Potential diagnostic utility of CD56 and claudin-1 in papillary thyroid carcinoma and solitary follicular thyroid nodules

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Received 30 August 2012; accepted 11 October 2012
Available online 6 November 2012

Abstract  Background and Aim: The pathological diagnosis of papillary thyroid carcinoma (PTC) is usually easily achieved. However distinguishing the follicular variant of papillary carcinoma (FVPC) from other follicular thyroid lesions is an area of controversy. In this study we investigated the role of CD56 and claudin-1 in the discriminating the FVPCs from other solitary follicular patterned nodules. We also evaluated the application of these two markers in reclassifying the controversial cases of the well differentiated tumors of unknown malignant potential (WDTs-UMP).

Materials and methods: The immunohistochemical expression of CD56 and claudin-1 was evaluated in 86 samples of thyroid lesions together with 10 samples of normal thyroid tissue. Thyroid lesions included: 29 PTCs [classic papillary carcinoma (n = 13) and FVPC (n = 16)], 47 solitary follicular patterned nodules [follicular adenomas (n = 12), hyperplastic nodules (n = 32) and follicular tumor of unknown malignant potential (n = 3)] and 10 WDTs-UMP.

Results: The statistical analysis showed significantly different expressions of each of CD56 and claudin-1 in the FVPCs versus other solitary follicular patterned nodules. Claudin-1 sensitivity (100%) was higher than CD56 sensitivity (81.3%). However claudin-1 specificity (80.9%) was < CD56 specificity (89.4%). The combined use of CD56 and claudin-1(Claudin-1+/CD56−) showed
Introduction

Papillary thyroid carcinoma (PTC) is the most common type of thyroid cancer comprising approximately 80% of thyroid epithelial malignancies [1,2]. The “gold standard” in diagnosis of thyroid nodules is pathologic evaluation using routine hematoxylin and eosin (H&E) staining. However, morphologic overlap between follicular lesions especially the follicular variant of papillary carcinoma (FVPC) is common. In such cases an objective consistent diagnosis based merely on morphologic assessment is sometimes impossible [3].

Consequently, immunohistochemical and molecular methods were investigated to aid in the diagnosis of these problematic cases [4–7]. Several immunohistochemical markers such as galectin-3, cytokeratin-19 and HBME-1 have been recommended to help in the discrimination between these controversial thyroid nodules [8]. Nonetheless up till now there is no agreed consensus about an immunohistochemical panel that would reliably overcome such diagnostic obstacles.

Claudin-1, a member of claudin family, is identified as a tight junction component [9]. Tight junctions are transmembrane and cytoplasmic proteins which mainly prevent the free diffusion of solutes and maintain cell polarity [10,11]. Increased claudin-1 expression has been reported in PTC [12,13]. On the other hand, CD56, a neural cell adhesion molecule (NCAM) [14], has been reported to be expressed in normal thyroid follicular cells with frequent low expression in malignant thyroid tumors especially PTC [15–18]. CD56 regulates cell motility and hemophilic binding between neurons, hence its expression may affect the migratory capacity of tumor cells [19].

Claudin-1 and CD56 have been candidates for investigations in several studies for the diagnosis of PTC. However none of these studies, to the best of our knowledge, applied the combination of both claudin-1 and CD56 in an immunohistochemical panel to test the probability of an additive statistical significant outcome.

Therefore, the present study investigated the expression of claudin-1 and CD56 separately and in combination in PTC, solitary thyroid nodules with follicular pattern, as well as normal thyroid tissue. Our aim was to identify the possible diagnostic role of these two markers in the follicular morphological mimics.

Materials and methods

The material of this retrospective study included 86 specimens of surgically removed, formalin-fixed and paraffin embedded thyroid lesions that were received at the pathology department of Ain Shams University Hospital during the period from January 2009 to January 2011. All specimens with residual specificity (100%), positive predictive value (100%) and sensitivity (81.3%) in the differentiation between the FVPCs and other follicular nodules. In the light of this statistical outcome, 5/10 cases of WDTs-UMP expressing the (claudin-1+ / CD56-) panel could be rediagnosed as PTC.

Conclusion: Combined utility of CD56 and claudin-1 is helpful in diagnosing the FVPC and its differentiation from other follicular patterned nodules. Application of these two markers may greatly aid in the reevaluation of the WDTs-UMP and interpretation of their expected behavior.

