

Original Research The effects of protocadherin 8 and WWOX in prostate adenocarcinoma

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Abstract

Objective: The protocadherin 8 (PCDH8) gene, located on chromosome 13q14.3 encodes an integral membrane protein. WWOX (fragile site FRA16D oxido-reductase) is a tumor suppressor gene located in region 16q23.324.1. The aim of this study was to investigate the staining pattern of protocadherin 8 and WWOX in adenomatous hyperplasia and prostate adenocarcinoma. **Methods**: Seventy adenomatous hyperplasia and 70 prostate adenocarcinoma preparations stored at the pathology department from 2013–2016 were retrospectively analyzed. Samples immunohistochemically stained with WWOX or protocadherin 8 were evaluated by two pathologists under a light microscope. After pathological investigation of the samples, the expression of WWOX and protocadherin 8 was scored. WWOX and PCDH8 expression was assessed semi-quantitatively according to staining intensity scored as none, mild, moderate, and strong (0 to 3+). For analysis of the data, the mild, moderate and strong staining scores were combined (as positive) and the data were classified as negative or positive. Differences in the WWOX or protocadherin 8 staining results between adenomatous hyperplasia and prostate adenocarcinoma cases were examined using a two-way chi-square test and binary logistic regression analysis. **Result**: Statistical analysis showed that WWOX expression was higher in adenomatous hyperplasia than in prostate adenocarcinomas. This difference was statistically significant (p = 0.035). There was no difference in PCDH8 expression between adenomatous hyperplasia and prostate adenocarcinoma samples (p = 0.217). **Conclusion**: In this study, the expression of WWOX decreased and the expression of PCDH8 remained unchanged in PCa cases. In terms of prognostic significance, it can be concluded that WWOX is a good prognostic parameter, while PCDH8 was an ineffective prognostic marker. In addition, the investigation of protocadherin 8 in larger series may provide more meaningful results.

Keywords: protocadherin 8; WWOX; prostate; logistic regression analysis

1. Introduction

The prostate gland consists of secretory ducts lined with epithelial cells within a stroma rich in smooth muscle tissue [1]. The prostate is the most common organ of neoplastic transformation in the human body. Benign or malignant transformation can be seen in prostate tissue [2] (Fig. 1).

Prostate cancer is the second leading cause of cancerrelated deaths among males [3]. Furthermore, prostate cancer is the fourth most common malignancy among European males [4]. Protocadherin 8 (PCDH8) is composed of six extracellular cadherin domains, a transmembrane domain, and different cytoplasmic domains [5]. PCDH8 is a member of the cadherin family and has an important function in cell adhesion, differentiation, migration, and proliferation [6], as well as in signal transduction and growth control [7]. Various members of the protocadherin family (PCDH10, 17 and 20) are usually suppressed by promoter methylation in nasopharyngeal carcinoma, stomach cancer, non-small cell lung cancer and colorectal cancer [8,9]. PCDH8-10, PCDH17 and PCDH20 have been reported to be candidate tumor suppressor genes [7]. The PCDH8 gene, located on chromosome 13q14.3, encodes an integral membrane protein [10]. Transcriptional suppression of PCDH8 can occur by genetic mutation [11] or epigenetic promoter hypermethylation [5]. Inactivation of PCDH8 by promoter methylation is an indicator of poor prognosis [12]. PCDH8 is usually inactivated by promoter methylation in bladder cancer, renal cell carcinoma, nasopharyngeal carcinoma, stomach cancer and breast cancer, which is associated with poor prognosis [6,12].

WWOX (fragile site FRA16D oxido-reductase) is a tumor suppressor gene located in region 16q23.324.1 [13, 14]. Loss of WWOX expression has been observed in ovarian, breast, testis, prostate, lung, pancreas, stomach, and hepatocellular carcinomas [15]. WWOX is thought to be a multifunctional protein associated with WW domain protein-protein interaction [14]. The human tumor suppressor gene encodes different mRNAs that are converted to WWOX, WWOX/WOX1, WOX2, and other isoforms. The role of WOX2 as a tumor suppressor is unknown [16,17]. Both WOX1 and its isoform WOX2 may be upregulated in the early stages of progression of breast, prostate and other cancers [18]. Changes in the human WWOX gene appear intensively in prostate and breast carcinomas [16,17].

A reduced or undetectable level of WWOX expression caused by deletion or epigenetic changes has been reported in approximately 70% of prostate cancer cases [19]. This study aimed to investigate whether PCDH8 and WWOX can be used as useful markers in the diagnosis and followup of prostate cancer.



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Fig. 1. Prostate tissue. (A) Adenomatous hyperplasia. Note the hyperplastic prostate glands (H&E, $\times 200$). (B) Prostate adenocarcinoma. The cancerous glands in this image show nuclear enlargement, hyperchromasia, prominent nucleoli, and the absence of a basal cell layer (H&E, $\times 200$).

