Original article
UDC: 616.441-006:576.385.5

Archive of Oncology 2003;11(1):5-8.

Immunohistochemical expression of CD44 in thyroid gland lesions

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BACKGROUND: *CD44 is a polymorphic family of cell surface proteoglycans and glycoproteins implicated in cell-to-cell and cell-to-matrix adhesion interactions and tumor metastasis. Its expression appears to be an indicator of invasive and metastatic behavior in carcinomas. The purpose of our study is to investigate the immunohistochemical expression of CD44 protein in thyroid lesions and its association to other histopathological parameters.*

METHODS: *Samples from thyroid lesions were obtained from 40 patients treated in our hospital. The material consisted of 5 cases of multinodular goiter, 7 cases of thyroiditis (Hashimoto type), 5 cases of follicular adenoma, 4 cases of Hurthle cell tumor, 15 cases of thyroid carcinoma (11 papillary carcinomas, and 4 myeloid tumors), and 4 cases of normal thyroid tissue. Immunostaining was performed using the Ventana ES automated immunostainer. A monoclonal antibody was used and avidin-biotin method was applied to paraffin-embedded samples. A membranous immunostaining pattern was considered positive.*

RESULTS: *CD44 expression was detected in three adenomas (60%), mostly of follicular type, and in eight carcinomas (72%). The CD44 immunostaining was especially apparent in papillary type of carcinomas, which showed high expression. In normal thyroid tissue, a reduced CD44 expression was observed.*

CONCLUSION: *The results of our study indicate that deregulated expression of CD44 contributes to the ability of thyroid carcinomas for invasion and metastasis and may constitute a prognostic factor for malignant biological behavior.*

KEY WORDS: *Antigens, CD44, Immunohistochemistry, Thyroid Diseases*

INTRODUCTION

The expression of CD44 molecule has been correlated to carcinogenesis and aggressive biological behavior of several malignant tumors. CD44 is a polymorphic family of cell surface proteoglycans and glycoproteins implicated in cell-cell and cell-matrix adhesion interactions, lymphocyte activation and homing, cell migration and tumor metastasis (1-3). CD44 protein is encoded by a gene located on 11p13 chromosome and consists of at least 20 exons. Polypeptide isoforms of CD44 are produced by alternative splicing of at least 10 of the 20 exons during mRNA processing (3). The simplest CD44 isoform (CD44 standard or CD44s) does not contain any additional exon product and is expressed by hematopoietic cells and most epithelial cells. Various combinations of variant isoforms (CD44v), containing the product of one or more additional exons, are expressed by normal epithelial cells and by leucocytes (4).

Interest has recently focused on the abnormal transcription of CD44 variants by cancer cells, and on their role in local invasion and metastatic dissemination (4). Alterations in the composition of CD44 protein and of its isoforms are associated with neoplastic transformation and metastasis in a number of different tissues. So, increased expression of CD44 has been implicated in melanoma metastasis (5), pancreatic adenocarcinomas, colorectal carcinomas (6), non-Hodgkin lymphomas and breast and lung carcinomas (7). On the other side, decreased expression of CD44v6 is related to tumor recurrence and unfavorable outcome in poorly differentiated squamous cell (7) and laryngeal carcinomas, as well as in superficial bladder (8) and prostate carcinomas

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The manuscript was received: 17. 02. 2003

Provisionally accepted: 24. 02. 2003

Accepted for publication: 06. 03. 2003

(9,10). CD44 variant isoforms are also expressed in normal and neoplastic thyroid tissue, but it is unclear whether they have any prognostic value in differentiated thyroid carcinoma (DTC) (9). DTC, which includes papillary and follicular carcinomas and their subtypes, is a rather common endocrine malignancy. Although the majority of patients have usually a mild clinical course and favorable prognosis, they often present with metastasis to regional lymph nodes and occasionally with invasion of local neck structures. Blood vessel invasion or distant metastases are uncommon. Despite these interesting biological properties, very little is known about the molecular factors involved in the pathogenesis and metastatic properties of this neoplasm (3).

In the present report, we investigated the immunohistochemical expression of CD44 protein in benign and malignant thyroid lesions, we evaluated the possible alternations in its expression and we associated our results with other classical clinicopathologic parameters.