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Potential diagnostic utility of CD56 and claudin-1 in papillary thyroid carcinoma

No evidence of papillary structures, capsular invasion or vascular invasion. There was no controversy regarding the revised diagnoses among both reviewers.

**Immunohistochemistry**

All 96 samples (86 thyroid lesions and 10 normal thyroid tissues) were subjected to immunohistochemical staining with claudin-1 and CD56 antibodies. The paraffin embedded tissue sections were deparaffinized in xylene and rehydrated through absolute alcohol. Antigen retrieval in citrate buffer (pH 9) was used after the sections were treated in a microwave at 8 W for 5–6 min, then at 3 W for 10 min, the sections were then left to cool for 20 min. Peroxidase and protein block were done. After that the slides were incubated overnight with each of the primary antibodies at room temperature using CD56 antibody (mouse monoclonal antibody 7 ml, Cat# MS-204-R7 Lab vision corporation, CA, USA) and claudin-1 rabbit polyclonal antibody 7 ml, Cat# 359A-18. Cell marque, CA, USA), followed by rinsing in PBS (pH 7.6). This was followed by the secondary biotin conjugated antibody for 1 h and finally the peroxidase conjugated streptavidin for another hour. Diaminobenzidine tetrachloride (DAB) (freshly prepared) was added for 25 min, then counterstained in Harris Hematoxylin, followed by dehydration, clearing and mounting. Positive control for CD56 antibody was neuroblastoma while positive control for claudin-1 was normal skin. Negative controls were done by excluding the primary antibody and its replacement with a non-immune antibody.

**Interpretation of immunohistochemical staining of CD56**

The results of immunohistochemical staining were assessed by both authors and a consensus regarding controversial cases was reached at a multtheaded microscope.

According to Park et al. [22], strong and complete membranous expression with or without cytoplasmic staining of the cells qualified the case as positive for CD56.

Results were expressed in a semiquantitative manner with respect to the percentage of positive tumor cells: 0, staining in <10% of the cells; 1, staining in 10–25% of the cells; 2, staining in 26–50% of the cells; 3, staining in >50% of the cells.

**Interpretation of immunohistochemical staining of claudin-1**

According to Nemeth et al. [23], cases exhibiting membranous claudin-1 staining in >5% of the cells were considered positive. Claudin-1 expression was scored as follows: 0, staining in <5% of the cells; 1, staining in 5–25% of the cells; 2, staining in 26–50% of the cells; 3, staining in >50% of the cells.

**Statistical analysis**

Data were analyzed using the SPSS program Version 15.

Qualitative data were presented using the frequency and related percentage. Comparison of qualitative variables between groups was done using the Chi-square or the Exact test. Sensitivity, specificity, predictive value positive and predictive value negative were calculated for the examined markers.

Percentage of agreement was done using the Kappa statistic (K).

A “P” value of 0.05 was chosen as the level of significance.

The K value can be interpreted as follows (Altman, 1991):

<table>
<thead>
<tr>
<th>Value of K</th>
<th>Strength of agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.20</td>
<td>Poor</td>
</tr>
<tr>
<td>0.21–0.40</td>
<td>Fair</td>
</tr>
<tr>
<td>0.41–0.60</td>
<td>Moderate</td>
</tr>
<tr>
<td>0.61–0.80</td>
<td>Good</td>
</tr>
<tr>
<td>0.81–1.00</td>
<td>Very good</td>
</tr>
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**Literature**


**Results**

**CD56 and claudin-1 expressions in normal thyroid tissue**

CD56 positive expression was found in the 10 samples of normal thyroid tissue (10/10 (100%), which showed strong membranous CD56 expression in >50% of the cells (score3). Meanwhile negative claudin-1 expression was observed in the 10 normal thyroid tissue samples (10/10 (100%)).

**CD56 in the studied thyroid lesions**

Among the solitary follicular patterned thyroid nodules; positive CD56 expression was observed in 42 out of 47 cases (89.4%) (Table 1), which included 11 out of 12 cases of follicular adenomas (91.7%) (Fig. 1a), 28 out of 32 cases of hyperplastic nodules (87.5%) (Fig. 2a) and 3 out of 3 cases of FTs-UMP (100%) (Fig. 3). All of the positive cases displayed strong CD56 expression in >50% of the cells (score 3), while the 5 negative cases showed CD56 expression in <10% of the cells.

