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Clinicopathological Analysis of the Expression of CD44v6 in Primary Thyroid Cancer and Recurrent Regional Lymph Nodes

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Although the prognosis of differentiated thyroid cancer is generally favorable, some patients develop progressive disease resulting in a life-threatening condition and occasionally fatal outcome. CD44v6 is known to be a marker linked to distant metastasis in several types of human malignancy. In the present study, we analyzed the expression of CD44v6 in primary tumors and recurrent disease in the regional lymph node and evaluated its correlation with clinical variables and prognostic factors in patients with differentiated thyroid cancer. CD44v6 expression in the primary tumor was immunohistochemically detected in 58 of 79 patients (73%). Among parameters investigated, none except patient age showed a correlation with CD44v6 expression; the rate of positivity for CD44v6 was significantly higher (p=0.023) in patients aged 50 years or less than in those aged over 50 years. During the follow-up period, 14 patients were found to have recurrent cancer in regional lymph nodes, and among these, distant metastases were later detected in bones and/or lungs in 6 patients. Although an overall correlation between the expression of CD44v6 in the regional recurrent site and in distant metastasis was not statistically significant, there was a tendency that patients with positive CD44v6 in the regional lymph nodes subsequently developed distant matastases. CD44v6 appears to play an important role in tumor progression in patients with differentiated thyroid cancer, particularly in terms of recurrent disease in regional lymph nodes.

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Keywords: CD44v6; Thyroid cancer; Metastasis

Introduction

The prognosis of differentiated thyroid cancer is generally favorable. However, some patients display an inauspicious course, such as repeated local and/or distant metastasis. From clinical and biological points of view, it is essential to gain insight into the mechanisms of the acquisition of such aggressive phenotypes.

CD44 is a cell surface glycoprotein that can be expressed as a standard receptor (CD44s) and as multiple splice isoforms (CD44v), the expression of which is altered during tumor growth and progression.[1] Correlations between CD44v6 expression and prognosis of various tumors remain controversial.[2-10] In thyroid tumors, some studies have suggested the possibility that CD44v6 expression can be used to distinguish between the malignant and benign conditions.[11,12]

The purpose of this study was to evaluate the associations between CD44v6 expression in the primary tumor, repeated recurrence in regional lymph nodes, distant metastasis, clinical course and prognostic factors in order to clarify the relationship between CD44v6 and malignant potential of thyroid cancer.

Patients and Methods

A total of 108 specimens from 79 patients with papillary thyroid cancer who underwent surgical resection at our clinic from 1980 to 1997 were investigated. Among them, 37 specimens in 14 patients were obtained from recurrent disease in regional lymph nodes. There were 10 males and 69 females, aged 33-78 years with the mean of 53.6 years. These patients were followed for a period of 9-28 years, with the mean duration of 17 years. Local recurrence or distant metastases were evaluated by regular check-ups in outpatient clinic for physical examination, chest x-ray, neck and chest CT, and annual bone scintigraphy.

Immunohistochemical analysis

Four-micron sections of paraffin-embedded tissue were deparaffinized, rehydrated and subjected to antigen retrieval in a microwave oven. Slides were blocked with 10% fetal bovine serum for 20 minutes and then incubated with 3 μg/mL anti-human CD44v6 monoclonal

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antibody, 2F10 (R&D System Inc, Minneapolis, MN), overnight at 4°C in a humidified chamber. After washing in phosphate buffered saline (0.01 M, pH 7.4), slides were incubated with an avidin-biotin complex immunoperoxidase reagent (Histofine ABC kit, Nichirei, Tokyo) and developed with 0.05% DAB (3, 3'-diaminobenzidene tetrahydrochloride)/0.03% hydrogen peroxide. Evaluation was considered positive when more than 10% of epithelial cells were stained.

Statistical analysis

The correlation between CD44v6 expression and clinical prognostic factors was analyzed using Fisher's exact test for factors with two categories, e.g., sex, while we used the Wilcoxon rank-sum test for factors with three or more ordinal categories, e.g., stage; we dichotomized continuous variables, e.g., age. StatView software (version 5.0 for PC; SAS Institute Inc., Cary, NC) was used for the calculations.

Results

In primary cancer of the thyroid gland, CD44v6 was positive in 58 of 79 (73%) patients (Figures 1 and 2). CD44v6 expressions were detected in primary tumors and metastatic lymph nodes, but not in normal thyroid tissue. In primary operation, 48 patients had metastatic lymph nodes, and CD44v6 expressions in primary tumor and metastatic lymph nodes were concordant in 33 of them, but were not concordant in 15 of them.

