# Prognostic significance of CD133 and ABCB5 expression in papillary thyroid carcinoma

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## ABSTRACT

Expression of CD133 and ABCB5 is associated with tumor aggressiveness, but evidence in papillary thyroid cancer (PTC) is lacking. We correlated CD133 and ABCB5 expression with pathological characteristics and factors of worse prognosis in PTC. Samples of 119 PTCs and 40 controls (goiters) were distributed in 8 tissue microarray blocks and evaluated with immunohistochemistry using anti-CD133 and anti-ABCB5 antibodies. The expression of each marker alone and combined was analyzed against pathological characteristics and factors of worse prognosis in PTC. Expression of CD133 alone (19 tumors, 16.0%) was more frequent in patients with *versus* without lymph node metastases (P=0.024). Expression of ABCB5 alone (n=95, 83.3%) was associated with larger tumor size (P=0.045). CD133-ABCB5 coexpression was not associated with pathological characteristics or factors of worse prognosis in PTC.

**Key words:** CD133 protein, human; prominin 1 protein, human; ATP-binding cassette transporter, subfamily B; ABCB5 protein, human; immunohistochemistry; cancer; papillary thyroid.

## Introduction

Survival rates of papillary thyroid carcinoma (PTC) are >90% over 10 years.[1-4] However, up to 90% of the patients with PTC have cervical lymph node metastases, which are associated with an increased risk of locoregional recurrence and distant metastases.[5,6] Recognition of PTCs with increased aggressive behavior allows for proper patient follow-up,[7] but new immunohistochemical biomarkers are needed to identify PTC cases with unfavorable prognosis. The pentaspan membrane glycoprotein CD133 (or prominin-1) has been used to identify cancer stem cells in various types of cancer[8,9] and has been associated with worse prognosis.[10,11] In young individuals with differentiated thyroid carcinoma, CD133 expression has been associated with tumor size, lymph node metastases, and BRAF mutation.[12] Expression of this protein is also associated with other markers, including the ATP-binding cassette subfamily B member 5 (ABCB5),[13,14] which has been associated with worse prognosis and vascular invasion in colorectal carcinomas.[15] To the best of our knowledge, the expression of ABCB5 and the coexpression of CD133 and ABCB5 have still not been evaluated in PTCs. The aim of this study was to correlate the expression of CD133 and ABCB5 with pathological characteristics and prognosis of PTC.

## Materials and Methods

### Cases

A search of pathology records identified 154 patients with PTC who had undergone thyroidectomy between January 1st, 2002, and December 31st, 2017, across three hospitals in Curitiba (Brazil). Forty cases of multinodular goiter served as controls. Clinical and histopathological data (including patients' age, presence of lymph node metastasis, and distant metastasis) were obtained from medical records and pathology reports. The 2017 American Joint Committee on Cancer (AJCC)/TNM staging system for differentiated thyroid carcinomas was used for tumor staging and classification.[4]

### Histological evaluation

Hematoxylin-eosin stained slides with tumor samples were reviewed by an independent pathologist blinded to the clinical history of the patients to confirm the diagnosis, identify the area of the tumor, and select representative paraffin block regions for the construction of tissue microarray (TMA) blocks.

Histological subtypes of PTC were identified according to the World Health Organization (WHO) 2017 criteria.[16] The evaluation of the tumors included the observation of the presence or absence of capsule, capsular invasion, tumor size, angiolymphatic invasion, microscopic and macroscopic extrathyroidal extension, and presence or absence of tumor multifocality (*i.e.*, presence of two or more tumor foci in the same lobe or in both lobes).[17] The total number of lymph nodes removed, and the presence of metastatic lymph nodes were recorded.

### Tissue microarray

For TMA assembly, we identified the best represented areas of the neoplasm in the original slides and matched these areas with the paraffin blocks. We then extracted a cylinder of tissue from the area selected using the Tissue-Tek Quick-Ray (Sakura Finetek USA Inc., Torrance, CA, USA) handheld device coupled with a 2-mm diameter forceps. The samples were then mapped and organized in TMAs, and control tissue (placenta fragment) was added to

the first spot in the TMA blocks to mark the starting point for slide reading. The TMA blocks were cut at 5 μm thickness using a microtome, placed on Superfrost Plus (hydrophilic) slides (ThermoFisher Scientific, Braunschweig, Germany) and counterstained with hematoxylin. The samples were then mapped and organized in TMAs, and control tissue (placenta fragment) was added to the first spot in the TMA blocks to mark the starting point for slide reading.

