33 male and 12 female with age ranging from 14 to 66 years (median, 42.2 years).

FHIT and WWOX mRNA expression

Significant difference was found in FHIT mRNA expression level between NPC tissues and non-cancerous nasopharyngeal tissues (P=0.049). Moreover, FHIT mRNA expression level of NPC tissues is just 0.23 times of non-cancerous nasopharyngeal tissues (Table 1). Similarly, the WWOX mRNA expression level detected in NPC tissues is only 0.15 times of non-cancerous nasopharyngeal tissues (P=0.045) (Table 2). We make classifications for NPC patients based on the clinical feature such as age, gender, histologic type, clinical stage, invasive range, Lymph node Metastasis and Distant Metastases. The relationship between clinicopathological characteristics and FHIT and WWOX expression in patients with NPC is summarized in Table 3. We did not find any significant association of FHIT and WWOX expression with age, sex, Lymph node Metastasis (N classification) or Distant Metastases (M classification) in 61 patients with NPC. However, we observed that the levels of FHIT and WWOX

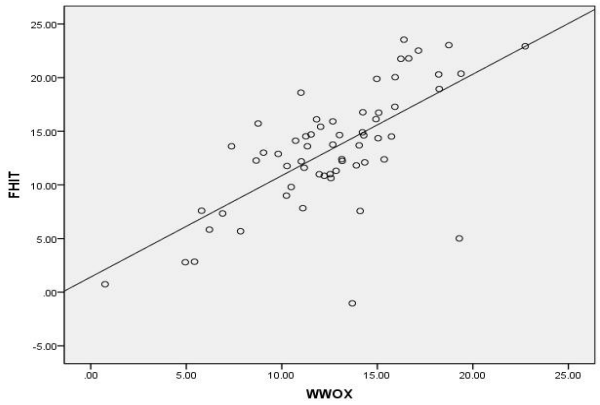

Figure 1. Correlation Analysis of WWOX and FHIT Expression in NPC Tissues

expression were closely correlated with the invasive range (T3-T4 vs. T1-T2) (P = 0.035, 0.048), histologic type (N classification, NKC vs. LDSC/ HDSC) (P =0.012, 0.016), and clinical stage (III-IV vs. I-II) (P = 0.026, 0.038). The FHIT was strongly corrected with WWOX in mRNA expression of NPC patients (r=0.681, P=0.00) (Figure 1)

Discussion

To control the relatively high morbidity and mortality of NPC in southern China and south-east Asia, more researchers explore the tumorigenesis mechanism of NPC on the molecular alterations along with the development of molecular biology, immunology and molecular pathology in recent years. Similar to other tumor types, NPC is also thought to arise following the genetic mechanisms that activate oncogenes and inactivate tumor suppressor genes. Among the two mechanisms, tumor suppressor genes have attracted considerable attention in recent years.

It is well accepted that chromosomal translocations, inversions, deletions, and amplifications play an important role in the pathogenesis of human cancer by affecting the expression of genes involved in cell growth regulation. In addition, there is evidence to show that several regions of chromosomes are more related to tumorigenesis than others.

It has been suggested that DNA instability at common fragile sites is associated with cancer and that such fragility may affect the function of genes located in those chromosomal regions (Yunis and Soreng, 1984). Previous studies have shown that primary tumors and carcinoma cell lines display hemizygous deletions with endpoints within fragile regions (Huebner and Croce, 2001), such as FRA3B, FRA16D, FRA6E, FRA7G as well as FRAXB. There is a great possibility that these deletions derive from misrepair of the fragile event represented by gaps in mitotic chromosomes. Common fragile regions have long been known as highly recombinogenic regions with elevated susceptibility to genotoxic agents, such as carcinogens found in tobacco smoke, and are involved nonrandomly in chromosomal alterations that occur in tumor cells (Popescu, 2003). These observations powerfully demonstrated that common fragile regions play an important role in the process of tumorigenesis. Among these fragile regions, FRA3B stands out as the most active fragile region in the human genome and FRA16D ranks the second.

FHIT and WWOX are two tumor suppressor genes that span the two most active common fragile sites FRA3B and FRA16D, respectively. In accordance with previous reports, lost or reduced FHIT and WWOX expression has been shown to be an important step in the initiation of tumorigenesis in a variety of tumors, including breast, lung, esophagus, kidney, cervix and other organs (Sozzi et al., 1997; Tseng et al., 1999; Mori et al., 2000; Connolly et al., 2000; Guler et al., 2005; Aqeilan et al., 2007). Moreover, loss of FHIT and WWOX expression has been repeatedly correlated with more advanced or worse clinicopathologic feature in many different types of tumors (Campiglio et al., 1999). Because no studies correlating FHIT and WWOX protein expression and cervical cancer progression have yet been reported previously, therefore, we believe it is worthwhile to analyze the expression of FHIT and WWOX in NPC and explore its association with clinicopathologic features.