When correlations between positivity for CD44v6 expression in primary cancer and clinico-pathological variables was investigated, only patient age was found to be closely linked (Table 1). The rate of positivity for CD44v6 expression was significantly higher in patients aged over 50 years than in those aged 50 years or less (p= 0.034). There were no significant correlations between CD44v6 expression in the primary tumor and other prognostic factors.

During the follow-up period, 14 patients exhibited evidence of repeated metastasis to regional lymph nodes, and 6 of them developed metachronous distant metastasis in the lungs and/or bones. Correlations between metachronous distant metastasis and clinical variables in patients with local recurrence are summarized in Table 2. No clear correlation of metachronous distant metastasis was seen with patient age, sex, tumor size, histopathological subtype, extracapsular invasion or lymph node metastasis. There were no correlations between expression of CD44v6 at the primary site and metachronous

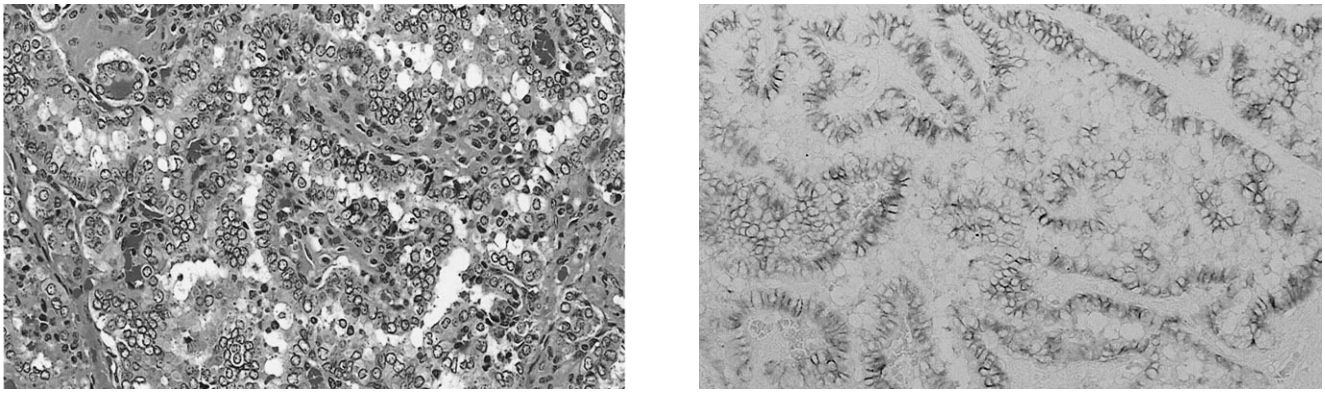

Figure 1. Hematoxylin and eosin stain, and immunohistochemical staining of CD44v6 in primary papillary thyroid carcinoma (×400).

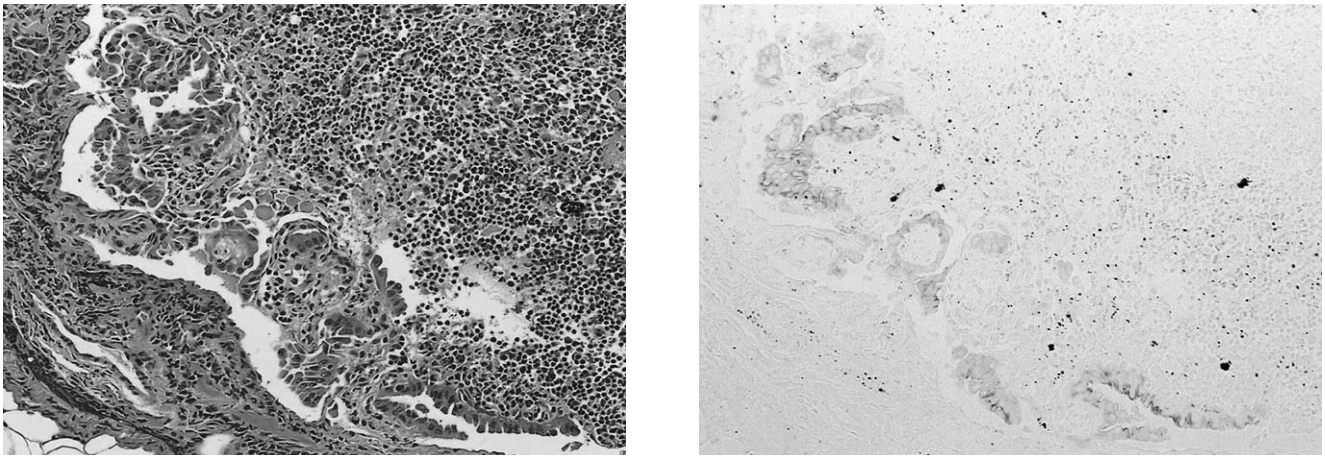

Figure 2. Hematoxylin and eosin stain, and immunohistochemical staining of CD44v6 in recurrent lymph node metastasis (×400).