### Immunohistochemistry

The slides with the TMA blocks were submitted to the immunoperoxidase technique (Benchmark Ultra, Roche Diagnostics, Indianapolis, IN, USA) with 3-in-1 integrated processing including deparaffinization, rehydration, and antigen recovery, and use of the Cell Conditioning 1 (high pH) and 2 (low pH) buffers (Roche Diagnostics GmbH, Mannheim, Germany). The slides were incubated with primary antibodies for 16-20 min at room temperature. Amplification was performed by ultraView Universal DAB Detection Kit (Roche Diagnostics GmbH). DAB was used as a chromogen and hematoxylin as a counterstain. The samples were processed on the Benchmark Ultra automated platform using an anti-CD133 polyclonal antibody (Biorbyt, Cambridge, UK) diluted at 1:100 and anti-ABCB5 antibody (clone 5H3C6; GeneTex, Irvine, CA, USA) diluted at 1:750.

Expression of CD133 and ABCB5 was defined by the presence (positive) or absence (negative) of immunoreactivity in the cell membrane or cytoplasm. The intensity scores were 0 (no staining), 1 (weak staining), 2 (moderate staining), and 3 (strong staining). Each slide was evaluated by two pathologists who separately observed 10 high power fields. A microscopic conference was held to discuss discrepancies and reach a consensus.

Categorical variables are summarized as absolute and percentage frequencies, and continuous variables as mean ($\pm$ SD) and median (minimum and maximum) values. Chi-square test or Fisher's exact test verified associations between two categorical variables, and the Mann-Whitney test compared groups of continuous variables. Results are expressed as odds ratios (OR) and 95% confidence intervals (95% CIs). The analyses were performed using Statistica (version 7; Statsoft Inc., Tulsa, OK, USA), SPSS (version 19; SPSS Inc., Chicago, IL, USA), and BioEstat (version 5; Instituto Mamirauá, Manaus, Brazil). The significance level was set at 5%.

## Results

Of the 154 samples, 35 were excluded due to detachment of the histological section from the slide during immunohistochemical processing, absence of tumor cells in the TMA section, low tumor volume, or absence of immunohistochemical reaction. The final analysis included 119 cases of PTC and 40 controls. In all, 35.3% (n=42) of the tumors were ≤1 cm, 24.4% (n=29) of the patients had metastases to locoregional lymph nodes (N1), and 5.0% (n=6) had distant (lung) metastases on ^{131}I whole-body scan (Table 1). The median follow-up was 36.5 months (0-241 months).

The most frequent PTC variants were classic, follicular, and papillary microcarcinoma. Uncommon variants (6.5%, n=8) included tall cell (n=3), oncocytic (n=3), Warthin-like (n=1), and macrofollicular (n=1).

### CD133 expression

CD133 expression was analyzed in all 119 cases and was positive in 19 (16.0%) samples (Table 2). The expression was observed in the cell membrane and cytoplasm (Figure 1) at low intensity (score 1, weak staining) and was more frequent among

patients with (n=9 of 29) *versus* without (n=10 of 90; P=0.024) lymph node metastases (Table 3). CD133 expression had no correlation with other markers of disease severity (Table 2).

### ABCB5 expression

ABCB5 expression was analyzed in 114 cases and was positive in 95 (83.3%) samples (Table 2), all at the cell membrane (Figure 1) and at different degrees of intensity. Compared with tumors without ABCB5 expression, those expressing ABCB5 were larger (1.23±0.85 cm *versus* 1.93±41.48 cm, respectively, P=0.045). No other association was found between ABCB5 expression and other markers of disease severity (Table 2).

### CD133-ABCB5 coexpression

Fifteen patients (12.6%) had tumors expressing both CD133 and ABCB5 (Table 4). Expression of both markers compared with expression of only one or none of the markers was not associated with increased frequency of any risk factor of disease severity.