On the other hand, assessment of CD56 staining in the 29 PTC cases showed negative CD56 expression in 24 out of the 29 cases (82.8%). These cases included 11 out of 13 cases of classic PTC (84.6%) and 13 out of 16 cases of FVPC (81.3%) (Fig. 4d). Positive CD56 expression was observed in only 5 cases of PTC (17.2%); two of which were classic PTC showing focal expression in 26–50% of the tumor cells (score 2), the other 3 cases were FVPCs and revealed focal expression in 10–25% of the tumor cells (score 1). No statistical significant difference was found between classic PTCs and FVPCs as regards CD56 expression (P = 1).

CD56 distinguished FVPCs from other follicular patterned nodules (follicular adenomas, hyperplastic nodules and FTs-UMP) with a high statistically significant difference (P < 0.001) (Table 1).

**Claudin-1 in the studied thyroid lesions**

In contrast to CD56, the solitary follicular patterned nodules showed negative claudin-1 expression (staining in <5% of the cells) in 38 out of 47 cases (80.9%) (Table 2), which included 9 out of 12 cases of follicular adenomas (75%) (Fig. 1b), 27 out of 32 cases of hyperplastic nodules (84.4%) (Fig. 2b) and 2 out of 3 cases of FTs-UMP (66.7%). Positive claudin-1 expression was observed in only 9 cases (19.1%). Five out of these 9 cases showed claudin-1 positivity in...
26–50% of the cells (score 2); two of which were hyperplastic nodules and 3 were follicular adenomas. Whereas 4 of the claudin-1 positive cases showed focal expression in 5–25% of the cells (score 1) and these comprised 3 cases of hyperplastic nodules and 1 case of FT-UMP.

Meanwhile regarding the 29 PTC cases, strong and diffuse claudin-1 expression in 80–90% of the tumor cells (score 3) was observed in all cases, including 13/13 cases of classic PTC (Fig. 4a) and 16/16 cases of FVPCs (Fig. 4c).
There was a high statistically significant difference between FVPCs and other follicular patterned nodules as regards claudin-1 expression \( (P < 0.001) \) (Table 2).

Results of immunohistochemical expression of (claudin-1+/CD56-/CD56–) panel in the studied thyroid lesions

(Claudin-1+/CD56–) expression panel was expressed only in 13 out of 16 cases (81.3%) of FVPCs. On the other hand, it was absent in all 47 cases of follicular patterned nodules (100%). There was a statistically significant difference between FVPC cases and other solitary follicular patterned thyroid nodules as regards the expression of (claudin-1+/CD56–) panel \( (P < 0.001) \) (Table 3).

Diagnostic validity of CD56, claudin-1 and (claudin-1+/CD56–) panel

CD56 was 81.3% sensitive and 89.4% specific in distinguishing FVPCs from other solitary follicular nodules, in comparison to claudin-1 which was 100% sensitive and 80.9% specific. Although not significant, the sensitivity of claudin-1 was higher than that of CD56, whereas its specificity was lower than that of CD56. The immunohistochemical panel of (claudin-1+/CD56–) distinguished FVPCs with diagnostic accuracy of 95.2% and specificity of 100% which were the highest compared to those yielded by CD56 and claudin-1 individual expression (Table 4).

Figure 3 Positive CD56 expression in follicular tumor of unknown malignant potential (CD56 × 100).

Figure 4 (a) Classic papillary thyroid carcinoma showing strong diffuse membranous claudin-1 immunostaining (claudin-1 × 400). (b) Follicular variant of papillary carcinoma (HE × 400). (c) Follicular variant of papillary carcinoma showing diffuse membranous claudin-1 immunostaining (claudin-1 × 200). (d) Follicular variant of papillary carcinoma showing negative CD56 immunostaining (CD56 × 400).
The results of CD56 and claudin-1 expressions in well differentiated tumors of unknown malignant potential (WDTs-UMP)

In the light of the previous results, ten cases of WDTs-UMP were then subjected to immunostaining using CD56 and claudin-1, where 3 out of 10 cases (30%) showed strong positive CD56 immunoreactivity in 26–50% of the cells (score 2) and the remaining 7 cases (70%) were CD56 negative.

Eight cases of WDTs-UMP (80%) showed strong positive claudin-1 expression in 80–90% of the cells (score 3) while 2 cases (20%) showed negative claudin-1 expression.

The results of combined expression of CD56 and claudin-1 in the 10 cases of WDTs-UMP showed: 2 cases (20%) negative for both claudin-1 and CD56 (claudin-1+/CD56−), 3 cases (30%) positive for both claudin-1 and CD56 (claudin-1+/CD56+) and 5 cases (50%) expressing the (claudin-1+/CD56−) panel (Fig. 5b and c).

