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THE UTILITY OF COMBINED WWOX, HBME-1 AND CK34BE12 AS A DIAGNOSTIC PANEL IN THYROID PAPILLARY CARCINOMA

Oncology

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ABSTRACT

OBJECTIVE:Thyroid papillary carcinoma (PTC) has a good prognosis, but making an accurate diagnosis remains an important issue. Borderline tumors in particular are difficult to determine. Immunohistochemical (IHC) staining is the first priority to assist in diagnosis in clinical practice. In the current study, we strive to confirm the diagnostic value of WWOX in thyroid cancer.

METHODS:We retrospectively selected 40 cases of nodular goiter (NG), 42 cases of follicular adenoma (FA), and 69 cases of PTC were collected in total.

RESULTS:WWOX positive rate was 100% in 69 cases of PTC. In addition, only 9 cases in totally 82 cases of benign tumor were positive for WWOX (4 cases in NG and 5 cases in FA). WWOX has the highest AUC, which showed significantly higher than CK19, CK34βE12, Galectin-3, and HBME-1. The combination of WWOX, HBME-1 and CK34BE12 was the best combination for diagnosis with 91.3% sensitivity and 97.6% specificity.

CONCLUSION:The malignant lesions of PTC had 100% of WWOX expression rate in IHC staining. The diagnostic sensitivity of WWOX is 100% and the specificity is 89.2%. WWOX might be a potential diagnostic or therapeutic target in thyroid differentiated thyroid carcinoma. At last, WWOX, HBME-1 and CK34BE12 was the best combination for thyroid malignancy diagnosis.

KEYWORDS

Thyroid; papillary carcinoma; WWOX; diagnosis

INTRODUCTION

The incidence of thyroid cancer continues to increase worldwide. Thyroid cancer is the fifth most common cancer in women in the United States, with more than 62,000 new cases in men and women in 2015 [1]. An international organization for cancer estimation research, the incidence of thyroid cancer in Korean women aged 50-59 years was 120 per 100,000 between 2003-2007 [2]. Normal thyroid histologically consists of two major parenchymal cell types. Follicular cells line the colloid follicles, concentrate iodine, and produce thyroid hormones. These cells induce both well-differentiated cancers (eg, papillae and follicular) and anaplastic thyroid cancers [3]. Papillary thyroid cancer (PTC) is the most common endocrine malignancies, accounting for 96.0% of all new endocrine cancers and 66.8% of deaths from endocrine cancers [4]. The rate of change in overall mortality was not significant in patients with PTC [5]. Local lymph node metastases are present in 20-90% of PTC patients at primary diagnosis and are less common in other histological types [6]. Factors associated with lymph node metastasis in PTC patients include tumor size, extracapsular invasion, and multifocality. Micrometastases are common [3]. A small number of patients have distant metastatic at the time of diagnosis. In a large series, 1-2% of PTC patients were metastasized to the neck or outside the mediastinum at diagnosis [7].

The tumor suppressor gene WW domain-containing oxidoreductase (WWOX) is a 414-amino acid protein, and the WW domain is used by tumor suppressants to suppress tumor growth through protein-protein interactions [8]. Numerous studies have found that the WWOX gene is a tumor suppressor gene in various types of malignancies and that WWOX expression is lost or reduced in various tumors [9]. PTCs have a good prognosis, but making an accurate diagnosis remains an important issue. Borderline tumors in particular are difficult to determine. Immunohistochemical (IHC) staining is the first priority to assist in diagnosis in clinical practice. However, none of the IHC markers are sufficient to distinguish between benign or malignant lesions. Of the markers in all papers, CK19, galectin-3 and HBME-1 are the most popular IHC markers to aid diagnosis. In the current study, we strive to confirm the diagnostic value of WWOX in thyroid cancer.

METHODS

PATIENTS

We retrospectively radomly seleted cases received thyroid surgery in our hospital between 2003 and 2004. All of these cases had no family history of thyroid cancer or malignancy in first-degree relatives as judged by interviews at the time of admission for surgery. All the patients with malignant disease had received routine total or subtotal thyroidectomy with regional lymphadenectomy concurrently during surgery. We further exclude subjects have been received chemotherapy or radiation therapy before resection. Finally, 40 cases of nodular goiter (NG), 42 cases of follicular adenoma (FA), and 69 cases of PTC were collected in total. All clinical charts and histopathology reports of patients were reviewed for data regarding age, gender, tumor size, bilateral, extra-capsule invasion, lymph nodes metastasis or distant metastasis and TMN staging. All enrolled cases were de-linked anonymously for protection and this study protocol was approved by the institutional review board of Cardinal Tein Hospital.