PATIENTS AND METHODS

Samples from thyroid lesions were obtained in 40 patients (34 women and 6 men) aged 45 through 50 years, treated in our hospital. The material comprised: 5 cases of multinodular goiter, 7 cases of thyroiditis (Hashimoto type), 5 cases of follicular adenoma, 4 Hurthle cell neoplasms and 15 cases of thyroid carcinoma (11 papillary and 4 medullary carcinomas) (Table 1).

Table 1. Histopathological diagnoses in 40 cases examined

Thyroid tissue	Number of cases
Papillary carcinoma	11
Medullary carcinoma	4
Adenoma	5
Multinodular goiter	5
Thyroiditis (Hashimoto t.)	7
Hurthle cell tumor	4
Normal thyroid tissue	4
Total no. of cases	40

At the same time, samples from 4 normal thyroid glands, excised for another reason, were examined. Avidin-biotin-peroxidase (ABC) method was applied to paraffin-embedded samples, using the Ventana ES automated immunostainer. The monoclonal antibody antihuman phagocytic glycoprotein-1, CD44 (DAKO), 1:50 was used.

The stained slides were examined microscopically. Counting of CD44 positive cells was performed on the most characteristic areas. The percentage of positive cells was evaluated by counting 10 high magnification power fields (x40) in three consecutive tissue sections, using semiquantitative criteria: 0= negative; 1+= less than 25% positive staining; 2+= 25%-50% positive staining; 3+= greater than 50% positive area. The cellular intensity of the stain was recorded as weak, moderate or strong. A continuous membranous immunoreactivity was considered positive. Neither cytoplasmic staining alone nor punctate membrane staining was considered positive (Figures 1-4).

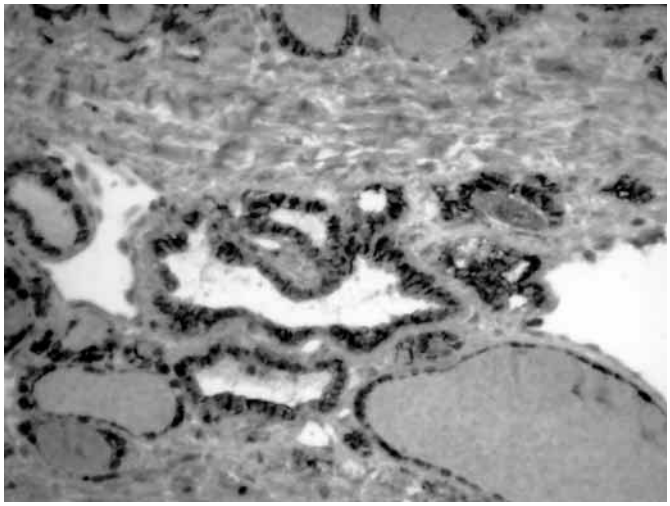

Figure 1. CD44 expression in multinodular goiter (x100)

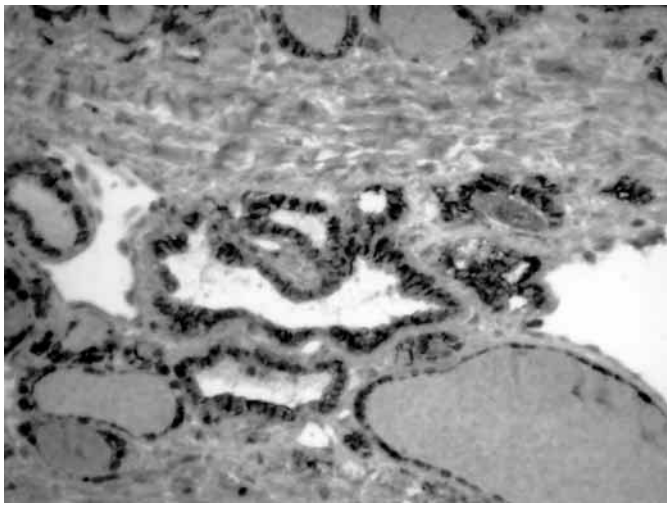

Figure 2. CD44 expression in a case of thyroiditis (Hashimoto type) (x100)