2. Material and methods

Before beginning the study, permission was granted by the Clinical Research Ethics Committee on 02/11/2015, numbered 2015/10. In the Ordu Training and Research Hospital Pathology Department, 70 adenomatous hyperplasia (AH) and 70 prostate adenocarcinoma (PCa) preparations diagnosed from 2013-2016 were analyzed retrospectively. New 3- μ m thick sections were obtained from paraffin blocks of the collected prostate tissues, and immunohistochemical staining was performed with a Leica Bond automatic tissue staining device for WWOX (polyclonal) C.Liq.0.1Ml (1:500) and protocadherin 8 (polyclonal) C.Liq.0.1Ml (1:1000). Samples stained with WWOX or protocadherin 8 were assessed under a light microscope (BX51, Olympus, Tokyo, Japan). WWOX and PCDH8 expression was assessed semi-quantitatively according to staining intensity scored as none, mild, moderate, and strong $(0 \text{ to } 3^+)$. For analysis of the data, the mild, moderate and strong staining scores were combined (as positive) and the data were classified as negative or positive.

Differences in the WWOX or PCDH8 staining results (negative and positive) between AH and PCa cases were examined using a two-way chi-square test and binary logistic regression analysis. The odds ratio (OR) with 95% confidence interval was computed to assess the strength of the association and statistical significance. A p value < 0.05 was accepted as significant. All statistical analyses were performed using SPSS v26.0 (version 26.0, IBM Corp., Armonk, NY, USA).

3. Results

First, pathological investigation of the samples taken from the patients was performed. The distribution of the WWOX staining results (negative and positive) in AH and PCa cases is given in Table 1. Expression of WWOX was higher in AH cases than in PCa cases. The chi-square test indicated a statistically significant difference in WWOX staining between AH and PCa cases (p = 0.035). In AH and PCa cases, the relationship between WWOX and staining was investigated with binary logistic regression analysis. The calculated OR showed that higher WWOX expression was 2.525 times more likely in AH compared to PCa samples (Fig. 2).

Table 1. Frequency distributions of patients according to WWOX staining results.

| | Staining | | Total | n | |
|-------|------------|-------------|--------------|--------------------|--|
| | Negative | Positive | Total | P | |
| AH | 9 (12.9%) | 61 (87.1%) | 70 (100.0%) | 0.035* | |
| PCa | 19 (27.1%) | 51 (72.9%) | 70 (100.0%) | $(\chi^2 = 4.464)$ | |
| Total | 28 (20.0%) | 112 (80.0%) | 140 (100.0%) | | |

 $\chi^2\colon$ Chi-square test, *p<0.05.

(AH, adenomatous hyperplasia; PCa, prostate adenocarcinoma).

The distribution of the PCDH8 staining results (negative and positive) in AH and PCa cases is given in Table 2 (Fig. 3).

Table 2. Frequency distributions of patients according toPCDH8 staining results.

| | Staining | | Total | n | |
|-------|------------|-------------|--------------|---------------------|--|
| | Negative | Positive | 10111 | P | |
| AH | 7 (10.0%) | 63 (90.0%) | 70 (100.0%) | 0.217 | |
| PCa | 12 (17.1%) | 58 (82.9%) | 70 (100.0%) | $(\chi^2 = 10.522)$ | |
| Total | 19 (13.6%) | 121 (86.4%) | 140 (100.0%) | | |

 χ^2 : Chi-square test.

(AH, adenomatous hyperplasia; PCa, prostate adenocarcinoma).



Fig. 2. Immunohistochemically stained with WWOX. (A) Adenomatous hyperplasia. Moderate cytoplasmic staining is observed (WWOX, \times 400). (B) Prostate adenocarcinoma. Mild cytoplasmic staining is seen (WWOX, \times 400).



Fig. 3. Immunohistochemically stained with PCDH8. (A) Adenomatous hyperplasia. Moderate cytoplasmic, membranous and nuclear staining is observed (PCDH8, ×400). (B) Prostate adenocarcinoma. Moderate cytoplasmic, membranous and nuclear staining is observed (PCDH8, ×400).

According to Table 2, there wasn't significant difference in PCDH8 expression between AH and PCa cases by the chi-square test (p = 0.217).

Binary logistic regression analysis showed that PCDH8 staining was not a diagnostic parameter in PCa (Table 3).

