The Expression of Sialic Fibronectin Correlates with Lymph Node Metastasis of Thyroid Malignant Neoplasmas

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Abstract. Background: Papillary thyroid carcinoma (PTC) is often accompanied by lymph node metastasis (LNM), compared with follicular thyroid carcinoma (FTC). Sialic acid is carried by fibronectin (sFN) as the antigen of monoclonal antibody (MoAb) JT-95 detected in 90% of PTC, and a few cases of FTC. Patients and Methods: JT-95 staining was performed in 9 PTC and 20 follicular type tumors to investigate the relationship between the expression of sFN and the frequency of LNM. Results: There were 11 cases with LNM from 23 malignant tumors, and no cases of LNM from 6 benign follicular type tumors. The staining scores by JT-95 of the 11 tumors with LNM were 5+ in 4 cases, and 6+ in 7 cases. On the other hand, the scores of 12 malignant tumors without LNM were <4+ in all cases. Conclusion: An increase of sFN expression in thyroid malignancies is correlated with LNM.

Differentiated carcinomas, known as papillary and follicular carcinomas, are the most common malignancies of the thyroid region and are often detected by physical examination. Among these malignancies, the rate of correct preoperative diagnosis of papillary thyroid carcinoma (PTC) is more than 90%, using physical examinations, such as fine-needle aspiration biopsy (FNA), ultrasound examination (US), and TI-Tc scintigram. On the other hand, follicular thyroid carcinoma (FTC) has a rate of correct diagnosis of only 20 to 42%, as it is histologically and morphologically difficult to differentiate between this tumor and benign follicular tumors (1-3). Moreover, the recurrence or metastasis pattern is different between PTC and FTC. Lymph nodes metastasis occurs in 80% of operated PTC cases, compared with 7-10% of FTC. However, the rate of distant metastasis, such as lung or bone via the blood stream, is higher in FTC than PTC (4-6). Thus, many studies have attempted to improve the diagnosis of FTC and to find the differences between PTC and FTC. Some studies have found that oncofetal fibronectin (OnfFN), which leads to the isoform of the III connecting segment (III-CS), and is alternatively spliced from fibronectin (FN) during tumor genesis, is detected in most cases of PTC and FTC (7-9).

A monoclonal antibody (MoAb) designated JT-95 was raised against the membrane fraction of PTC, as well as recognizing glycochain-containing sialic acid, which is carried by fibronectin (sFN) as antigen. The antigen of JT-95 was detected in more than 90% of PTC, and 10-15% of FTC in our series (10, 11).

Here we investigated the relationship between the expression of sFN and lymph node metastasis in thyroid malignancies, performing JT-95 staining of the original tumor of PTC and follicular type tumor including FTC, and for the lymph node metastases.

Patients and Methods

Patients. Twenty follicular-type tumor patients (9 males and 11 females) and nine PTC patients (9 females), who were operated on for thyroidectomy with sentinel lymph node biopsy (SNB) at the Jikei University in 2009, were examined. At the time of surgery their ages ranged from 24 to 61 years, with a mean age of 47 and median age of 42. The 20 follicular-type tumor cases were suspected of malignancy based on one or more positive results from preoperative examinations involving ultrasonography (US), TI-Tc scintigraphy, fine-needle aspiration cytology (FNA). These tumors
were solid and isolated, with a size of more than 40 mm. The results of FNA in 9 PTC cases were class IV or V, and the results of US were also diagnosed as carcinoma preoperatively in all cases.

SNBs were performed with thyroidectomy, because even though the malignancy of a thyroid follicular-type tumor cannot always be preoperatively determined, metastasis in lymph nodes outside of the thyroid itself is definitive evidence of follicular malignancies.

\textbf{MoAb JT-95 preparation and immunohistochemical staining.} The production and characterization of MoAb JT-95 was as previously described (10). Briefly, mice were immunized with a soluble membrane extract of human PTC, and the isotype of JT-95 was IgM. JT-95 recognized glycochain-containing sialic acid, carried by both FN and ganglioside as antigens (11). The formalin-fixed tumor and lymph node sections (3 μm) were stained with 1 μg/ml MoAb JT-95 using the Vectastain Kit (Vector Laboratories, Burlingame, CA, USA) for immunohistochemical study.