Discussion

The diagnosis of PTC is, usually but not always, easily achieved with almost minimal interobserver variability. However, in the absence of papillary architecture, distin-
guishing the FVPCs from cellular adenomatous nodules may be challenging [24]. Therefore this study investigated the possible role of CD56 and claudin-1 in resolving such problem.

CD56 has been reported to be an antigen related to the differentiation of the follicular epithelium [25] and many previous studies reported high CD56 expression in normal thyroid tissue and benign thyroid follicular lesions as follicular adenomas and nodular hyperplasias [3,18,22,26–28].

In accordance with those studies, we currently reported a high positive CD56 expression in normal thyroid tissue compared to PTC cases. The present study also confirmed the strong and diffuse positive CD56 expression in 89.4% of the solitary follicular patterned thyroid nodules (Follicular adenomas, FTs-UMP and hyperplastic nodules).

On the other hand, the current study showed negative CD56 expression in 82.8% of all PTC cases. Similarly, previous studies reported negative CD56 expression in all or most of their studied PTC cases [3,16–18,22,27,28].

The underlying molecular context of CD56 expression in thyroid cancer remains to be elucidated. However it is speculated that CD56 expression might be involved in the activation of epithelial mesenchymal transition (EMT) (which leads to more migratory and invasive cancers), and modulation of genes regulating metastasis as the vascular endothelial growth factor (VEGF) [29–32]. This could explain the maintained elevated CD56 expression in some PTC cases which may acquire later a more aggressive and metastatic phenotype.

Based on the previously mentioned results and in the light of our finding that there was no statistically significant difference between CD56 expression in FVPCs and its expression in classic PTCs ($P > 0.05$), we investigated the applicability of using CD56 as a marker to differentiate between FVPCs and other solitary follicular nodules. As a result a statistically significant difference between these two groups as regards CD56 expression was found ($P < 0.001$). Therefore we were able to emphasize that lack of CD56 expression in the FVPCs was very helpful in their discrimination from other follicular nodules. The sensitivity and specificity of CD56 as a negative marker of FVPCs was 81.3% and 89.4% respectively and its diagnostic accuracy was 87.3%. On the other hand, Etem el al. [33] found no statistically significant difference between his studied group of FVPCs and the other group of follicular tumors (FTs-UMP, follicular adenomas and follicular carcinomas) as regards CD56 expression.

In addition to CD56, claudin-1 immunohistochemistry was previously evaluated in various thyroid lesions [34].

The role of caludin-1 as a tight junction protein in cancer initiation and progression has been intensively investigated.
Reduced expression, elevated levels or subcellular relocalization of tight junction proteins have been reported in various human malignancies and are variably associated with tumor differentiation and survival [35–41]. The overexpression of these proteins in cancers (which typically lose their tight junctions) is unexpected but may be related to roles that are unrelated to tight junction formation [42].

The upstream signaling pathways influencing claudin-1 expression in thyroid tumors remain elusive. Tzlepi et al. [34] demonstrated that papillary carcinoma being a well differentiated thyroid carcinoma shows high claudin-1 expression, and that dedifferentiation of thyroid tumors involves tight junction impairment via claudin-1 down regulation. In addition, loss of tight junction integrity leads to an increased influx of growth factors, nutrients and other tumor promoting molecules, therefore providing an advantage for tumor development and progression. On the other hand, Hucz et al [12] and Nemeth et al. [23] associated claudin-1 expression with the invasive and metastatic phenotype of PTC due to preserved claudin-1 strong expression in the lymph node metastasis.

In the current study all cases of PTCs (100%) showed strong and diffuse claudin-1 expression. While all normal thyroid tissue samples (100%) and 80.9% of the solitary follicular patterned nodules showed negative claudin-1 expression. Similar results were elucidated by Hucz et al. [12] and Nemeth et al. [23] who reported high claudin-1 expression in PTC cases compared to negative expression in normal thyroid tissue and in follicular adenomas. This differential expression in the normal versus neoplastic tissues may provide new opportunities for targeted cancer therapy [42]. In addition, our results showed a high statistically significant difference between FVPCs and other solitary follicular patterned nodules as regards claudin-1 expression ($P < 0.001$). Thus claudin-1 immunohistochemistry is proved to be very useful in differentiating FVPCs from other follicular nodules with 100% sensitivity and 80.9% specificity, 100% NPV and 85.7% diagnostic accuracy. Although the specificity of claudin-1 is lowered compared to that of CD56, claudin-1 negative expression can rule out the possibility of papillary carcinoma in any suspicious follicular nodule.