### CD133 and ABCB5 expression in papillary tumors compared with goiter

Among samples obtained from patients with goiter in the control group, 5 (12.5%) expressed CD133 and 15 (37.5%) expressed ABCB5 (Table 5, Figure 1). Expression of ABCB5 was more frequent in patients with PTC *versus* goiter (OR 8.33, 95% CI 3.71-18.69, P<0.0001), but the same was not observed for CD133 (OR 1.33, 95% CI 0.46-3.83, P=0.78).

### Discussion

Increased CD133 expression in PTC has been associated with early lymph node metastases and advanced TNM stages in some[18] but not all studies,[19] and with poor prognosis in medullary thyroid carcinoma.[20] Additionally, CD133 has been shown *in vitro* to promote the self-renewal capacity of anaplastic thyroid cancer cell lines.[21] The prevalence of positive CD133 among our patients was low, but expression of this marker increased by 3.6 times the odds of metastatic lymph nodes. If this finding is confirmed in larger samples and extended to cytopathological analysis, CD133 may emerge as a pivotal presurgical diagnostic tool to identify patients benefiting from a more extensive neck dissection. Interestingly, the suppression of ABCB5 sensitizes cells for uptake of doxorubicin and cell apoptosis,[22] implying that the expression of both CD133 and ABCB5 may be associated with worse clinical course.

**Table 1.** Clinical characteristics of the patients evaluated for CD133 and ABCB5 expression

|  | CD133 (n=119) | ABCB5 (n=114) |
|---|---|---|
| Age on diagnosis (years) | 45.45±13.32 | 44.92±12.71 |
| Gender |  |  |
| Female | 83.19% (n=99) | 82.45% (n=94) |
| Male | 16.80% (n=20) | 17.54% (n=20) |
| Tumor size |  |  |
| ≤ 1cm | 35.29% (n=42) | 35.96% (n=41) |
| > 1cm | 64.7% (n=77) | 64% (n =73) |
| Distant metastases on diagnosis | 5.04% (n=6) | 5.26% (n=6) |
| Lymph node metastases (N1) | 24.36% (n=29) | 23.52% (n=28) |
| Age ≥ 55 years on diagnosis | 23.52% (n=28) | 21.05 (n=24) |
| AJCC/TNM 8th edition (2017) |  |  |
| I + II > 55 years | 22.68% (n=27) | 20.17% (n=23) |
| III + IVa + IVb >55 years | 0.84% (n=01) | 0.87% (n=1) |
| I < 55 years | 72.26% (n=86) | 74.56% (n=85) |
| II<55 years | 4.20% (n=5) | 4.38% (n=5) |

**Table 2.** Pathological characteristics of papillary thyroid carcinoma in relation to CD133 and ABCB5 immunoreactivity.