Table 1. Classification of patients with differentiated thyroid cancer by CD44v6 expression in primary tumor and clinico-pathological variables

Variable	Category	CD44v6 expression		Row-sum	*p*-value
		Positive (n=58)	Negative (n=21)		
Age (years)	≤50	41	9	50	0.034
	>50	17	12	29	
Sex	Male	7	2	9	1.000
	Female	51	19	70	
Tumor size (cm)	≧5	5	0	5	0.317
	<5	53	21	74	
Distant metastasis	Yes	2	1	3	1.000
	No	56	20	79	
Capsular invasion	Yes	30	7	37	0.203
	No	28	14	42	
Regional lymph node metastasis	Yes	39	10	49	0.125
	No	19	11	30	
Stage	I	18	7	25	0.754
	II	6	3	9	
	III	32	10	42	
	IV	2	1	3	
Cancer related mortality	Dead	5	3	8	0.432
	Alive	53	18	71	

Table 2. Classification of 14 patients with primary thyroid cancer patients who developed local recurrence by metachronous distant metastasis and clinico-pathological variables

Variable	Category	Metachronous distant metastasis		Row-sum	*p*-value[a]
		Yes (n=6)	No (n=8)		
Age (years)	≤50	0	1	1	1.000
	>50	6	7	13	
Sex	Male	1	2	3	1.000
	Female	5	6	11	
Tumor size (cm)	≧5	2	1	3	0.539
	<5	4	7	11	
Histological diagnosis	Papillary carcinoma	5	8	13	0.429
	Follicular carcinoma	1	0	1	
Capsular invasion	Yes	6	5	11	0.209
	No	0	3	3	
Lymph node metastasis	Yes	6	8	14	NA[b]
	No	0	0	0	

[a]Based on Fisher's exact test.
[b]NA=Not available because of no patients in one row.

distant metastasis. In addition, there were no significant correlations between the expression of CD44v6 at the local recurrent site and metachronous distant metastasis. However, there was a tendency for patients with CD44v6-positive local recurrent sites to have distant metastases (Table 3). The expression patterns of CD44v6 in the primary and recurrent sites are summarized in Figure 3. Seven primary sites exhibited expression of CD44v6, while 2 primary sites were negative, and 5 primary tumors were not available for immunohistochemistry. Of note, among 7 cases showing CD44v6 expression in recurrent sites, 5 had metachronous distant metastasis.

Table 3. Classification of 14 patients with differentiated thyroid cancer who developed local recurrence by CD44v6 expression in primary tumor and metachronous distant metastasis

Metachronous distant metastasis	CD44v6 expression		Row-sum	*p*-value
	Positive (n=7)	Negative (n=7)		
Yes	5	1	6	0.103
No	2	6	8	

