CD56 Expression in Papillary Thyroid Carcinoma is Highly Dependent on the Histologic Subtype: A Potential Diagnostic Pitfall

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Research

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Abstract

Background

Loss of CD56 expression has been regarded as a diagnostic marker of papillary thyroid carcinoma (PTC). However, certain variants of PTC can aberrantly express CD56.

Methods

Using a digital-image analysis tool, we evaluated H-scores of CD56 immunostaining in 216 thyroid tumors.

Results

The H-score of the CD56 of all PTCs was lower than that of noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) ($p < 0.001$). The H-scores of CD56 were lower in classic PTC, the infiltrative follicular variant, and the diffuse sclerosing variant than in other PTC variants ($p < 0.001$), whereas the H-scores were higher in tall cell variant, Warthin-like variant, and cribriform-morular variant than in classic PTC ($p < 0.001$). The optimal cut-off value of H-scores for the CD56 expression was 180 for differentiating the NIFTP from the follicular adenoma and 30 for the differential diagnosis of NIFTP and infiltrative follicular variant PTC.

Conclusions

CD56 expression is predominantly lost in classic and infiltrative follicular variants of PTCs and more preserved in the other histologic subtypes of PTC and NIFTP. CD56 is particularly useful for differentiating PTC from follicular-pattern thyroid neoplasms, but the aberrant expression in uncommon variants of PTC could be a diagnostic pitfall.

Background

Papillary thyroid carcinoma (PTC) is the most common thyroid cancer and has more than ten histologic variants with distinct architectural and cytologic features [1]. Classic PTC comprises more than 50% of PTC, and the follicular variant is the second most common subtype. Most noninvasive encapsulated follicular variants of PTC are now reclassified as noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP), which is considered to be a borderline tumor instead of cancer [2, 3]. NIFTP has the molecular profile of RAS and other RAS-like mutations, as is similar to those of follicular adenoma, follicular carcinoma, and the follicular variant of PTC [2]. Although the criteria for the histologic diagnosis of the follicular-patterned tumors are well defined and produce good consensus, the same tumor may receive different diagnoses, mainly because of intraobserver and interobserver differences in

applying the histologic criteria in daily pathology practice [4–6]. Because of difficulty in distinguishing the follicular variant of PTC from NIFTP and follicular neoplasms, immunohistochemical markers can be useful in diagnosing difficult cases.

CD56 is a neural cell adhesion molecule and is characteristically expressed in normal thyroid follicular cells and benign thyroid nodules but not in PTC [7–10]. That CD56 works well as a diagnostic marker for PTC against benign thyroid lesions has been shown in several previous studies [11–13]. However, little is known about the CD56 expression in NIFTP and the uncommon variants of PTC, such as the tall cell variant. A few studies have shown that follicular variant PTC (FVPTC) has reduced expression of CD56 expression compared to benign thyroid nodules, suggesting the utility of CD56 immunostaining for the differential diagnosis [14]. Although most authors agree that CD56 expression is reduced in the process of thyroid carcinogenesis [14–17], there is still a lack of data about the CD56 expression in different variants of PTC other than classic PTC or about the utility of CD56 as a diagnostic marker in rare PTC variants [13].

To find the diagnostically relevant cut-off values of CD56 expression, we investigated the CD56 expression levels in various thyroid tumors, focusing on uncommon variants of PTC and NIFTP, by employing a computer-aided image-analysis system.

Materials And Method

Patients

We selected 174 PTCs, 22 NIFTPs, and 20 follicular adenomas from the database of the Department of Pathology, Seoul St. Mary's Hospital, diagnosed from January 2012 to December 2015. The institutional review board approved this study. All the patients underwent total thyroidectomy or lobectomy and were followed up at the same hospital. The histological review was conducted by an endocrine pathologist (Chan Kwon Jung), and diagnoses were reassessed according to the 4th World Health Organization classification of thyroid malignancy [3]. Classic PTC with tall cell features was defined as a PTC harboring 10–30% tall cells [18]. Tall cell variant PTC was defined as tumors that harbor more than 30% tall cells [3, 18]. Tumors were staged according to the 7th American Joint Commission on Cancer (AJCC) [19]. Clinical and pathologic characteristics were collected from the hospital medical records.

