Clinicopathological features and significance of CD26 expression in papillary thyroid carcinoma

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Abstract

Purpose

This study aimed to evaluate the potential significance of CD26 expression in papillary thyroid cancer (PTC) tissues and to investigate their relationship with classical clinicopathological characteristics and prognosis.

Methods

Immunohistochemistry (IHC) staining was used to explore the expression pattern of CD26 in PTC tissues and corresponding adjacent tissues in 86 patients. In addition, we searched GEPIA database to estimate the expression difference of CD26 mRNA in thyroid cancer and normal thyroid tissues, and download the expression bar graph of mRNA and Kaplan Meier curve of CD26 in PTC compared to normal thyroid tissues.

Results

We found that 89.53% (77/86) of PTC overexpressed CD26, on the contrary, we observed that CD26 was not expressed in normal thyroid tissues adjacent to the tumor. The expression of CD26 was strongly associated with lymph node metastasis (2 = 7.59, P = 0.006), tumor size (2 = 7.59, P = 0.006) and patients' age (2 = 3.95, P = 0.047), but there had no association between CD26 expression and patients' gender, capsular invasion (P > 0.05). Moreover, CD26 mRNA level was extremely low in normal thyroid tissues and significantly increased in thyroid cancer tissues (P < 0.01). Survival analysis presented that the patients with higher CD26 mRNA expression owned lower disease-free survival (DFS) and higher recurrence risk (HR = 1.8, P = 0.048).

Conclusion

Our data demonstrated that CD26 might be promising biomarkers and therapeutic target for PTC.

Introduction

Thyroid cancer is one of the most common malignant tumors in the endocrine system. Its prognosis is good, but recurrence and metastasis are very easy to occur. Its incidence is on the rise, and it may be projected to become the fourth leading type of cancer across the globe. From 1990 to 2013, the global age-standardized incidence rate of thyroid cancer increased by 20%[1]. The statistical data of thyroid cancer from Chinese Cancer Registry Annual Report presented that the average annual percent change (AAPC) of incidence rate was 12.4% and AAPC of age-standardized mortality was 2.9% in the period...
2005–2015[2]. Papillary thyroid carcinoma (PTC) is the most common thyroid cancer, accounting for 80% of all cases, and the prognosis of PTC is good, with the 5-year survival rate being more than 97%[3]. PTC has a relatively indolent clinical course despite a high incidence of lymph node (LN) metastasis[4], but LN metastasis to the lateral cervical compartment is associated with shorter disease-free survival[5]. Furthermore, extranodal extension (ENE) of metastatic LNs is recognized as an important prognosticator of increased nodal persistence, distant metastasis, and cancer-related death. Hence, ENE of metastatic LNs has been considered as a critical prognostic indicator of recurrence and survival in PTC[6]. Therefore, it is particularly important to explore effective biomarkers for the diagnosis and metastasis risk assessment of thyroid cancer.

Dipeptidyl peptidase 4 (DPP4) is expressed as both a type II cell surface protein (CD26) and as a soluble protein lacking intracellular and transmembrane domains[7], playing numerous biological functions including glucose metabolism, immunomodulation, and tumorigenesis[8]. While CD26 expression in normal tissues is rather ubiquitous, in neoplasms, CD26 is aberrantly expressed, which is involved in tumorigenesis and may serve as a tumor suppressor or activator, depending on its tumor microenvironment[9]. Abnormal expression and glycosylation of CD26 were found in a variety of malignant tumors such as thyroid cancer[10], esophageal neoplasms[11], malignant mesothelioma[12], pancreatic cancer[13] and colorectal cancer[14]. On the contrary, loss or alteration of membrane CD26 expression has been described in breast cancer[15], endometrial endometrioid adenocarcinoma[16] and prostate cancer[17]. Hence, CD26 has been frequently studied as a tumor biomarker and therapeutic target. In this study, we focused on exploring the expression and clinicopathological features of CD26 in PTC. The results showed that CD26 expression was closely correlated with lymph node metastasis ($\chi^2=7.59, P=0.006$), tumor size ($\chi^2=7.59, P=0.006$) and patients' age ($\chi^2=3.95, P=0.047$), on the contrary, there had no correlation between CD26 expression and patients' gender, capsular invasion ($P>0.05$).