In our study, we analyzed the mRNA expression of FHIT and WWOX in NPC. As the best of our knowledge, this is the first study which focuses on such a subject. The novelty of our study is just that the study of FHIT and WWOX expression in NPC has not been reported previously. We found that lower mRNA expression level of WWOX and FHIT in NPC tissues than levels in non-cancerous nasopharyngeal tissues. In addition, there is a significantly lower mRNA expression level in NPC patients with T3-T4 invasive range, non keratin carcinoma histologic type and III-IV clinical stage. In this point of view, many authors have shown that suppressed transcription of WWOX is associated with the more aggressive phenotype of breast cancer (Macias et al., 2002), non-small cell lung cancer (Donati et al., 2007) and ovarian cancer (Nunez et al., 2005). Here, we show that relatively lower FHIT and WWOX expression corresponds with the larger invasive range of NPC patients (T3-T4 vs. T1-T2, P = 0.035, 0.048, respectively), more advanced clinical stage (III-IV vs. I-II) (P = 0.026, 0.038, respectively) and NKC histologic type (NKC vs. LDSC/ HDSC, P =0.012, 0.016, respectively). This supports the view that loss of FHIT and WWOX expression is associated with tumourigenesis and more advanced stage in different types of cancers (Pluciennik et al., 2006; Cantor et al., 2007). Interestingly, no significant difference was found between FHIT and WWOX expression and lymph node metastasis or distant metastasis. Gulnur Guler, et al demonstrated that the coordinate reduced expression of both FHIT and WWOX was significantly related to metastasis in breast carcinoma (Guler et al., 2004). We thought this inconsistent result was just caused by the difference between breast carcinoma and NPC. Our correlation analysis between FHIT and WWOX demonstrated that the two important tumor suppressor genes were positively associated in mRNA expression of NPC tissues (Figure 1). Therefore, we speculate that synergies between FHIT and WWOX may exist in the development of NPC and the association between the altered expression of FHIT and WWOX may be a critical event in the progression of NPC

WWOX exhibits many features similar to FHIT. FHIT and WWOX are most active fragile genes and they are large genes with the length of more than 1 Mb. Both genes are located in common fragile sites and lie in a region of homozygous deletions and present a high frequency of aberrant RT-PCR products in tumors. The fragile sites FRA3B and FRA16D that the two genes live in are likely to cause chromosome break, rearrangement and recombination. High frequency of allele loss of FHIT and WWOX has been found in many human tumors. It is mentioned above that functional study showed that FHIT causes cell apoptosis (Qin et al., 2007) and inhibits tumorigenic activity. Likewise, WWOX which interacts with other proteins by WW domain is involved in the regulation of cell growth and apoptosis (Ishii et al., 2003). Expression of both FHIT and WWOX in cancer cells can result in induction of proapoptotic signal pathways in vitro experiments (Cantor et al., 2007). FHIT and WWOX are regulators of signal pathways although the clear mechanisms of signal pathway have not been defined in detail. Generally, carcinogens can lead to DNA damage that may lead to inactivation of tumor suppressor genes such as FHIT and WWOX. In our study, WWOX and FHIT show similar declines in expression of NPC and the two genes may exist in synergisms in the pathological process of NPC. Guler, et al demonstrated coordinate absence or reduction of FHIT and WWOX expression in 55 and 63% of invasive breast tumors was reported (Guler et al., 2004). Coordinate loss of FHIT and WWOX has also been reported in other tumors (Dumon et al., 2001; Guler et al., 2004; Bloomston et al., 2009). These investigators provided some evidence for our study to a certain extent. From the experimental results, we come to the part conclusion that analyzing the mRNA expression of FHIT and WWOX in NPC may be significant in evaluating and predicting tumor progression, although the viewpoint that whether loss of FHIT and WWOX expression could predict tumor progression remains controversial. Most studies approve of this idea but not all the literatures agree (Michael et al., 1997).

Some limitations we need to pay attention to. Firstly, immunohistochemistry was not applied to achieve the protein expression of FHIT and WWOX gene. However, the study of gene expression is composed of transcriptomics which studies mRNA expression and proteomics which studies protein expression. Gene transcript for mRNA first and then translate for protein. In addition, mRNA and protein products of FHIT and WWOX have been reported to show identical decrease in various tumors (Bednarek et al., 2001; Wang et al., 2008). Wang T, et al demonstrated that within each histological category in breast epithelial abnormalities including 51 invasive ductal carcinoma and 33 ductal carcinoma in situ, differences among fractions of specimens showed that FHIT and WWOX mRNA and protein expression were explainable by chance (Wang et al., 2008).