Immunohistochemistry and evaluation of immunohistochemical staining

We did immunohistochemical staining for CD56 using the automated Ventana Benchmark XT platform (Ventana Medical Systems, Tucson, Arizona, USA). A representative formalin-fixed paraffin-embedded block from each case was cut into four- μ m-thick tissue sections. Antigen retrieval was done for 48 min using the Ventana Benchmark CC1 standard program. Tissue sections were incubated with primary mouse anti-CD56 monoclonal antibody (clone CD564, 1:200 dilution, Dako, Glostrup, Denmark) for 16 min at 37 °C. Immunoreaction was visualized using Ventana OptiView DAB Immunohistochemical

Detection Kit (OptiView HQ Linker for 8 min and OptiView HRP Multimer for 8 min) and OptiView Amplification Kit. We did counterstaining using Ventana Hematoxylin II for 4 min, followed by bluing reagent (Ventana) for 4 min. Normal follicular cells were used as an internal positive control.

CD56 immunostaining score was calculated as the product of intensity and percentage of membranous positivity using a computer-aided image-analysis system, GenASIs HiPath (Applied Spectral Imaging, Carlsbad, California, USA). We captured images at $\times 20$ magnification in the area of highest immunostaining and manually annotated the tumor areas for inclusion in the image analysis. More than 2000 cells per case were analyzed. The image-analysis system quantified the intensity (0, no staining; 1, weak staining; 2, moderate staining; 3, strong staining) and the percentage of membranous positivity. The formula obtained Histoscore (H-score) is: $H\text{-score} = (1 \times \% \text{ of weakly staining cells}) + (2 \times \% \text{ of moderately staining cells}) + (3 \times \% \text{ of strongly staining cells})$, giving a range of 0 to 300.

Molecular analysis of BRAF gene

We did molecular analysis of the *BRAF* gene as previously described [20]. Briefly, genomic DNA was extracted from the tumor tissue sections using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) and subsequently amplified via PCR using specific primers (forward 5'-TCATAATGCTTGCTCTGATAGGA-3' and reverse 5'-GGCCAAAAATTTAATCAGTGGA-3') producing a 224-bp product. Sanger sequencing of PCR amplicons was done using the same PCR primers.

The Cancer Genome Atlas (TCGA) data analysis

We obtained RNAseq-based, gene-level normalized RSEM (RNA-Seq by Expectation Maximization) scores for PTC from the Genomic Data Commons of the National Cancer Institute (<https://gdc.cancer.gov>). The clinicopathological data for each TCGA samples were obtained from the article by the TCGA research network [21].

Statistical Analysis

Clinical and pathologic characteristics of the patients were compared using the chi-square test or Fisher's exact test where appropriate. The Mann-Whitney U test was used to compare the median H-scores of CD56 expression. All the p values are two-sided, and those less than 0.05 were considered to indicate statistical significance. The ROUT method (robust regression followed by outlier identification) setting Q to 1% was used to identify outliers from nonlinear regression [22]. We did all the analyses using GraphPad Prism (version 6.05, GraphPad Software, La Jolla, CA, USA)

Results

Expression pattern of CD56 in different thyroid tumors

Normal thyroid follicular cells adjacent to tumors showed strong membranous expression of CD56 (Fig. 1a). Most tumor cells in all follicular adenomas showed diffuse strong membranous reactivity for

CD56 (Fig. 1b). In NIFTPs and PTCs, the expression levels of CD56 ranged from totally absent (H-score 0) to diffuse strong positive (H-score 288.0) (Fig. 1c-h, Fig. 2). The mean H-score of the whole series was 28.9 ± 52.7 , and the median H-score was 0.

The H-scores for CD56 expression in all PTCs were significantly lower than those in NIFTP (median 0 vs. 76.5, $p < 0.001$). The H-scores of classic, infiltrative follicular, and diffuse sclerosing variants were significantly lower than those of the tall cell variant, classic PTC with tall cell features, Warthin-like variant, and cribriform-morular variant (Fig. 3). NIFTP showed a significantly higher level of CD56 expression than did all variants of PTCs, excluding Warthin-like and cribriform-morular variants ($p < 0.001$, Fig. 3).

Selecting the optimal cut-off H-scores of CD56 positivity for diagnostic purposes

Since the main differential diagnoses of follicular-patterned tumors of the thyroid gland include follicular adenoma, NIFTP, and follicular variant of PTC in daily pathology practice, we used the receiver operating characteristic (ROC) curve analysis to select the cut-off scores for CD56 expression to differentiate NIFTP from follicular adenoma and follicular variant of PTC. The areas under the ROC (AUC) were 0.866, 0.935, and 0.885 for the diagnosis of NIFTP vs. follicular adenoma, NIFTP vs. follicular variant of PTC, and NIFTP vs. all types of PTC, respectively (Fig. 4). The H-score cut-off values of 180 and 30 had the best diagnostic performance for the differential diagnosis of NIFTP from follicular adenoma and PTC, respectively (Fig. 4).