|  | CD133 negative | CD133 positive | P | ABCB5 negative | ABCB5 positive | P |
|---|---|---|---|---|---|---|
| Samples, % (n) | 84% (n=100) | 16.0% (n=19) | 0.00001 | 16.7% (n=19) | 83.3% (n=95) | 0.00001 |
| Age (years), mean ± SD | 45.9±13.7 | 43.1±11 | - | 46.2±10.4 | 44.7±13.1 | - |
| Male sex, % (n) | 14 (14%) | 6 (31.58%) | 0.067 | 5.6% (n=1) | 20% (n =19) | 0.107 |
| Variants |  |  |  |  |  |  |
| Classic | 36% (n=36) | 42.10 % (n=8) | 0.61 | 31.58% (n=6) | 37.89% (n=36) | 0.60 |
| Follicular | 22% (n=22) | 15.79% (n=3) | 0.40 | 10.53% (n=2) | 22.10% (n=21) | 0.21 |
| Papillary microcarcinoma | 35% (n=35) | 36.84% (n=7) | 0.88 | 47.37% (n=9) | 33.68% (n=32) | 0.26 |
| Others | 7% (n=7) | 5.26% (n=1) | 0.62 | 10.53% (n=2) | 6.31% (n=6) | 0.40 |
| Tumor size (cm) | 1.79±1.38 | 1.86±1.11 | 0.84 | 1.23± 0.85 | 1.93±1.48 | 0.045 |
| T staging |  |  |  |  |  |  |
| T1a | 35% (n=35) | 36.84% (n=7) | 0.88 | 47.37% (n=9) | 33.68% (n=32) | 0.26 |
| T1b | 38% (n=38) | 21.05% (n=4) | 0.16 | 42.10% (n=8) | 33.68% (n=32) | 0.48 |
| T2 | 22% (n=22) | 36.84% (n=7) | 0.17 | 10.53% (n=2) | 25.26% (n=24) | 0.16 |
| T3 | 5% (n=5) | 5.26% (n=1) | 0.66 | 0% (n=0) | 7.37% (n=7) | 0.27 |
| N staging |  |  |  |  |  |  |
| N1 | 20% (n=20) | 47.37% (n=9) | 0.011 | 21.05% (n=4) | 25.26% (n=24) | 0.47 |
| M1 | 5% (n=5) | 5.26% (n=1) | 0.66 | 10.53% (n=2) | 4.21% (n=4) | 0.26 |
| Unifocal | 57% (n=57) | 63.16% (n=16) | 0.62 | 68.42% (n=13) | 57.89% (n=55) | 0.39 |
| Multifocal | 43% (n=43) | 36.84% (n=7) | 0.62 | 31.58% (n=6) | 41.11% (n=40) | 0.39 |
| Microscopic extension | 14% (n=14) | 26.31% (n=5) | 0.16 | 15.79% (n=3) | 17.89% (n=17) | 0.82 |
| Macroscopic extension | 3% (n=3) | 5.26% (n=1) | 0.51 | 0% (n=0) | 5.26% (n=5) | 0.39 |
| Capsular invasion | 1% (n=1) | 0% (n=0) | 0.85 | 15.79% (n=3) | 7.37% (n=7) | 0.22 |
| Angiolymphatic invasion | 27% (n=27) | 36.84% (n=7) | 0.38 | 31.58% (n=6) | 33.68% (n=32) | 0.86 |

However, coexpression of CD133 and ABCB5 in our study was not useful in recognizing markers of disease severity in PTC when compared with expression of each marker alone or none of the markers.

Although limited by the retrospective design and small number of patients with metastatic lymph nodes, our study showed that patients with PTC expressing CD133 have higher odds of lymph node metastases, while tumors expressing ABCB5 are larger than those not expressing this marker. CD133-ABCB5 coexpression was no more helpful than expression of each marker alone or none of the markers in identifying patients with markers of disease severity.

**Table 3.** Analysis of the association between the presence (+) or absence (-) of expression of each marker (CD133 and ABCB5) alone or in combination *versus* presence or absence of lymph node metastasis.

|  | Lymph node affected | | Odds ratio | |
|  | Yes (n = 29) | No (n = 90) | (95% confidence interval) | P |
|---|---|---|---|---|
| CD133+ | 31.03% (n=9) | 11.11% (n=10) | 3.60 (1.29-10.03) | 0.024 |
| CD133- | 68.97% (n=20) | 88.89% (n=80) | 0.28 (0.10-0.77) | |
| CD133+/ABCB5+ | 24.14% (n=7) | 8.42% (n=8) | 3.26 (1.07-9.98) | 0.067 |
| CD133+/ABCB5- | 6.90% (n=2) | 0% (n=0) | - | - |
| CD133-/ABCB5+ | 58.62% (n=17) | 65.56% (n=59) | 0.74 (0.32-1.75) | 0.65 |
| CD133-/ABCB5- | 6.90% (n=2) | 14.44% (n=13) | 0.44 (0.09-2.07) | 0.46 |
| ABCB5+ | 85.71% (n=24) | 85.56% (n=71) | 1.27 (0.38-4.9) | 0.92 |
| ABCB5- | 14.29% (n=4) | 17.44% (n=15) | 0.79 (0.24-2.61) | |

**Table 4.** Pathological characteristics of the tumors in relation to the presence (+) or absence (-) of expression of each marker (CD133, ABCB5) alone or in combination.