Thus, detecting the mRNA expression could approximately reflect corresponding gene expression. Furthermore, several mechanisms such as deletions, point mutations, methylation, loss of a whole chromosome and genetic recombination contributed to loss of FHIT and WWOX expression, we did not explore deeply on the mechanisms. However, some evidence demonstrated that loss of FHIT and WWOX expression was proportionally more frequent than abnormalities in p53, p16, or SMAD4 expression (Bloomston et al., 2009). It is well-known that aberrant p53 expression which happens at early event in carcinogenesis. Even more, WWOX loss was the most common molecular alteration with a reduction in expression from 92% in benign samples to only 28% in the pancreatobiliary cancers (Mark et al., 2009). Additionally, FHIT changes have been observed in early stage such as in situ carcinoma of breast, Barrett's esophagus and borderline ovarian tumors (Man et al., 1996; Michael et al., 1997; Ozaki et al., 2001). These studies strongly demonstrate that the loss of both FHIT and WWOX often occurs prior to other genetic alterations and is likely to be a very early event in tumor development. So it is of great value for detecting the expression of FHIT and WWOX in the development of NPC.

In conclusion, FHIT and WWOX may be considered as important genetic markers of NPC and the synergies between FHIT and WWOX may exist in the development of NPC. Detecting the expression of FHIT and WWOX probably provides clinical significance in tumor diagnosis, progression and treatment modalities for NPC.

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References

Aqeilan RI, Croce CM (2007). WWOX in biological control and tumorigenesis. *J Cell Physiol*, **212**, 307-10.

Aqeilan RI, Donati V, Palamarchuk A, et al (2005). WW domain-containing proteins, WWOX and YAP, compete for interaction with ErbB-4 and modulate its transcriptional function. *Cancer Res*, **65**, 6764-72.

Aqeilan RI, Hagan JP, Aqeilan HA, et al (2007). Inactivation of the Wwox gene accelerates forestomach tumor progression in vivo. *Cancer Res*, **67**, 5606-10.

Aqeilan RI, Hassan MQ, de Bruin A, et al (2008). The WWOX tumor suppressor is essential for postnatal survival and normal bone metabolism. *J Biol Chem*, **283**, 21629-39.

Aqeilan RI, Pekarsky Y, Herrero JJ, et al (2004). Functional association between Wwox tumor suppressor protein and p73, a p53 homolog. *Proc Natl Acad Sci USA*, **101**, 4401-6.

Aqeilan RI, Palamarchuk A, Weigel RJ, et al (2004). Physical and functional interactions between the Wwox tumor suppressor protein and the AP-2gamma transcription factor. *Cancer Res*, **64**, 8256-61.

Aqeilan RI, Trapasso F, Hussain S, et al (2007). Targeted deletion of Wwox reveals a tumor suppressor function. *Proc Natl*

