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 relevant to tumor diagnosis, progression and treatment modalities for NPC.

Keywords: WWOX - FHIT - nasopharynx carcinoma - prognosis

Asian Pacific J Cancer Prev, 14 (1), 165-171

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., 1999).
Several loss of heterozygosity (LOH) studies have 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/reductase domain (SRD) domain, and a nuclear localization sequence between these domains (Macias et al., 2002). These different domains of WWOX each have 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. Conservatory domain of the sequences were analyzed and chosen. Forward primer: 5’- TCGCAGCTGGTGGTGTTAC - 3’, reverse primer: 5’- AGCTCTCTGTGATGCTCTGT - 3’, forward primer: 5’- CCACATCTCATCAAGCCCCTTCT - 3’, reverse primer: 5’- TCCACACTGTCTCCGACT - 3’ for FHIT gene and 18S gene was used as an internal control using the forward primer 5’-GACCGTCAAGGCTGAGAC-3’ and reverse primer 5’-TGGTGAAAGACCGCAGTGGA-3’. All the primers were synthesized (ShengGong Inc, China) and

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

<table>
<thead>
<tr>
<th>Tissue</th>
<th>n</th>
<th>FHIT mRNA (Δ CT)</th>
<th>2^{-ΔCT}</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>non-cancerous nasopharyngeal tissues</td>
<td>45</td>
<td>11.24±5.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPC tissues</td>
<td>61</td>
<td>13.36±5.51</td>
<td>0.23</td>
<td>0.049</td>
</tr>
</tbody>
</table>

NPC, nasopharynx carcinoma; CT, cycle threshold

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

<table>
<thead>
<tr>
<th>Tissue</th>
<th>n</th>
<th>WWOX mRNA (Δ CT)</th>
<th>2^{-ΔCT}</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>non-cancerous nasopharyngeal tissues</td>
<td>45</td>
<td>11.24±5.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPC tissues</td>
<td>61</td>
<td>13.36±5.51</td>
<td>0.15</td>
<td>0.045</td>
</tr>
</tbody>
</table>

NPC, nasopharynx carcinoma; CT, cycle threshold

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 mRNA expression were significantly lower in patients with distant metastasis compared to those without distant metastasis (P=0.003). The results of statistical analysis are presented in Table 3.

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.
Fragile regions play an important role in the process of tumorigenesis. 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 located in those fragile sites. It is 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 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. Previous studies have shown that primary tumors and carcinoma cell lines display hemizygous deletions with endpoints within fragile regions, such as FRA3B, FRA16D, FRA6E, FRAXB, and FRA7G. There is great possibility that these deletions derive from misrepair of the fragile event represented by gaps within fragile regions. 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. 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. FHIT and WWOX have been repeatedly demonstrated that common fragile regions play a nonrandom role in chromosomal alterations that occur in tumor cells. These observations have been shown to be an important step in the initiation of tumorigenesis in a variety of tumors. Moreover, the loss of FHIT and WWOX expression has been repeatedly correlated with more advanced or worse clinicopathologic feature in many different types of tumors. 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. In this point of view, many authors have shown that suppressed transcription of WWOX is associated with the more aggressive phenotype of breast cancer. In our study, we showed 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). In addition, we showed that relatively lower FHIT and WWOX expression was associated with the 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. Interestingly, no significant difference was found between FHIT and WWOX expression and lymph node metastasis or distant metastasis.

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.

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. In this point of view, many authors have shown that suppressed transcription of WWOX is associated with the more aggressive phenotype of breast cancer. In our study, we showed 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). In addition, we showed that relatively lower FHIT and WWOX expression was associated with the 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. 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. 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 probe 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 pancreaticobiliary 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.

Acknowledgements

This work was supported by a grant from the Guangxi Nature Science Fund named “Research of WWOX in Nasopharynx Carcinoma” (2010GXNSFA013184). The authors declare that they have no conflict of interest relating to the publication of this manuscript. This study was supported by some students (Xiuli Huang, Zhe Wang) of First Affiliated Hospital of Guangxi Medical University in acquisition of data and searching background information relevant to our study. We would like to thank them for their help which have led to improvement of this article.

References