\textbf{Data interpretation. Histological diagnosis:} The final tumor diagnosis was determined by hematoxylin-eosin (H-E)-stained histological sections.

\textbf{MoAb JT-95 staining:} As sFN is present in the extracellular matrix (ECM) of cells, the cell localization was classified into three categories: (a) membranous only; (b) membranous and cyttoplasmic (heterogeneous); and (c) cyttoplasmic staining only. If the tumor cells were stained at the membrane or heterogeneous, the cells were considered positive for MoAb JT-95. Absence of staining, or cyttoplasmic staining was considered a negative result and given a score of (0+). Positive specimens were further evaluated for the cell intensity and the range of staining area by two pathologists and one physician. The staining intensity was classified as weak (1+), moderate (2+) and strong (3+). The staining range of the entire set of tumors was also scored from (1+) to (3+). When fewer than 10% of cells in tumor were only stained, the range score allocated was (1+); when 10-50% of the tumor cells were positive, the score was (2+); when more than 50% of the cells in tumor were stained, the range score allocated was (3+). Tumor was evaluated using the combined intensity and range scores for MoAb JT-95 staining.

\textbf{Statistical analysis.} The statistical difference in staining score of JT-95 between benign tumors and malignant tumors was calculated using the Mann-Whitney U-test as a non-parametric method. The statistical difference between tumors with lymph node metastasis and without metastasis was also calculated (N=29).

\section*{Results}

\textbf{Final pathological diagnosis of tumors.} The final diagnoses of 29 tumors were FTC in 6, follicular variant of papillary thyroid carcinoma (FVPTC) in 8, PTC in 9, follicular adenoma (FA) in 5, and adenomatous goiter (AG) in 1 (Table I).

\textbf{MoAb JT-95 immunohistochemical staining.} The results of the range, intensity, and combined total staining scores of 29 cases are summarized in Table I (see also Figures 1 and 2). The total staining scores were from 0+ to 6+ in the malignant lesions. Concerning the metastatic lymph nodes, the total staining score was 6+ in all 11 cases (Figure 3).

\begin{table}[h]
\centering
\caption{Table I. The relationship between lymph node metastasis and JT-95 staining.}
\begin{tabular}{|c|c|c|c|}
\hline
\textbf{Pathological diagnosis of follicular type tumor} & \textbf{No. of sentinel lymph node metastasis} & \textbf{Range and intensity score of JT-95 staining} \\
\hline
FTC & 0/2 & 1+ & 2+ & 3+ \\
FTC & 0/1 & 1+ & 3+ & 4+ \\
FTC & 1/1-1/12 & 2+ & 3+ & 5+ \\
FTC & 0/1 & 0+ & 0+ & 0+ \\
FTC & 0/1 & 1+ & 1+ & 2+ \\
FVPTC & 0/3 & 1+ & 3+ & 4+ \\
FVPTC & 1/2-6/18 & 2+ & 3+ & 5+ \\
FVPTC & 0/2 & 1+ & 2+ & 3+ \\
FVPTC & 0/4 & 1+ & 2+ & 3+ \\
FVPTC & 2/2-2/9 & 2+ & 3+ & 5+ \\
FVPTC & 1/1-2/3 & 3+ & 3+ & 6+ \\
FVPTC & 0/1 & 0+ & 0+ & 0+ \\
PTC & 1/2-6/8 & 3+ & 3+ & 6+ \\
PTC & 1/2-9/18 & 3+ & 3+ & 6+ \\
PTC & 0/2 & 2+ & 3+ & 5+ \\
PTC & 2/2-9/17 & 3+ & 3+ & 6+ \\
PTC & 1/2-9/10 & 3+ & 3+ & 6+ \\
PTC & 0/1 & 2+ & 3+ & 5+ \\
PTC & 3/3-9/11 & 3+ & 3+ & 6+ \\
PTC & 1/2-5/18 & 2+ & 3+ & 5+ \\
PTC & 1/1-9/10 & 3+ & 3+ & 6+ \\
FA & 0/2 & 1+ & 1+ & 2+ \\
FA & 0/2 & 0+ & 0+ & 0+ \\
FA & 0/2 & 2+ & 3+ & 6+ \\
FA & 0/4 & 0+ & 0+ & 0+ \\
FA & 0/1 & 0+ & 0+ & 0+ \\
AG & 0/2 & 2+ & 1+ & 3+ \\
\hline
\end{tabular}
\end{table}

SND, Sentinel lymph nodes not detected; FTC, follicular thyroid carcinoma; FVPTC, follicular variant of papillary thyroid carcinoma; PTC, papillary thyroid carcinoma, FA, adenoma; AG, adenomatous goiter.