Clinicopathologic significance of CD56 expression in PTC

The H-scores of CD56 expression were grouped into low (< 30) and high (≥ 30) levels by the ROC analysis. The expression level of CD56 was associated with the histologic variant, but not with other clinicopathologic characteristics (Table 1).

Table 1
Correlation of CD56 expression with clinicopathologic features in 174 patients with papillary thyroid carcinoma.

Characteristics	H-score			Pvalue
	n	Low (< 30)	High (≥ 30)	
Histologic variant				
Classic	61	59 (96.7%)	2 (3.3%)	< 0.001
Infiltrative follicular	29	26 (89.7%)	3 (10.3%)	
Diffuse sclerosing	3	3 (100.0%)	0	
Tall cell	30	27 (90.0%)	3 (10.0%)	
Classic with tall cell feature	30	23 (76.7%)	7 (23.3%)	
Warthin-like	18	5 (27.8%)	13 (72.2%)	
Cribriform-morular	3	0	3 (100.0%)	
Age				
< 55	127	107 (84.3%)	20 (15.7%)	0.241
≥ 55	47	36 (76.6%)	11 (23.4%)	
Sex				
Female	142	117 (82.4%)	25 (17.6%)	0.879
Male	32	26 (81.3%)	6 (18.8%)	
Pathologic (p) T category				
pT 1–2	89	73 (82.0%)	16 (18.0%)	0.955
pT 3–4	85	70 (82.4%)	15 (17.6%)	
Extrathyroidal extension				
Absent	90	74 (82.2%)	16 (17.8%)	0.989
Present	84	69 (82.1%)	15 (17.9%)	
Gross extrathyroidal extension				
Absent	164	134 (81.7%)	30 (18.3%)	0.999
Abbreviation: PTC, papillary thyroid carcinoma; AJCC, American Joint Committee on Cancer				
^a Seven cases were not evaluated for lymph node metastasis.				
^b Four cases were not evaluated for <i>BRAF</i> mutation.				

Characteristics	H-score			P value
	n	Low (< 30)	High (≥ 30)	
Present	10	9 (90.0%)	1 (10.0%)	0.812
pN category ^a				
pN0	88	72 (81.8%)	16 (18.2%)	
pN1	79	64 (81.0%)	15 (19.0%)	
AJCC stage, 7th edition				
I, II	115	94 (81.7%)	21 (18.3%)	0.830
III, IV	59	49 (83.1%)	10 (16.9%)	
<i>BRAF</i> V600E mutation ^b				0.306
Absent	35	27 (77.1%)	8 (22.9%)	
Present	135	114 (84.4%)	21 (15.6%)	
Abbreviation: PTC, papillary thyroid carcinoma; AJCC, American Joint Committee on Cancer				
^a Seven cases were not evaluated for lymph node metastasis.				
^b Four cases were not evaluated for <i>BRAF</i> mutation.				

Interesting outliers in CD56 expression levels

All outlier cases were reviewed. Although most of the follicular adenomas had a high H-score of CD56, there were three outliers (H-score 0, 5, and 10) in the lower boundary of a follicular adenoma case (Fig. 1). The tumors revealed no vascular or capsular invasion and had no typical nuclear features of PTC (Fig. 5). In 174 PTCs, outliers of H-score in the upper boundary were found in 11 (18%, range 2.5–38.2) of 61 classic variants, six (21%, range 9.5–90.2) of 29 infiltrative follicular variants, four (13%, range 79–199.4) of 30 classic variants with tall cell features, and four (13%, range 28.4–136.2) of 30 tall cell variants (Fig. 1). These outliers had similar histologic features with their corresponding variants of PTC that had a typical CD56 expression pattern.

CD56 (NCAM1) mRNA expression in PTC

When the expression levels of *NCAM1* mRNA from TCGA dataset were correlated with histologic variants of PTC, *NCAM1* mRNA expression levels were the highest in the follicular variant ($p < 0.001$), followed by tall cell and classic variants (Fig. 6). *NCAM1* mRNA expression levels were significantly higher in normal thyroid tissue than in all types of PTC ($p < 0.001$). Although the TCGA study did not subcategorize the

follicular variants of PTC into NIFTP, encapsulated follicular variant, and infiltrative follicular variant, these findings suggest that NIFTP was included in the follicular variant.