Acad Sci USA, **104**, 3949-54.
Baffa R, Veronese ML, Santoro R, et al (1998). Loss of FHIT expression in gastric carcinoma. *Cancer Res*, **58**, 4708-14.
Barnes LD, Garrison PN, Siprashvili Z, et al (1996). Fhit, a putative tumor suppressor in humans, is a dinucleoside 50,50 00-P1, P3-triphosphate hydrolase. *Biochemistry*, **35**, 11529-35.
Bednarek AK, Keck-Waggoner CL, Daniel RL, et al (2001). WWOX, the FRA16D gene, behaves as a suppressor of tumor growth. *Cancer Res*, **61**, 8068-73.
Bednarek AK, Laflin KJ, Daniel RL, et al (2000). WWOX, a novel WW domain-containing protein mapping to human chromosome 16q2.33-2.41, a region frequently affected in breast cancer. *Cancer Res*, **60**, 2140-5.
Bloomston M, Kneile J, Butterfield M, et al (2009).Coordinate loss of fragile gene expression in pancreatobiliary cancers:correlations among markers and clinical features. *Ann Surg Oncol*, **16**, 2331-8.
Campiglio M, Pekarsky Y, Menard S, et al (1999). FHIT loss of function in human primary breast cancer correlates with advanced stage of the disease. *Cancer Res*, **59**, 3866-9.
Cantor JP, Iliopoulos D, Rao AS, et al (2007). Epigenetic modulation of endogenous tumor suppressor expression in lung cancer xenografts suppresses tumorigenicity. *Int J Cancer*, **120**, 24-31.
Cho WC (2007). Nasopharyngeal carcinoma: molecular biomarker discovery and progress. *Mol Cancer*, **6**, 1.
Connolly DC, Greenspan DL, Wu R, et al (2000). Loss of Fhit expression in invasive cervical carcinomas and intraepithelial lesions associated with invasive disease. *Clin Cancer Res*, **6**, 3505-10.
Donati V, Fontanini G, Dell'Omodarme M, et al (2007). WWOX expression in different histologic types and subtypes of non-small cell lung cancer. *Clin Cancer Res*, **13**, 884-91.
Driouch K, Prydz H, Monese R, et al (2002). Alternative transcripts of the candidate tumor suppressor gene, WWOX, are expressed at high levels in human breast tumors. *Oncogene*, **21**, 1832-40.
Dumon KR, Ishii H, Fong LY, et al (2001). FHIT gene therapy prevents tumor development in Fhit-deficient mice. *Proc Natl Acad Sci USA*, **98**, 3346-51.
Guler G, Uner A, Guler N, et al (2004). The fragile genes FHIT and WWOX are inactivated coordinately in invasive breast carcinoma. *Cancer*, **100**, 1605-14.
Huebner K, Croce C (2001). FRA3B and other common fragile sites: the weakest links. *Nat Rev Cancer*, **1**, 214 -21.
Guler G, Uner A, Guler N, et al (2005). Concordant loss of fragile gene expression early in breast cancer development. *Pathol Int*, **55**, 471-8.
Huebner K, Druck T, Siprashvili Z, et al (1998). The role of deletions at the FRA3B/FHIT locus in carcinogenesis. *Recent Results Cancer Res*, **154**, 200-15.
Huebner K, Garrison PN, Barnes LD, Croce CM (1998). The role of the FHIT/FRA3B locus in cancer. *Annu Rev Genet*, **32**, 7-31.
Huebner K, Hadaczek P, Siprashvili Z, Druck T, Croce CM (1997). The FHIT gene, a multiple tumor suppressor gene encompassing the carcinogen sensitive chromosome fragile site, FRA3B. *Biochim Biophys Acta*, **1332**, M65-70.
Iliopoulos D, Guler G, Han SY, et al (2005). Fragile genes as biomarkers: epigenetic control of WWOX and FHIT in lung, breast and bladder cancer. *Oncogene*, **24**, 1625-33.
Ilsley JL, Sudol M, Winder SJ (2002). The WW domain: linking cell signaling to the membrane cytoskeleton. *Cell Signaling*, **513**, 30-7.
Ishii H, Vecchione A, Furukawa Y, et al (2003). Expression of FRA16D/WWOX and FRA3B/FHIT genes in hematopoietic malignancies. *Mol Cancer Res*, **1**, 940-7.
Kurek KC, Del Mare S, Salah Z, et al (2010). Frequent attenuation of the WWOX tumor suppressor in osteosarcoma is associated with increased tumorigenicity and aberrant RUNX2 expression. *Cancer Res*, **70**, 5577-86.
Kuroki T, Trapasso F, Shiraishi T, et al (2002). Genetic alterations of the tumor suppressor gene WWOX in esophageal squamous cell carcinoma. *Cancer Res*, **62**, 2258-60.
Kuroki T, Yendamuri S, Trapasso F, et al (2004). The tumor suppressor gene WWOX at FRA16D is involved in pancreatic carcinogenesis. *Clin Cancer Res*, **10**, 2459-65.
Ludes-Meyers JH, Bednarek AK, Popescu NC, Bedford M, Aldaz CM (2003). WWOX, the common chromosomal fragile site, FRA16D, cancer gene. *Cytogenet Genome Res*, **100**, 101-10.
Ludes-Meyers JH, Kil H, Bednarek AK, et al (2004). WWOX binds the specific proline-rich ligand PPXY: identification of candidate interacting proteins. *Oncogene*, **23**, 5049-55.
Macias MJ, Wiesner S, Sudol M (2002). WW and domains, two different scaffolds to recogniaz praline-rich ligands. *FEBS Lett*, 513, 30-7.
Man S, Ellis IO, Sibbering M, Blamey RW, Brook JD (1996). High levels of allele loss at the FHIT and ATM genes in non-comedo ductal carcinoma in situ and grade I tubular invasive breast cancers. *Cancer Res*, **56**, 5484-9.
Mark B, Jeffrey K, Matthew B, et al (2009). Coordinate Loss of Fragile Gene Expression in Pancreatobiliary Cancers: Correlations Among Markers and Clinical Features. *Ann Surg Oncol*, **16**, 2331-8
Michael D, Beer DG, Wilke CW, Miller DE, Glover TW (1997). Frequent deletions of FHIT and FRA3B in Barrett's metaplasia and esophageal adenocarcinomas. *Oncogene*, **15**, 1653-9.
Mori M, Mimori K, Shiraishi T, et al (2000). Altered expression of Fhit in carcinoma and precarcinomatous lesions of the esophagus. *Cancer Res*, **60**, 1177-82.
Nakayama S, Semba S, Maeda N, et al (2008). Role of the WWOX gene, encompassing fragile region FRA16D, in suppression of pancreatic carcinoma cells. *Cancer Sci*, **99**, 1370-6.
Noguchi T, Muller W, Wirtz HC, Willers R, Gabbert HE (1999). FHIT gene in gastric cancer: association with tumour progression and prognosis. *J Pathol*, **188**, 378-81.
Nunez MI, Ludol-Meyer J, Abba MC, et al (2005). Frequent loss of WWOX expression in breast cancer correlation with estrogen receptor status. *Breast Cancer Res Treat*, **89**, 99-150.
Nunez MI, Ludes-Meyers J, Aldaz CM (2006). WWOX protein expression in normal human tissues. *J Mol Histol*, **37**, 115-25.
Nunez MI, Rosen DG, Ludes-Meyers JH, et al (2005).WWOX protein expression varies among ovarian carcinoma histotypes and correlates with less favorable outcome. *BMC Cancer*, **5**, 64.
Ohta M, Inoue H, Cotticelli MG, et al (1996). The FHIT gene, spanning the chromosome 3p14.2 fragile site and renal carcinoma-associated t (3; 8) breakpoint, is abnormal in digestive tract cancers. *Cell*, **84**, 587-97.
Ozaki K, Enomoto T, Yoshino K, et al (2001). Impaired FHIT expression characterizes serous ovarian carcinoma. *Br J Cancer*, **85**, 247-54.
Paige AJ, Taylor KJ, Taylor C, et al (2001). WWOX: a candidate tumor suppressor gene involved in multiple tumor types. *Proc Natl Acad Sci USA*, **98**, 11417-22.
Pluciennik E, Kusinska R, Potemski P, et al (2006).WWOX-the FRA16D cancer gene: expression correlation with breast cancer progression and prognosis. *Eur J Surg Oncol*, **32**,