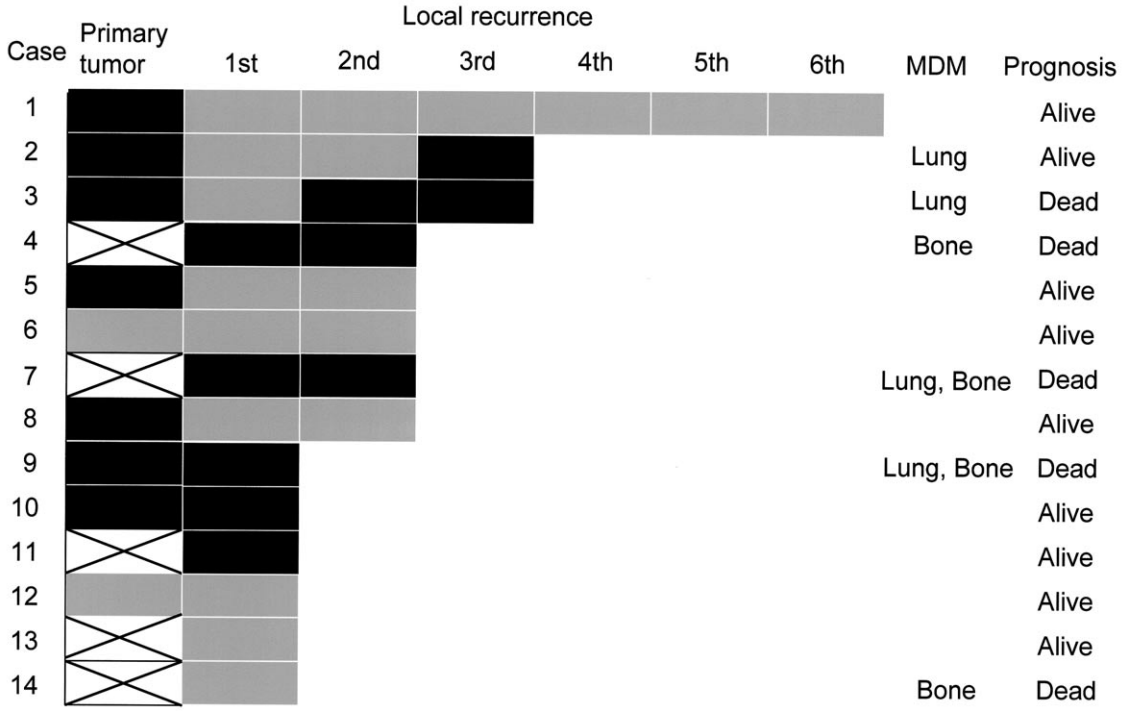

Figure 3. Recurrent lymph node metastases and their expression of CD44v6 in relation to metachronous distant metastasis and prognosis. ■ : CD44v6 positive; ▒ : D44v6 negative; ⊠ : unknown; MDM: metachronous distant metastasis.

Discussion

Lung and bone metastases have been documented in the patients with repeated local recurrence of thyroid cancer. Distant metastasis may arise due to cancer cells developing into a more aggressive phenotype enabling dissemination.

CD44 is a polymorphic family of immunologically related cell surface glycoproteins implicated in cell-cell and cell-extracellular matrix interaction, lymphocyte activation, and tumor growth and progression.[13] The homing receptor CD44 has a complex structure with 10 variant exons that can be spliced into the extracellular domain. The receptor molecule containing splice variant exon 6 is often aberrantly expressed on malignant tumors, including lymphomas, and colon, pancreas and breast cancers.[1]

In the present study, CD44v6 expression in the primary tumor of thyroid gland was significantly lower in older (≥50 years old) patients than in younger patients (<50 years old). However, no association was found between CD44v6 expression and other clinico-pathological factors, such as sex, size, distant metastasis and capsular invasion. Age is thought to be one of the most important variables related to prognosis of patients with papillary thyroid cancer. The AGES, AMES, MACIS and Cancer Institute Hospital scoring systems use age as a factor to predict prognosis.[14-17] CD44v6 expression in the primary tumor was observed more frequently in our younger patients. Furthermore, even though CD44v6 expression in the primary tumor was detected in 7 patients with local recurrence, 5 of the 7 patients lost CD44v6 positivity at the recurrent sites. Therefore, CD44v6 expression in the primary tumor is not always closely linked to the risk of developing distant metastasis.

CD44v6 impact on prognosis of various tumors is currently controversial. It has been claimed that overexpression of CD44v6 is associated with metastasis of prostate cancer,[4,18] and prognosis of

thymic tumors,[7] and breast[5] and gastric cancers.[6] On the other hand, some reports have suggested that CD44v6 might be negatively associated with the progression of several tumor types. Reduced CD44v6 expression was correlated with manifestations of aggressiveness in lung cancer.[8] In addition, CD44v6 does not appear to be implicated in the progression and metastasis of endometrial cancer.[9]

In the present study, statistically significant correlations between CD44v6 expression in the recurrent lymph node and distant metastasis were not documented. Yet, 5 of the 6 patients with distant metastasis had CD44v6-positive recurrent lymph nodes. In 2 of the 5 patients, distant metastases occurred after CD44v6 restoration had been noted in the later-onset recurrent lymph node metastasis (patients 2 and 3 in Figure 3).

As ectopic expression of CD44v6 has been found to confer metastatic potential in a non-metastasizing rat pancreatic carcinoma cell line, CD44v6 is apparently linked to the increase in malignant potential of cancer cells.[9] Our findings are in line with the notion that if CD44v6 expression is upregulated during cancer progression, it may promote the development of distant metastasis.

In conclusion, our data suggest that CD44v6 plays an important role in the acquisition of aggressive phenotypes of differentiated thyroid cancer, and potentiates the formation of distant metastasis from repeated local lymph node recurrence in a subset of patients.

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