Histology, Immunohistochemistry and Scoring

The constructed tissue paraffin embedded blocks would be cut in 5μm-thick sections to perform H&E stain. IHC were performed, using the Ventana BenchMark XT automated stainer (Ventana, Tucson, AZ). The primary antibodies of WWOX (1:50, novus, Missouri, USA), CK34ßE12 (1:100, Dako), CK19 (1:100, Dako), Galectin-3 (1:100, Sigma-Aldrich) and HBME-1 (1:100, Dako) were performed. Ventanna UltraView DAB detection kit was applied and slides will be counterstained with hematoxylin, dehydrated, and mounted.

Statistical analyses

Statistical analyses were all performed using SPSS-18.0 software (SPSS Inc., Chicago, IL). To test for differences between positive and negative of IHC expression, chi-square analysis would be performed for categorical variables. To evaluate which IHC marker is better, receiver operating characteristic (ROC) curves was applied (MedCalc Software, Broekstraat 52, 9030 Mariakerke, Belgium). By comparing with the area under curve (AUC) of ROC, the best IHC marker is found. All statistical tests were two-sided and considered statistically significant when $p < 0.05$.

Table 1 Demographic data of the study cases

	Papillary carcinoma	Nodular goiter	Follicular adenoma
n	69	40	42
Sex (M/F)	12/57	18/22	21/21
Age (year)	46.2 ± 3.7	48.8 ± 4.2	45.6 ± 4.7
Tumor Size (cm)*	1.92 ± 0.53	1.49 ± 0.45	2.42 ± 0.17
Lymph nodes metastasis			
No	52 (75.3%)	---	---
Yes	17 (24.7%)	---	---
Stage			
I and II	59 (85.5%)	---	---
III and IV	10 (14.5%)	---	---

* The largest adenomatous nodule was measured.

Table 2 Immunoreactive rate of different Immunohistochemistry markers

	WWOX	CK19	CK34βE12	Galectin-3	HBME-1	p value
Tumor subtype						
Nodular goiter	4 (10.0)	31 (77.5)	0 (0)	10 (25.0)	2 (5.0)	< 0.001
Follicular adenoma	5 (11.9)	33 (78.5)	1 (2.3)	2 (4.7)	6 (14.2)	
Papillary carcinoma	69 (100)	68 (98.5)	46 (66.6)	60 (86.9)	58 (84.0)	
Total Lesions						
Benign lesions	9 (10.9)	64 (78.0)	1 (1.2)	12 (14.6)	8 (9.7)	< 0.001
Malignant lesions	69 (100)	68 (98.5)	46 (66.6)	60 (86.9)	58 (84.0)	

Represented by number (percentage).

Table 3 Sensitivity, specificity, positive predict value and negative predict value in different immunochemistry markers

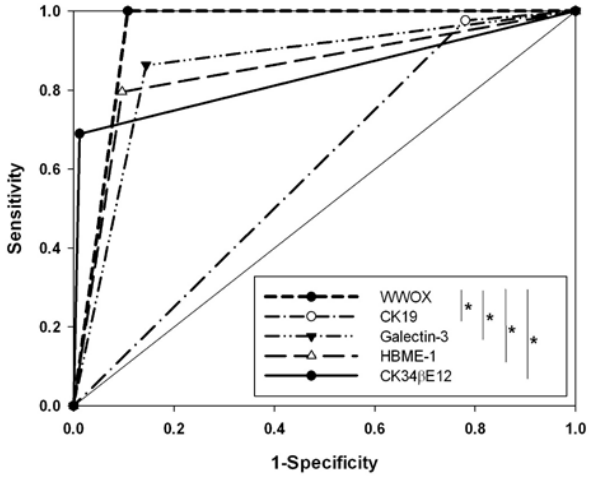
	Sensitivity	Specificity	Positive predict value	Negative predict value
WWOX	100.0%	89.0%	88.5%	100.0%
CK19	98.6%	22.0%	51.5%	94.7%
CK34BE12	66.7%	98.8%	97.9%	77.9%
Galectin-3	87.0%	85.4%	83.3%	88.6%
HBME-1	84.1%	90.2%	87.9%	87.1%

Table 4 Compare different combined markers and different cut-off value

	WWOX and HBME-1	WWOX and HBME-1	WWOX, HBME-1 and CK34BE12	WWOX, HBME-1 and CK34BE12	WWOX, HBME-1 and CK34BE12
Cut-off value	Either one marker	Both markers	Either one marker	Any two markers	All three markers
Sensitivity	100.0%	84.1%	100.0%	91.3%	59.4%
Specificity	80.5%	98.9%	80.5%	97.6%	100.0%
PPV	81.2%	98.3%	81.2%	96.9%	100.0%
NPV	100.0%	88.0%	100.0%	93.0%	74.5%

PPV = Positive predict value; NPV = Negative predict value

Figure 1 Receiver operating characteristic curve of the different Immunohistochemistry markers