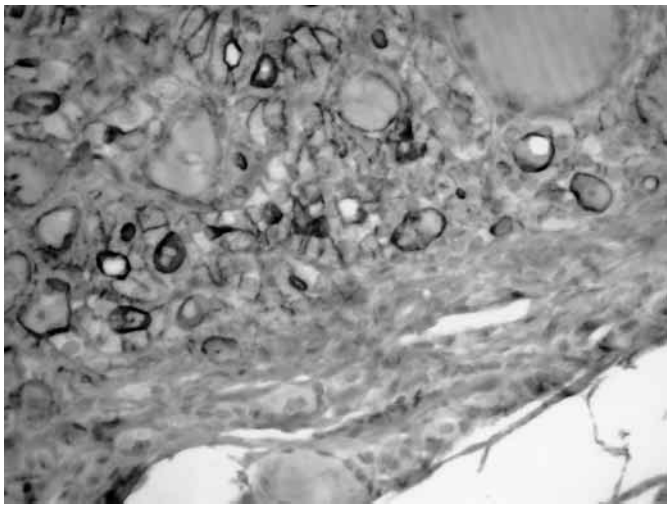

Figure 3. Follicular adenoma, CD44 expression (x100)

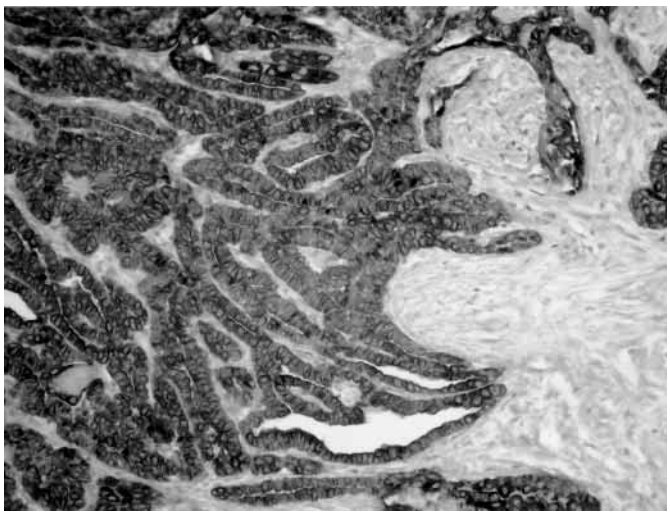

Figure 4. Papillary thyroid carcinoma, CD44 expression (x100)

RESULTS

The results of our study are summarized in Table 2.

Table 2. Relation between CD44 expression and histopathological diagnoses in the examined cases

Thyroid tissue	0+	(%)	1+	(%)	2+	(%)	3+	(%)
Papillary carcinoma	2	(18)	1	(19)	4	(36)	4	(36)
Medullary carcinoma	4	(10)	-	-	-	-	-	-
Adenoma	-	-	2	(40)	1	(20)	2	(40)
Multinodular goiter	1	(20)	2	(40)	1	(20)	1	(20)
Thyroiditis (Hashimoto type)	4	(57)	2	(28)	-	-	1	(14)
Hurthle cell tumor	3	(75)	-	-	-	-	1	(25)
Normal thyroid tissue	2	(50)	2	(50)	-	-	-	-

Intense immunoreactivity with anti-CD44 was observed in 4 of the 11 thyroid papillary carcinomas (36%) and moderate immunostaining was also observed in 4 cases (36%), while in the rest 3 cases (27%) there was weak or no membranous staining. None of the 4 medullary carcinomas (100%) expressed CD44. The 5 adenomas presented a variety of immunoreactivity; two of them (40%) expressed intense immunostaining, one adenoma (20%) presented moderate staining and the rest of them (40%) presented weak immunoreactivity. In the 5 cases of multinodular goiter a wide range of CD44 expression was present; in two of them (40%) a weak immunostaining was observed, while the rest three cases presented intense (20%), moderate (20%) and no staining (20%) correspondingly. Six of seven cases of thyroiditis (85%) presented weak or no staining at all and only in one case (14%) an intense positivity with anti-CD44 was observed. A similar expression of CD44 was present in the 4 Hurthle cell tumors; three of them (75%) were not immunoreactive for anti-CD44 and only one case (25%) presented an intense staining. In the 4 cases of normal thyroid tissue (100%) a weak or negative CD44 expression was observed.

DISCUSSION

The present study concerns material of archives, which was immunohistochemically examined. Immunohistochemistry is an easily applicable and relatively fast and cheap procedure (10). We analyzed CD44 expression in benign and malignant thyroid lesions and we correlated it to other histopathological parameters. Four from 11 papillary thyroid carcinomas presented an intense immunostaining and in 4 cases we observed a moderate staining. This fact is according to mild biological behavior and excellent prognosis of these neoplasms. The 5 cases of adenoma expressed a variety of staining, with a propensity to moderate and intense immunoreactivity. In the 5 cases of multinodular goiter we also observed a width of CD44 expression, a finding that is probably related to the functional condition of follicular cells. Six of seven cases of thyroiditis (Hashimoto type) presented weak or negative CD44 expression and only in one case we observed intense immunostaining. Similar expression was present in the 4 Hurthle cell tumors, three of which showed negative expression and only one showed intense immunoreactivity. This fact is probably related to metaplastic epithelial alterations observed in these lesions. In the samples of normal thyroid tissue we observed a reduced expression of CD44.