|  | CD133+/ABCB5+ | CD133+ /ABCB5- | P | CD133-/ABCB5+ | P | CD133-/ABCB5- | P |
|---|---|---|---|---|---|---|---|
| Sample, % (n) | 12.6% (n=15) | 1.68% (n=2) | 0.001 | 63.86% (n=76) | 0.00001 | 12.6% (n=15) | 1 |
| Variant | | | | | | | |
|   Classic | 53.33% (n=8) | 0.00% (n=0) | 0.051 | 36.84% (n=28) | 0.22 | 33.33% (n=5) | 0.27 |
|   Follicular | 13.33% (n=2) | 0.00% (n=0) | 0.55 | 23.68% (n=18) | 0.39 | 13.33% (n=2) | 0.70 |
|   Papillary microcarcinoma | 33.33% (n=5) | 20.00% (n=1) | 0.52 | 31.57% (n=24) | 0.87 | 46.66% (n=7) | 0.46 |
|   Others | 0.00% (n=0) | 20.00% (n=1) | 0.25 | 7.89% (n=6) | 0.33 | 6.66% (n=1) | 0.31 |
| Tumor size (cm) | 1.89 ± 1.17 | 1.10±0.57 | 0.34 | 1.91±1.47 | 0.84 | 1.24±0.93 | 0.15 |
| T staging | | | | | | | |
|   T1a | 33.33% (n=5) | 50.00% (n=1) | 0.52 | 31.58% (n=24) | 0.56 | 45.66% (n=7) | 0.46 |
|   T1b | 20.00% (n=3) | 50.00% (n=1) | 0.43 | 37.66% (n=29) | 0.15 | 40% (n=6) | 0.21 |
|   T2 | 40.00% (n=6) | 0.00% (n=0) | 0.40 | 23.37% (n=18) | 0.15 | 13.33% (n=2) | 0.11 |
|   T3 | 6.67% (n=1) | 0.00% (n=0) | 0.75 | 6.49% (n=5) | 0.67 | 00% (n=0) | 0.31 |
| N staging | 60.00% (n=9) | 40.00% (n=2) | 0.40 | 44.15% (n=34) | 0.26 | 33.33% (n=5) | 0.14 |
|   Nx | 40.00% (n=6) | 60.00% (n=3) | 0.40 | 55.84% (n=43) | 0.26 | 66.66%(n=10) | 0.14 |
|   N0 | 13.33% (n=2) | 0.00% (n=0) | 0.55 | 22.07% (n=17) | 0.35 | 20% (n=3) | 0.50 |
|   N1 | 46.66% (n=7) | 40% (n=2) | 0.60 | 22.07% (n=17) | 0.053 | 13.33% (n=2) | 0.054 |
|   M1 | 0.00% (n=0) | 20% (n=01) | 0.25 | 5.19% (n=4) | 0.48 | 6.66% (n=1) | 0.31 |
|   Unifocal | 66.67% (n=10) | 50% (n=1) | 0.60 | 56.38% (n=43) | 0.47 | 66.67% (n=10) | 1.0 |
|   Multifocal | 33.33% (n=5) | 50% (n=1) | 0.52 | 42.85% (n=33) | 0.49 | 33.33% (n=5) | 1.0 |
| Microscopic extension | 33.33% (n=5) | 0.00% (n=0) | 0.29 | 14.28% (n=11) | 0.08 | 20% (n=3) | 0.34 |
| Macroscopic extension | 6.67% (n=1) | 0.00% (n=0) | 0.75 | 3.89% (n=3) | 0.52 | 00% (n=0) | 0.31 |
| Capsular invasion | 0.00% (n=0) | 20.00% (n=1) | 0.25 | 7.79% (n=6) | 0.33 | 20% (n=3) | 0.11 |
| Angiolymphatic invasion | 40.00% (n=6) | 40.00% (n=2) | 0.70 | 31.16% (n=24) | 0.35 | 20% (n=3) | 0.21 |