4. Discussion

Similar to studies reported in the literature, WWOX expression was higher in AH than PCa in the present study. PCDH8 was found to be an ineffective diagnostic parameter. Lzycka *et al.* [20] reported a significant decrease in PCDH9 expression during progression to the advanced or metastatic stage in prostatic cancer. He *et al.* [21] identified PCDH17 as a tumor suppressor of nasopharyngeal carcinoma and suggested that its methylation can be used as an epigenetic biomarker. PCDH10 has been shown to be inactivated by promoter methylation in various types of cancer [non-small cell lung cancer [22], gastric cancer [23], colorectal cancer [24], nasopharyngeal cancer,

esophageal cancer [25], endometrioid endometrial cancer [26], and bladder cancer [27]. Furthermore, the methylation of PCDH10 is believed to be associated with poor prognosis in patients with gastric cancer [28]. Methylation of PCDH8 was found to be significantly associated with high Gleason score, advanced pathological stage, and positive lymph node metastasis [12].

Researchers have reported that PCDH8 can also be considered a risk factor for the progression of bladder cancer. In addition, PCDH8 methylation may be a useful prognostic biomarker in bladder carcinomas without muscle invasion. PCDH8 is frequently inactivated by promoter methylation in bladder carcinoma, renal cell carcinoma, nasopharyngeal carcinoma, stomach carcinoma and breast carcinoma, and this inactivation is associated with poor prognosis [6,29,30]. PCDH8 is a helpful prognostic biomarker and may also serve as a potential therapeutic target in patients with hypopharyngeal carcinoma [31]. However, its clinical significance in prostate cancer is still unclear [32]. In another study, researchers reported that

Table 3. Logistic regression analysis of predictive factor for predicting PCa.

| | | В | SE | Wald | р | OR (95% CI) | | |
|--|---------------------|-------|-------|-------|--------|---------------------|--|--|
| WWOX | Staining (Positive) | 0.926 | 0.447 | 4.295 | 0.038* | 2.525 (1.052-6.063) | | |
| PCDH8 | Staining (Positive) | 0.622 | 0.509 | 1.491 | 0.222 | 1.862 (0.686–5.052) | | |
| OP Odds Patia $*n < 0.05$ Pafaranaa astagary: Nagatiya | | | | | | | | |

OR, Odds Ratio. *p < 0.05, Reference category: Negative.

(PCa, prostate adenocarcinoma).

PCDH8 methylation in PCa may be an important marker for use in the early diagnosis and prediction of prognosis in PCa [33]. Niu *et al.* [12] found that methylation of protocadherin 8 often occurs in PCa tissues but not in normal prostate tissues. Unlike other studies, PCDH8 expression was found to be an ineffective prognostic parameter in our study.

The WWOX gene is mapped to chromosomal region 16q23.324.1. Changes in WWOX expression are associated with very different cancers such as breast [34], ovary [35], prostate [19], stomach [36], and hepatocellular carcinoma [37]. The WWOX gene is known to play a role in breast cancer. Increased WWOX expression inhibits cell proliferation and reduces tumor growth rates in xenografts [38]. A reduction in WWOX protein expression can be detected early during cancer development [16,17]. Low expression alleles of WWOX were observed in patients with lung cancer [39] and glioma [40]. Also, acquisition of chemoresistance in squamous cell carcinoma, breast cancer, and osteosarcoma cells was found to be associated with WWOX deficiency [41]. Hughes et al. [42] reported reduced WWOX mRNA and protein expression in PCa cells and tissues. Again, Wen et al. [43] found that WWOX can inhibit invasion and angiogenesis in osteosarcoma. Researchers noted that WWOX expression differs between cell lines with varying degrees of tumorigenicity and metastasis [44]. In addition, Fabbri et al. [45] observed that WWOX-deficient mice developed osteosarcoma, an aggressive bone tumor with a poor prognosis. Another study found decreased expression of WWOX in pancreatic intraductal mucinous neoplasm [46]. A decrease in WWOX gene expression was observed in 35% of oral leukoplakias [47]. It is known that WWOX expression is reduced in different types of cancer [36], and this protein has also been shown to participate in various cellular events, including cancer cell apoptosis and tumor suppression [17].

In our study, WWOX expression was higher in AH than in PCa, suggesting that it may be a useful parameter in this cancer. Similar to the results of this study, Hong *et al.* [48] reported that WWOX suppresses tumor formation by inducing apoptosis in prostate cancer and breast cancer. In addition to prostate cancer cells, C1q/WOX1 has also been shown to induce the death of breast and neuroblastoma cells [49].

5. Conclusions

In this study, the expression of WWOX decreased and the expression of PCDH8 remained unchanged in PCa cases. In terms of diagnostic significance, it can be concluded that WWOX is a useful parameter, while PCDH8 was an ineffective diagnostic marker. To fully clarify this issue, comparison of PCDH8 with other useful parameters in larger series may provide more meaningful results.

Author contributions

MAÇ—Creating a Hypothesis, Article writing, Material supply (biological, technical), Data collecting; HE— Planning and organization, Creating a Hypothesis; SÇ, YKA—Data analysis, Statistics. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was by the Ordu University Clinical Research Ethics Committee (2015/10).

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Conflict of interest

The authors declare no conflict of interest.

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