CD44 and its isoforms have been described in normal epithelia such as the basal layer of esophagus, the myoepithelial cells of the breast, the pneumocytes and bronchial epithelium, the base of the crypts of the small and large intestine, but also in various human cancers, including mammary, esophageal, gastric, lung and colon cancers. In human epithelial tumors, CD44 variants, although preferentially localized to the neoplastic cells, are also weakly expressed by the adjacent normal tissues (6). The presence of CD44 isoforms on cancerous cells has been correlated to tumor stage and prognosis. Elevated expression of CD44 is associated with the metastatic potential of malignant melanoma (Regauer et al.) (5). Durai et al. and Saegusa et al. show that malignant as well as borderline tumors of the ovary usually express a large spectrum of CD44 isoforms, in contrast to benign tumors (4,12). Several authors (6) have noted the correlation between CD44v6 expression and high metastatic potential with poor outcome in mammary, nasopharyngeal, non-small cell lung carcinoma and non-Hodgkin's lymphoma. Later on, it became evident that CD44v6 may be a good marker of tumor progression and metastasis in some types of tumors, but it can have no correlation or inverse correlation in other settings, like squamous-cell carcinomas (Hudson et al.) (7). In colorectal tumors, the significance of CD44v6 expression remains to be defined. The association of CD44 expression with higher stage of disease and metastatic potential is contradictory. Coppola et al. (6), using immunohistochemistry and Western-blot analysis, described the

predominant CD44v6 expression in colorectal adenocarcinomas and its loss at the metastatic site. Because little is known about the functions of the CD44 isoforms, additional experiments are required to exactly define relevant isoforms and their biological role (3). In differentiated thyroid carcinoma it is unclear whether the deregulated expression of CD44 has any prognostic value (9). Most of our bibliographic reports are based on PCR studies. Ermak et al. applying PCR and hybridization technique, describe that papillary thyroid carcinomas, adenomas and multinodular goiters exhibit a generalized increase in CD44mRNA isoforms, distinguishing them from histologically normal thyroid tissue (3,13). Studies have also been made about CD44 immunostaining in thyroid fine-needle aspirates of papillary carcinoma. Ross et al. conclude that the majority of thyroid papillary carcinomas express CD44 immunoreactivity in a membranous pattern, in contrast to normal follicular cells and nonpapillary carcinoma thyroid lesions, even those with cytologic features suggestive of papillary carcinoma (11,14).

Abnormalities in CD44 isoform expression are associated generally with a disordered growth state of the follicular cells. The deregulated expression of CD4 may be involved directly in the pathogenesis of these disordered growth states, given the known role of CD44 in cell-cell and cell-matrix adhesion. Alterations in CD44 isoform expression on the cell surface could change cell-cell interactions or interactions with macromolecules, such as collagen, fibronectin, and hyaluronate, in the extracellular matrix, resulting in a disordered growth pattern. Therefore, it is possible that deregulation of CD44mRNA processing is a very early step in the pathway of successive molecular events that lead to development of papillary cancer (13). The selective expression of CD44 cell-adhesion molecule in thyroid papillary carcinoma, in addition to the potential diagnostic value, may have relevance in predicting disease progression. The expression of this lymphocyte homing and attachment molecule may explain the propensity for this tumor to involve regional lymph nodes (11).

CONCLUSION

In conclusion, the results of our study indicate that most papillary carcinomas of the thyroid express the cell-adhesion molecule CD44, in contrast to other neoplastic and nonneoplastic thyroid lesions and normal follicular cells. It seems that deregulated expression of CD44 plays a role in the carcinogenesis of thyroid gland and possibly contributes to the mild biological behaviour of differentiated papillary carcinomas. However, a greater number of cases and further studies of CD44 activity are needed in order to evaluate CD44 expression in thyroid gland lesions and determine this molecule as a prognostic marker.

Acknowledgments

The authors thank E. Velkopoulou, technician, Department of Pathology, for technical assistance.

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