**Materials And Methods**

**Clinical data**

In total, we have analyzed 86 samples from 80 papillary thyroid carcinoma (PTC) patients (2020 to 2021) from Department of Pathology of Sinopharm Dongfeng General Hospital of Hubei University of Medicine by immunohistochemistry to assess the expression significance of CD26. As controls, we have included 86 adjacent normal thyroid tissues in this study. All patients with complete case data underwent thyroid surgery for the first time and were diagnosed as PTC by pathological examination. All the specimens were fixed in 10% neutral formalin. Conventional dehydrated and paraffin embedded, sliced 4µm thick and adhered to slides, and then Immunohistochemistry (IHC) experiments were conducted. This study was approved by the medical ethics committee of the Sinopharm Dongfeng General Hospital of Hubei University of Medicine, and all the patients signed informed consent.
512 cases of thyroid cancer and 337 normal thyroid tissues were collected through GEPIA database (http://gepia.cancer-pku.cn) to compare the expression difference of DPP4 mRNA in thyroid cancer and normal thyroid tissues and analyze the relationship between the expression level and the prognosis of patients with thyroid cancer. In addition, the expression bar graph of mRNA and Kaplan Meier curve of CD26 in PTC compared to normal thyroid tissues were downloaded from GEPIA database.

**Immunohistochemistry**

Immunohistochemical (IHC) staining procedure was performed as previously described. Serial sections of 4µm were cut from paraffin blocks and deparaffinized in xylene and hydrated in a graded series of alcohol. After hot repaired by citrate, endogenous peroxidase was suppressed by incubation with 3% \( \text{H}_2\text{O}_2 \). Tissue sections were incubated overnight in CD26 (D6D8K) rabbit antibody (1:300, Cell Signaling, #40134) at 4°C and incubated with an anti-rabbit secondary antibody (1:1000, HRP, Cell Signaling, #8114) for 30 min at 37°C on the following day. Finally, the tissue sections were washed with buffer and then incubated with Signalstein® DAB for color rendering. CD26 was positively located in cell membrane and cytoplasm, and the color was light yellow to tan. Immunohistochemical results were interpreted by two pathologists who randomly selected five high-power lenses (400×) containing at least 100 cells under double-blind conditions. The percentage of positive cells and staining intensity were observed as follows: (1) Staining intensity: 0 point if there is no positive color or the cell color cannot be distinguished from the surrounding stroma; Light yellow is 1 point; Yellow or brownish yellow is 2 points; Tan is 3 points; (2) Percentage of positive cells: 0 point if the number of positive cells is less than 5%; 5% ~ 25% is 1 point; 26% ~ 75% is 2 points; >75% is 3 points. Multiply the above two scores for nal interpretation: 0 points are negative, ≥ 1 points are positive, 1 ~ 3 points are low expression, 4 ~ 6 points are medium expression, and ≥ 7 points are high expression.

**Statistical analyses**

Statistical analyses were performed using Prism (GraphPad Software, Inc., La Jolla, CA). The counting data was expressed in percentage (%), and \( \chi^2 \) test was conducted to compare the expression difference of DPP4 between PTC and adjacent tissues, and then analyze the association between CD26 expression and clinicopathological features of PTC. Kaplan Meier method was used to analyze the relationship between the expression of CD26 and the prognosis of patients. \( P < 0.05 \) was statistically significant.