Discussion

In the current study, we observed that CD56 expression level was useful to differentiate PTC from FA and NIFTP using different H-score cut-off values, but the CD56 expression levels were significantly different depending on the histologic types of PTC. Most previous studies reported loss of CD56 expression in PTCs without further specifying their histologic variants [9–17, 23, 24]. In our study, classic, infiltrative follicular, and diffuse sclerosing variants of PTC showed a considerably reduced CD56 expression. However, tall cell variant PTC, classic PTC with tall cell features, Warthin-like PTC, and cribriform-morular PTC had significantly higher H-scores of CD56 expression than did classic PTC.

CD56 *NCAM* mRNA expression in TCGA data of PTC supports the differential expression of CD56 according to the histologic types. The reason for CD56 expression being different for each PTC variant has not yet been clarified. Many previous studies have focused on the usefulness of CD56 in differentiating between malignant, especially PTC, and benign thyroid tumors [9, 12]. Only a few studies have analyzed CD56 expression by specific PTC variants. Scarpino et al. [8] reported relatively high rates of CD56 expression in various PTC variants: 63% (12/19)% of the classic variant, 90% (19/21) of the follicular variant, 20% (1/5) of the diffuse sclerosing variant, 60% (3/5) of the encapsulated variant, 67% (4/6) of the oncocytic variant, and 80% (4/5) of the tall cell variant. Although the level of CD56 expression is lower in PTC than in adjacent normal tissue, the positive expression of CD56 may be more frequently found in the tall cell variant than in the classic variant. Since the studied cases are too few, conclusions based on these data could be a hasty generalization. Likewise, there are very limited subgroup analysis data on the CD56 in the other uncommon variants of PTC [13]. However, CD56 expression differs from subtype to subtype of PTC, as our data show, and more data on this subject will be needed.

CD56 regulates cell motility and affects the migration capacity of the carcinoma cells. Reduced expression of CD56 in tumor cells has been implicated for aggressive tumor behavior or poor prognosis in some other cancer types, such as colorectal cancer, cholangiocarcinoma, and pancreatic cancer [25–28]. In a transgenic mouse model, the loss of CD56 function increased lymph-node metastasis in pancreatic β cell tumors and also increased lymphangiogenesis [25, 26]. An in vitro study of the PTC cell line demonstrated that silenced CD56 is related to the increased adhesive capacity of PTC cells to the extracellular matrix, but the migratory capacity of the tumor cells was decreased [8]. These results enabled us to hypothesize that aggressive subtypes of the PTC, e.g., tall cell variant PTC and classic PTC with tall cell features [18], would have a lower expression of CD56 than did other subtypes. Instead, CD56 expression levels were significantly higher in tall cell variant PTC and classic PTC with tall cell features than in classic PTC. Also, our present study showed no significant correlation between reduced CD56 expression and aggressive clinicopathologic characteristics. Therefore, we suggest that the CD56 expression occurs early during PTC development but may not be associated with the progression of PTC.

In a recent study, researchers demonstrated that CD56 is a handy screening marker in thyroid cytology. CD56 showed high sensitivity (90%) and diagnostic accuracy (88.4%) in the diagnosis of the indeterminate lesion (Bethesda category III) [29]. Although the overall diagnostic performance of CD56 is good [16], our results reveal the possible pitfall when using CD56 for preoperative diagnosis. Expression of CD56 in the tall cell variant, classic PTC with tall cell features, Warthin-like, or cribriform-morular PTCs will not cause misdiagnosis in the surgical specimen, because the histological characteristics of these variants are evident. However, the preoperative prediction based on immunostaining of CD56 on cytology cell blocks or core-needle biopsy may require careful attention. Looking at our data, it will need careful consideration of the different expression levels between the subtypes of PTC to avoid misdiagnosis.

Diagnoses of follicular adenoma, NIFTP, and PTC are strictly morphology-based. But still, when the nuclear features of encapsulated follicular-patterned tumors are questionable for the determination of PTC, the differential diagnosis ranges from benign FA to malignant PTC depending on the pathologists' subjective diagnostic thresholds. In this regard, our data discovered the excellent performance of CD56 immunostaining in distinguishing NIFTP from PTC and FA with high AUC values (0.866 and 0.885, respectively) using different cut-off values for the loss of CD56 expression. High H-scores of CD56 expression in NIFTP were also useful in discriminating NIFTP from FVPTC (AUC 0.935). These results are consistent with a previous study on distinguishing encapsulated follicular variant PTC from infiltrative follicular variant PTC or classic PTC [23]. The previous study had 90.9% sensitivity and 59.8% specificity in distinguishing infiltrative follicular variant PTC and encapsulated follicular variant PTC. Therefore, loss of CD56 expression could be useful for differential diagnosis of follicular-patterned neoplasm including NIFTP.