Our recent observations encouraged us to assess the possible value of the (claudin-1+/CD56−) panel in the differential diagnosis of the studied thyroid nodules with a better statistical significant outcome. As a result the sensitivity and NPV of (claudin-1+/CD56−) combination were lowered compared to those of claudin-1 alone. However this panel was able to discriminate FVPCs among other follicular patterned nodules with diagnostic accuracy (95.2%), specificity (100%), and PPV (100%), which were the highest compared to those of CD56− and claudin-1+. Therefore adding CD56 to claudin-1 especially in claudin-1 positive cases could greatly aid in reaching the final diagnosis.

Based on the previous results of CD56 and claudin-1 immunoreactivities in our studied cases, this work proposed the following protocol in the evaluation of the follicular thyroid nodules:

(a) Diagnosis of papillary carcinoma (FVPC) in any controversial case should be definitely excluded if it shows negative claudin-1 expression (claudin-1 sensitivity is 100% and its NPV is 100%).

(b) Diagnosis of papillary carcinoma (FVPC) is definite in any controversial case if it shows (claudin-1+/CD56−) immunoreactivity (this panel has 100% specificity and 100% PPV).

(c) The diagnosis of PTC (FVPC) is excluded by 94% in any controversial case if it shows (claudin-1+/CD56+) panel [NPV of (claudin-1+/CD56−) is 94%].

Williams et al. [21] have proposed the term WDT-UMP for an encapsulated tumor composed of well differentiated follicular cells with questionable PTC type nuclear changes, whether capsular invasion is absent or questionable and in absence of vascular invasion. The problem with this term is that it doesn’t really solve the interobserver variability between pathologists regarding the diagnosis of FVPC. Instead it allows safe expression of differences in opinion and help in avoiding excessive treatment in controversial cases that cannot be readily placed in a definite benign or a definite malignant category.

Therefore the application of the above mentioned protocol on the atypical category of WDTs-UMP might be helpful in the reclassification and interpretation of the biologic behavior of these tumors.

Accordingly, by evaluating the claudin-1 and CD56 immunoreactivities in the 10 studied cases of WDTs-UMP, the diagnosis of papillary carcinoma was likely to be excluded in the two cases which showed negative claudin-1 expression. Moreover, the diagnosis of papillary carcinoma would probably be excluded by 94% in the 3 cases which showed (claudin-1+/CD56+) panel. Hence we could suggest that the focal and incompletely developed PTC nuclear changes observed in these cases may be due to a mere artifact of fixation. On the other hand, the diagnosis of papillary carcinoma could be suggested in the 5 cases which showed (claudin-1+/CD56−) panel.

The more apparent nuclear pseudoinclusions and grooves seen in these 5 cases may prove to be the expression of a pathological abnormality rather than a technical error. In this situation the nuclear features argue in favor of rediagnosis of the (claudin-1+/CD56−) cases as PTC or at least suggest a link between WDT-UMP and PTC as the former may possibly be a precursor of the latter.

In conclusion, although the differential diagnoses of thyroid follicular nodules are based on histologic and cytomorphologic criteria, combined utility CD56 and caludin-1(caludin1+/CD56−) might be useful in the diagnosis of PTC. However a question is raised “Does (claudin-1+/CD56+) expression panel has a practical diagnostic role in the sense of absolute pushing of the WDT-UMP cases into the benign category?” Our study demonstrated that this could be achieved by 94%. However the satisfactory answer to this question will require long term follow up in order to see whether the biologic effects of malignancy will influence these cases or not. Therefore the current study recommends the evaluation of the role of claudin-1 and CD56 panel in more extensive studies including other variants of follicular cell derived nodules especially the borderline category of WDT-UMP to evaluate their expected behavior.

Competing interests

The authors declare that they have no competing interests.
Authors' contributions

RMA conceived, designed and coordinated the study, reviewed the histologic diagnosis, evaluated immunohistochemistry, carried out photographing and drafted the manuscript. LSS performed data collection, reviewed the histological diagnosis, evaluated immunohistochemistry, helped in the study design, helped to draft the manuscript and critically reviewed the manuscript. Both authors read and approved the final manuscript.

References