153-7.
Popescu NC (2003). Genetic alterations in cancer as a result of breakage at fragile sites. *Cancer Lett*, **192**, 1-17.
Pylkkanen L, Wolff H, Stjernvall T, et al (2002) Reduced Fhit protein expression and loss of heterozygosity at FHIT gene in tumours from smoking and asbestos-exposed lung cancer patients. *Int J Oncol*, **20**, 285-90.
Qin HR, Iliopoulos D, Nakamura T, et al (2007). WWOX suppresses prostate cancer cell growth through modulation of ErbB2-mediated androgen receptor signaling. *Mol Cancer Res*, **5**, 957-65.
Ramos D, Aldaz CM (2006). WWOX, a chromosomal fragile site gene and its role in cancer. *Adv Exp Med Biol*, **587**, 149-59
Solomon E, Borrow J, Goddard AD (1991). Chromosome aberrations and cancer. *Science*, **25**, 1153-60.
Sozzi G, Pastorino U, Moiraghi L, et al (1998). Loss of FHIT function in lung cancer and preinvasive bronchial lesions. *Cancer Res*, **58**, 5032-37.
Sozzi G, Tornielli S, Tagliabue E, et al (1997). Absence of Fhit protein in primary lung tumor and cell lines with FHIT gene abnormalities. *Cancer Res*, **57**, 5207-12.
Tseng JE, Kemp BL, Khuri FR, et al (1999). Loss of Fhit is frequent in stage I non-small cell lung cancer and in the lungs of chronic smokers. *Cancer Res*, **59**, 4798-803.
Tuner BC, Ottey M, Zmonjic DB, et al (2002). The fragile histidine triad/common chromo some fragile site 3B locus and repair-deficient cancer. *Cancer Res*, **62**, 4054-60.
Wang TT, Frezza EE, Ma R, et al (2008) Loss expression of active fragile sites genes associated with the severity of breast epithelial abnormalities. *Chin Med J*, **121**, 1969-74.
Yendamuri S, Kuroki T, Trapasso F, et al (2003). WW domain containing oxidoreductase gene expression is altered in non-small cell lung cancer. *Cancer Res*, **63**, 878-81.
Yunis JJ, Soreng AL (1984). Constitutive fragile sites and cancer. *Science*, **226**, 1199-204.