*p<0.05

RESULTS

There were 69 cases of PTC, 40 cases of NG, and 42 cases of FA cases in the current study. The mean age at diagnosis is 46.2, 48.8, and 45.6 years old in PTC, NG, and FA, respectively (Table 1). WWOX positive rate was 100% in 69 cases of PTC (Table 2). In addition, only 9 cases in totally 82 cases of benign tumor were positive for WWOX (4 cases in NG and 5 cases in FA). When comparing with the traditional PTC markers of CK19, CK34βE12, Galectin-3, and HBME-1, WWOX has the highest sensitivity and negative predict value (Table 3). Moreover, WWOX has the highest AUC, which showed significantly higher than CK19, CK34βE12, Galectin-3, and HBME-1 (Figure 1). If we combined these markers for diagnosis, we found the combination of WWOX, HBME-1 and CK34BE12 was the best combination. Positive expression any two markers as the cut-off value showed 91.3%, 97.6%, 96.9%, and 93.0% of sensitivity, specificity, positive predictive value, and negative predictive value, respectively (Table 4).

DISCUSSION

In the current study, we demonstrated increased expression of WWOX in human thyroid cancer epithelium. WWOX is a 46 kDa protein composed of two N-terminal WW domains and a C-terminal short chain dehydrogenase / reductase domain [10] and spans one of the most active common domains, FRA16D [11]. Vulnerable sites associated with cancer. The genomic position of the Wwox-encrypted gene creates a locus that is vulnerable to heterozygous and homozygous fruiting that reduces gene expression [12]. Loss of Wwox expression was observed in cancers of many organs, including breast, lung, esophageal, and gastric cancers [11, 13] WWOX was partially via Cterminal short-chain alcohol dehydrogenase / reductase (SDR), localized to mitochondria [14]. WWOX maintains mitochondrial respiratory function and [15] induces mitochondrial apoptosis when overexpressed [16]. In the early stages of cancer progression, WWOX is Tyr33 phosphorylated, which is significantly upregulated and limits the development of cancer in vivo [17].

WWOX is often deleted or altered due in many malignancies. Among the triple negative breast cancer and invasive breast cancer, the decreased expression of WWOX related to high local recurrence rate, lack of effective targeted therapy, distant metastasis and poor disease-free survival [18]. Previous studies have found that there is a significant correlation between the expression of WWOX and lymph node metastasis, liver metastasis, differentiation, depth of invasion, and staging in colorectal cancer, indicating WWOX is an important biomarker in predicting prognosis of colorectal cancer [19]. Another study found that there is a negative correlation between CD133 expression and WWOX and E-cadherin, and there is a positive correlation between WWOX and E-cadherin in colorectal cancer. This shows a clear relationship between the three biomarkers in colorectal cancer and allows physicians to predict progression and prognosis [20].

WWOX is known to limit the growth and metastasis of cancer [21]. WWOX regulates the migration and metastasis of cancer cells [22]. The loss of WWOX in triple-acoustic breast cancer cells leads to the activation of the deformation of the JAK2/STAT3 pathway [22]. In the early stages of cancer development, WWOX is up-regulated and then decreased, thereby promoting the growth of cancer [23]. Over-expressed WWOX exerts pro-apoptotic pressure to support the forming capillary microtube by bFGF-secreting basal cell carcinoma cells, thereby promoting the growth and metastasis of cancer cells. Ferguson et al. described tissue specific targeted ablation of WWOX gene in adult mouse tissues. The mouse model showed that WWOX was significantly down-regulated in breast epithelial cells, and the loss of WWOX expression was found to be related to tumor progression, although WWOX may not be a classic tumor suppressor gene. However, knockdown of WWOX in the mouse model caused the impaired mammary branching morphogenesis [24].

Nunez et al. found that the expression of WWOX in normal thyroid tissue was consistent with our result [25], showing strong positive cytoplasmic staining in the cuboidal follicular cells and weak staining in follicular cells representative of inactive glandular activity (flat follicular cells). In all cases, it is worth noting that the WWOX staining on the cytoplasm [26]. Dias et al. [27] found that 91% of PTC subjects had reduced or insufficient WWOX expression in their cancer tissues. Based on these results, it is indicated that low or non-expression of WWOX in PTC is the cause of increased cell proliferation, leading to further genomic instability, with the overlap of genetic and epigenetic events. This would give the follicular epithelial cells a clonal advantage, thereby helping the carcinogenesis process of PTC [27]. But this is a result that is inconsistent with our findings. Different antibody clone and different stages of the inclusion subjects might be the reason for this opposite results.

To this end, since our results show significant association of WWOX protein expression with relevant clinicopathological variables, our data are important and suggest a novel diagnostic marker in thyroid cancer. In conclusion, the malignant lesions of PTC had 100% of WWOX expression rate in IHC staining. The diagnostic sensitivity of

WWOX is 100% and the specificity is 89.2%. WWOX might be a potential diagnostic or therapeutic target in thyroid differentiated thyroid carcinoma. At last, WWOX, HBME-1 and CK34BE12 was the best combination for thyroid malignancy diagnosis.

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Conflict of interest statement

All authors declare no conflict of interest.

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