**Results**

**The expression of CD26 in thyroid papillary carcinoma**

The mRNA expression level of CD26 in 512 thyroid cancer tissues and 337 normal thyroid tissues were collected from GEPIA database. CD26 mRNA level was extremely low in normal thyroid tissues and significantly increased in thyroid cancer tissues. The difference between the two groups was statistically significant (\( P < 0.01 \), Fig. 1).
In order to further verify the expression of CD26 in PTC, the expression of CD26 protein in PTC and adjacent normal thyroid tissues was detected by immunohistochemistry. The results showed that the positive rate of DPP4 in PTC was 89.53% (77/86), DPP4 expression was not found in normal thyroid tissues adjacent to cancer (0/86), and the difference between the two groups was statistically significant ($P<0.0001$, Table 1, Fig. 2).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>CD26</th>
<th>2</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjacent normal thyroid tissues</td>
<td>86</td>
<td>86</td>
<td>0</td>
<td>139.4</td>
</tr>
<tr>
<td>Papillary thyroid carcinoma</td>
<td>86</td>
<td>9</td>
<td>1</td>
<td>12</td>
</tr>
</tbody>
</table>

**Table 1**
The expression level of CD26 in PTC and adjacent normal thyroid tissues

**Association between CD26 expression and clinicopathological features in thyroid papillary carcinoma**

The results of immunohistochemical studies of the association between CD26 expression and clinicopathological features in thyroid papillary carcinoma were summarized in Table 2.
Table 2
The relationship between CD26 expression and clinicopathological features in PTC

<table>
<thead>
<tr>
<th>Clinicopathological parameters</th>
<th>Cases ((n = 86))</th>
<th>CD26</th>
<th>(\chi^2)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>Low expression</td>
<td>Medium expression</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>20</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Female</td>
<td>66</td>
<td>8</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>Age(years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 50</td>
<td>46</td>
<td>2</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>≥ 50</td>
<td>40</td>
<td>7</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>49</td>
<td>9</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Present</td>
<td>37</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Capsular invasion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>41</td>
<td>7</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Yes</td>
<td>45</td>
<td>2</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Tumor diameter(cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1.0</td>
<td>49</td>
<td>9</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>≥ 1.0</td>
<td>37</td>
<td>0</td>
<td>1</td>
<td>6</td>
</tr>
</tbody>
</table>

The results showed that CD26 expression was closely correlated with lymph node metastasis \(\chi^2=7.59, P = 0.006\), tumor size \(\chi^2=7.59, P = 0.006\) and patients' age \(\chi^2=3.95, P = 0.047\). The positive rate of PTC patients with lymph node metastasis, larger tumor diameter or age less than 50 increased significantly. On the contrary, there had no correlation between CD26 expression and patients' gender, capsular invasion \((P > 0.05, Table 2\). These results suggested that lymph node metastasis, larger tumor diameter and smaller age might be the risk factors of thyroid papillary carcinoma.

**Association between CD26 expression and prognosis of thyroid papillary carcinoma patients**

Lymph node metastasis is an important factor in postoperative recurrence of thyroid cancer. Therefore, GEPIA database was used to analyze the relationship between DPP4 expression and disease-free survival (DFS) of patients with thyroid cancer. The results showed that DFS in patients with higher CD26 mRNA expression group was significantly shorter than that in patients with lower expression group (Fig. 3), and
the recurrence risk of thyroid cancer in higher expression group was 1.8 times of that in lower expression group (HR = 1.8, \( P = 0.048 \)).

**Discussion**

At present, thyroid lobectomy is recommended as the primary treatment for thyroid cancer. However, recurrence and hypothyroidism may develop after lobectomy, necessitating thyroid hormone supplementation\[18\]. But, serval factors including the patients' age, education level, fear of taking hormones and concern about the progress of PTC may affect patients' choice of treatment plan for PTC, a survey presented that approximately three-quarters of the participants chose active surveillance (AS) over surgery\[19\]. In addition, macroscopic ENE has a significant adverse impact on recurrence and survival after treatment for PTC, ENE is considered as a high-risk factor for recurrence\[6\]. Although, distant metastases (DM) are a rare occurrence in well-differentiated thyroid carcinoma, and when DM occur in primary thyroid carcinoma with low-risk histology, they are almost always found at presentation\[20\]. Worryingly, the incidence rate of thyroid cancer is ever-growing and the age of patients is gradually younger. The treatment of thyroid cancer is also facing more and more challenges. Consequently, seek effective therapeutic targets to balance the choice between thyroidectomy and AS is particularly important and urgent.