\textbf{Relationship between JT-95 staining score and SN metastasis.} There were 11 cases with SN metastasis in 23 malignant tumors, and no cases in 6 benign follicular type tumors. The staining scores by JT-95 of the 11 tumors with SN metastasis were 5+ in 4 cases, and 6+ in 7 cases. The score 5+ cases were in 1 of FVPTC, in 2 of FVPTC, and in 1 of PTC. The score 6+ cases were in 1 of FVPTC, and in 6 of PTC. Thus the JT-95 scores for all of these 11 malignant tumors with lymph node metastasis were ≥ 5+.

\textbf{Statistical analysis.} With regard to tumor staining, a statistical difference was not suggested in the relationship of
JT-95 staining scores between 23 malignant tumors and 6 benign follicular type tumors, when the staining scores of ≥4+ were considered as a positive proof in the JT-95 test. On the other hand, according to the presence or absence of lymph node metastasis for the 23 malignant tumor cases, statistical analysis revealed a significant difference between patients with JT-95 staining scores of ≥4+ and those with lower scores (calibration, $p=0.00860$).

Discussion

Many studies have attempted to improve the diagnosis of thyroid tumors. FN in the cytoplasm of follicular thyroid cells is reported to be associated with transformation, and the co-expression of FN, galectin-3, and Hector Battifora mesothelin 1 (HBME1) is commonly observed in carcinomas but restricted in benign lesions (12, 13).

Moreover, FN is alternatively spliced, leading to isoforms such as the extracellular domains A/B and III-CS, especially during tumorigenesis. These uniquely glycosylated isoforms (III-CS) are predominantly expressed by fetal and neoplastic cells and have been designated as OnfFN (14).

OnfFN has a wide spectrum of tumor specificity that includes hepatocellular carcinoma, breast carcinoma, and sarcoma; the molecular weight (MW) of OnfFN is recognized as 310-335 kDa (14, 15). With regard to thyroid carcinoma, OnfFN is successful in detecting PTC and FTC using the RT-PCR method (7-9).

The MoAb designated JT-95, which reacts with an antigen in thyroid carcinoma, has been produced with membrane fractions of human PTC. For other carcinomas, JT-95 responds to pancreatic and ovarian carcinomas, but does not react with hepatocellular carcinoma or breast carcinomas. The antigen detected by JT-95 in thyroid carcinomas has an apparent MW of 250 kDa (10).

Antigenic analysis of JT-95 reveals that two antigens are present: glycosylated FN including sialic acid, and glycosylated ganglioside (11). Taking into account tumor specificity, differences in molecular size, and the two
antigens, we believe that the epitope structure of JT-95 is distinct from that of OnfFN.

It is thought that epithelial–mesenchymal transition (EMT) occurs in carcinoma cells at the first stage of metastasis or invasion by the effects of transforming growth factor β (TGF-β) or retinoblastoma (Rb) gene (16, 17). EMT being induced reduces the quantity of cadherin between cells, and causes some ECM molecules to be secreted abundantly in carcinoma cells. Accordingly, carcinoma cells move easily in tissues with interactions of ECM and integrin focal adhesion kinase (18).

In this study, the increasing amount of sFN detected by JT-95, which exists in the ECM, correlated to lymph node metastasis in thyroid carcinomas. In addition, B and T lymphocytes, which are found in lymph nodes, hold integrin α in their cell surface as the receptor of FN (19, 20). We hypothesize that the interactions of sFN and integrin α may cause lymph node metastasis of thyroid malignancy.

There were many PTC of score >5 compared with FTC. This is in accordance with the fact that PTC has more frequent lymph node metastasis than follicular carcinoma clinically (4–6). In conclusion, increasing sFN expression in a thyroid tumor correlates with lymph node metastasis, and may suggest malignancy of thyroid tumors.

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References