**Table 5.** Expression of CD133 and ABCB5 in the papillary thyroid cancer (PTC) and goiter (control) groups.

|  | PTC group | Control group | Odds ratio (95% confidence interval) | P |
|---|---|---|---|---|
| Positive CD133, % (n) | 16.0% (n=19) | 12.5% (n=5) | 1.33 (0.46-3.83) | 0.78 |
| Positive ABCB5, % (n) | 83.3% (n=95) | 37.5% (n=15) | 8.33 (3.71-18.69) | <0.0001 |
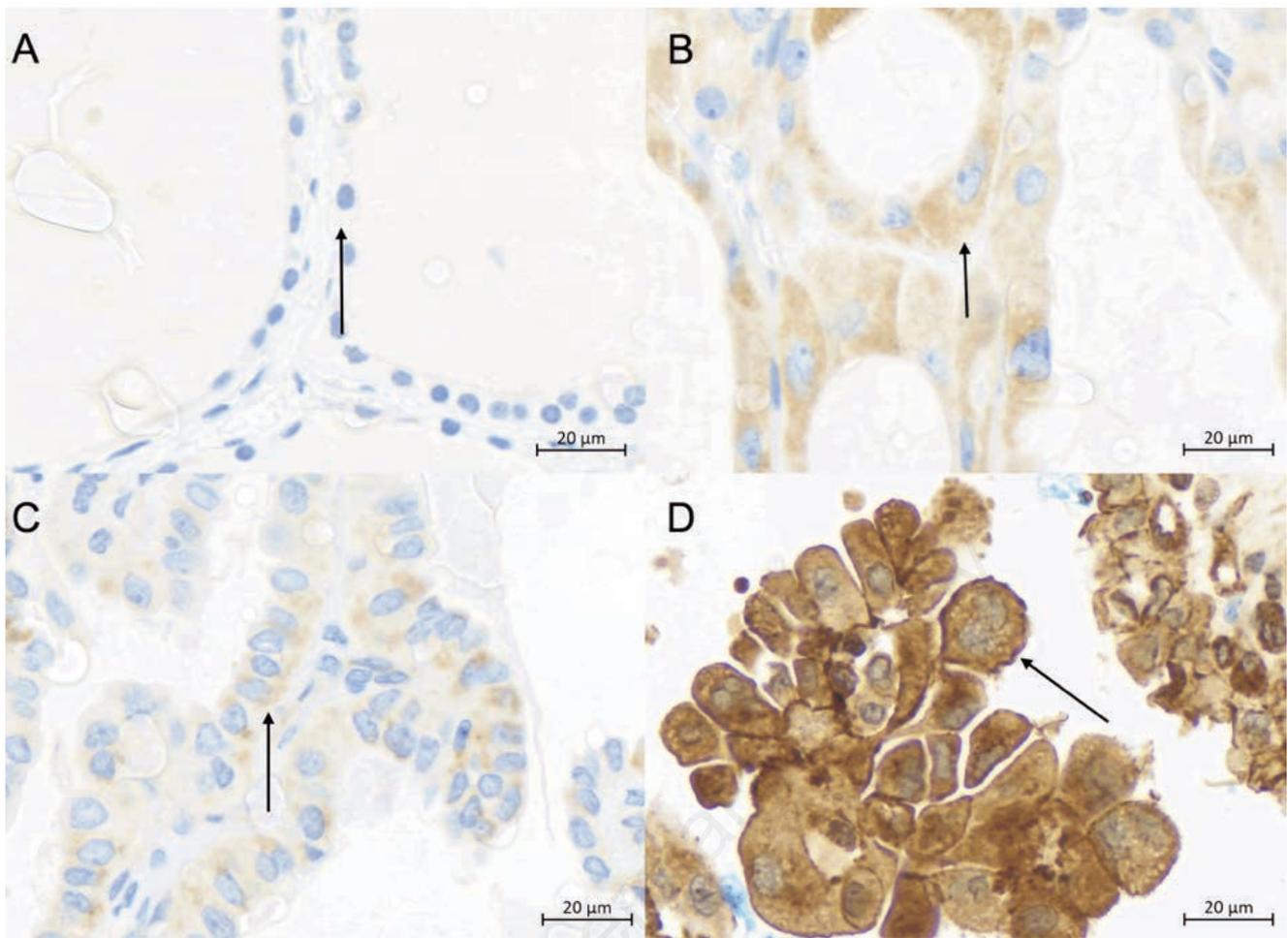
**Figure 1.** Immunohistochemical features of CD133 and ABCB5 in goiter and papillary carcinoma. A-D) Representative photomicrographs of goiter and papillary carcinoma, including (A) negative CD133 expression (arrow) in goiter; B) weak ABCB5 cytoplasmic expression (arrow) in goiter; C) weak CD133 cytoplasmic expression (arrow) in papillary carcinoma, and (D) strong ABCB5 cytoplasmic and membrane positivity (arrow) in papillary carcinoma.

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