In our study, we used the H-scoring method to evaluate the degree of CD56 expression. Using this method has disadvantages that it is not routinely used in the pathologic diagnosis, it can be subjective to interpret the intensity, and the result can vary depending on the detection system. Despite the disadvantages, the derivation of the H-score using computer-aided image-analysis system retains consistency between the cases and can more objectively quantify the degree of CD56 expression when expression pattern is heterogeneous.

Conclusions

Three levels of H-scores for CD56 expression were useful for differential diagnosis of follicular patterned thyroid tumors. Low (< 30), intermediate (30–180), and high (> 180) H-scores were specific for classic or follicular variants of PTC, NIFTP, and follicular adenoma, respectively. However, PTCs with papillary architecture may aberrantly express CD56. Follicular adenoma can occasionally show the complete loss of CD56 expression. Therefore, CD56 immunostaining should not be interpreted in isolation but should be done in the context of histological findings to avoid diagnostic pitfalls.

Abbreviations

AJCC: American Joint Committee on Cancer; AUC: areas under the receiver operating characteristic; FA: follicular adenoma; FVPTC: follicular variant papillary thyroid carcinoma; H-score: histoscore; NIFTP: noninvasive follicular thyroid neoplasm with papillary-like nuclear features; PTC: papillary thyroid carcinoma; ROC: receiver operating characteristic; TCGA: The Cancer Genome Atlas.

Declarations

Acknowledgments

Not applicable.

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Availability of data and materials

All data analyzed during this study are included in this published article.

Ethical declarations

This study was approved by the institutional review board of Seoul St. Mary's Hospital, Seoul, Korea (KC16RISI0040). This study was conducted in accordance with the Declaration of Helsinki.

Consent for publication

Informed written consent was obtained from all the patients.

Competing interests

The authors declare that they have no competing interests.

Author Contributions

Conceptualization, U.C. and C.K.J.; methodology, U.C., Y.K., S.J. and C.K.J.; software, U.C. and C.K.J.; validation, U.C. and C.K.J.; formal analysis, Y.K., S.J., and C.K.J.; investigation, U.C., Y.K., S.J. and C.K.J.; resources, C.K.J.; data curation, U.C., Y.K., S.J. and C.K.J.; writing—original draft preparation, U.C. and C.K.J.; writing—review and editing, U.C., Y.K., S.J. and C.K.J.; visualization, U.C. and C.K.J.; supervision, C.K.J.; project administration, C.K.J.; funding acquisition, C.K.J. All authors have read and agreed to the published version of the manuscript.

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Figures

Figure 1

Representative images of CD56 immunostaining. Intense membranous staining in normal thyroid tissue (a, ×200) and follicular adenoma (b, ×200). The expression of CD56 is partially lost in noninvasive follicular thyroid neoplasms with papillary-like nuclear features (NIFTP) (c, ×200) and completely absent in infiltrative follicular (d, ×200), classic (e, ×200), and diffuse sclerosing (f, ×200) variants of papillary thyroid carcinoma. Strong expression of CD56 is shown in the tall cell (g, ×200), Warthin-like (h, ×200), and cribriform-morular (i, ×200) variants of papillary thyroid carcinoma.

Figure 2

Distributions of H-scores of CD56 expression, histologic variants, and BRAFV600E mutation in 174 papillary thyroid carcinomas and 22 noninvasive follicular thyroid neoplasms with papillary-like nuclear features (NIFTP). All thyroid tumors were ranked in ascending order of the H-scores of CD56 expression.

Figure 3

Comparison of H-scores (median with interquartile range) of CD56 expression in follicular adenoma (FA), noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP), and different variants of papillary thyroid carcinoma (PTC).

	AUC (95% CI)	H-score	Sensitivity	Specificity
NIFTP vs FA	0.866 (0.758 – 0.974)	180	80%	82%
NIFTP vs FVPTC	0.935 (0.859 – 1.010)	30	93%	91%
NIFTP vs all PTC	0.885 (0.817 – 0.953)	30	83%	91%

Figure 4

ROC curves for H-scores of CD56 expression for the differential diagnoses of NIFTP vs. follicular adenoma (FA) (a), NIFTP vs. follicular variant of PTC (b), and NIFTP vs. all types of papillary thyroid carcinoma (PTC) (c). ROC, receiver operating characteristic; AUC, area under ROC.

Figure 5

Follicular adenoma with complete loss of CD56 expression. H&E (a, $\times 40$; b, $\times 400$), CD56 immunostaining (c, $\times 40$; d, $\times 400$).

Figure 6

Expression of NCAM1 mRNA in TCGA data of papillary thyroid carcinoma.