CD26/DPP4 is mainly expressed on cells of various solid organs as well as on most hematopoietic cells, apart from this constitutive expression however, CD26/DPP4 expression is altered in numerous solid tumors\[21\]. CD26/DPP4 can be expressed on the surface of tumor cells and tumor-infiltrating immune cells. In the circulation, it is found as a soluble form (sCD26/DPP4) and on the surface of immune cells\[22\]. The multifunctional roles of CD26 may account for its varied roles in different cancers, previous studies on CD26 have yielded various results in different cancers. CD26 presence has been associated with more aggressive variants in certain cancers through its regulation of metastasis and local invasion, while its absence has also been linked to the development of other cancers due to its ability to regulate cancer progression\[9, 23\]. Preclinical studies showed that increased CD26 expression inhibited metastasis in ovarian cancer\[24\], whereas suppression of CD26 promoted metastasis in prostate cancer\[25, 26\]. On the contrary, inhibition of CD26 in renal cell carcinoma decreased tumor growth and reduced binding of the cancer cells to fibronectin and collagen\[27\]. Moreover, clinical studies in thyroid cancer, gastrointestinal stromal tumor (GIST), and T-cell non-Hodgkin lymphoma/leukemias suggested that CD26 expression was associated with distant metastasis, recurrence after resection, or poor survival\[28–30\]. Our present study investigated the potential association between CD26 expression and the clinicopathological features and prognosis of PTC. Fortunately, we found that the positive rate of PTC patients with lymph node metastasis, larger tumor diameter or age less than 50 was significantly higher, but there had no correlation between CD26 expression and patients' gender, capsular invasion. Furthermore, the patients with lower CD26 mRNA expression had longer DFS.
As a surface glycoprotein with intrinsic dipeptidyl peptidase IV (DPPIV) activity, CD26 has both enzymatic dependent activity and enzymatic independent activity. Thus, DPP4 inhibitors can target both the enzymatic and non-enzymatic functions of DPP4. CD26/DPPIV regulates the activities of a number of cytokines and chemokines, and it also has other non-enzymatic functions that are unrelated to its dipeptidase activity, in which it interacts with different partners and sustains tumor growth, invasion, and metastasis \[31\], such as CD45 \[32\], CXCR4 \[33\], adenosine deaminase \[34\]. Furthermore, researches have reported that CD26 expression was closely linked to cell-cycle regulation, apoptosis, and chemotherapy resistance \[35\]. CD26/DPPIV also increases cell sensitivity to apoptosis in response to topoisomerase II inhibitors, such as doxorubicin and etoposide, in in vitro and in vivo studies \[36–38\].

In conclusion, approximately 90% PTCs expressed CD26. CD26 may considered as promising biomarkers and therapeutic target for PTCs. As a pilot study, the potential mechanism of CD26 in the occurrence and development of cancer is not clear at present time, our data demonstrated that the clinical significances of CD26 on PTCs should be further studied to help exploring the regulatory mechanism of CD26 in PTC.

**Declarations**

**Author contributions**

Lan Li and Zhengpeng Zhu: Conceptualization, Lan Li: writing and editing, Jin Luo; Jingyi Fang: methodology and validation, Rui Zhang; Huanhuan Zhou: sample collection. All authors have read and agreed to the published version of the manuscript.

**Conflict of interest:**

The authors declare that no competing financial interests exist.

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Figures
mRNA expression level of CD26 in PTC and adjacent normal thyroid tissues was collected and analyzed from GEPIA database. A Gene Expression Profile, B Expression on Box Plots.
Figure 2

Immunohistochemical studies on conventional tissue sections of PTC and adjacent normal thyroid tissues. A, CD26 was negatively expressed in adjacent normal thyroid tissues (magnification, 100×). B, CD26 was negatively expressed in some PTC (magnification, 100×). C, CD26 was low expressed in a small part of PTC (magnification, 100×). C, CD26 was moderately expressed in a part of PTC.
E, CD26 was highly expressed in most PTC (magnification, 100×). F, CD26 was highly expressed in most PTC (magnification, 100×).

Figure 3

Relationship between DPP4 mRNA expression and DFS in thyroid carcinoma