S100A10 Expression in Thyroid Neoplasms Originating from the Follicular Epithelium: Contribution to the Aggressive Characteristic of Anaplastic Carcinoma

YASUHIRO ITO1, KAZUMORI ARAI2, RYUSHI NOZAWA3, HIROSHI YOSHIDA1, TAKUYA HIGASHIYAMA1, YUUKI TAKAMURA1, AKIHIRO MIYA1, KAORU KOBAYASHI3, KANJI KUMA1 and AKIRA MIYAUUCHI1

1Kuma Hospital, Kobe; 2Department of Pathology, Shizuoka General Hospital; 3Laboratory of Host Defense, University of Shizuoka, Japan

Abstract. Background: S100A10, a member of the S100 family, forms a heterotetramer with annexin II and promotes carcinoma invasion and metastasis by plasminogen activation. In this study, S100A10 and annexin II expression in thyroid neoplasms were demonstrated. Patients and Methods: The expression levels of S100A10 and annexin II in 193 thyroid neoplasms were immunohistochemically investigated. Results: S100A10 and annexin II were not expressed in normal follicular cells or any follicular adenomas. Cells stained positively in 14.6% and 20.8% of follicular carcinomas for S100A10 and annexin II, respectively, but their expression levels were always low. S100A10 and annexin II were expressed in all papillary carcinomas, but 88.2% and 82.8% of papillary carcinomas were classified in the low group. These expression levels were not linked to any clinicopathological features. S100A10 and annexin II were also expressed in all anaplastic carcinomas, with 83.3% of these lesions were classified in the high group. Conclusion: These findings suggest that S100A10 and annexin II contribute to the aggressive characteristics of anaplastic carcinoma, while playing a constitutive role in papillary carcinoma.

S100A10 belongs to the S100 family comprised of calcium-binding proteins with a common EF-hand, helix-loop-helix motif (1, 2). S100A10 is known to form a heterotetramer with annexin II, which is located in the cell membrane. These complexes regulate the stimulation of plasminogen activation that is related to tumor invasion, metastasis and angiogenesis. Indeed, S100A10 knock-down cells showed a loss in extracellular matrix degradation and invasiveness (3-5). Recently, S100A10 gene overexpression was observed in renal cell carcinoma cells and S100A10 and annexin II protein expressions were immunohistologically positive in all tissues of renal cell carcinoma (6, 7). However, Ji et al. (8) demonstrated that the S100S10 mRNA expression level is likely to be reduced in esophageal carcinoma, indicating that S100A10 expression in carcinoma is organ-specific.

Thyroid carcinoma originating from follicular cells is a representative malignancy of endocrine organs. This carcinoma consists of two prominent histological types: papillary and follicular carcinomas. Follicular carcinoma histologically resembles benign adenoma and the differential diagnosis is often difficult and even confusing. Although thyroid carcinoma has a mild characteristic and generally grows slowly, it occasionally becomes dedifferentiated and transforms into anaplastic carcinoma, which is one of the most aggressive malignancies (9). The mechanism and trigger of dedifferentiation remains unclear except for the p53 gene alteration (10).

We have previously demonstrated four S100 proteins, S100A2, S100A4, S100A6, and S100A9, in thyroid carcinoma and demonstrated that these isforms contribute to carcinoma progression in various ways (11-13). In this study, we investigated S100A10 expression together with annexin II expression in thyroid neoplasms to elucidate their clinical significance.

Patients and Methods

Tissue specimens. Tissue specimens of thyroid neoplasms were obtained from 193 patients who underwent surgery at the Kuma Hospital. These specimens consisted of 36 anaplastic carcinomas, 93 papillary carcinomas, 48 follicular carcinomas and 16 follicular adenomas. This project was approved by the ethics committee of our hospital and written informed consent was obtained from the participating patients. For immunohistochemistry, tissues were fixed with 10% formalin and paraffin-embedded.
Antibodies. A mouse antihuman S100A10 antibody provided by our coworkers and a mouse antihuman annexin II antibody (BD Bioscience, Tokyo, Japan) were adopted. They were applied at dilutions of 1:200 and 1:100, respectively.

Immunohistochemistry. Immunohistochemistry was performed using a simple staining method (Nichirei, Tokyo, Japan). Briefly, tissue sections 4 μm-thick were dewaxed and endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in methanol for 15 min. For antigen retrieval, sections were immersed in 0.03 mol/L citrate buffer (pH 6.0) and incubated at 95°C for 40 min. After rinsing in phosphate-buffered saline (PBS) pH 7.2, 10% bovine serum (Wako, Osaka, Japan) was applied for 20 min to block nonspecific reactions. The sections were then incubated overnight at 4°C with a primary antibody. After rinsing in PBS, these specimens were treated with peroxidase-labeled anti-mouse and anti-rabbit immunoglobulins (Nichirei, Tokyo, Japan) for 30 min. After washing with PBS, the peroxidase reaction was visualized by incubating the sections with 0.02% 3,3’-diaminobenzidine tetrahydrochloride in 0.05 M Tris buffer with 0.01% hydrogen peroxide. The sections were counterstained with hematoxylin. The immunospecificity of these antibodies has been described previously.

Immunohistochemical evaluation. Immunoreactivity was observed in cell membranes. The cells were regarded as positive for S100A10 or annexin II when immunoreactivity was clearly observed in their cell membranes as described elsewhere (7). The labeling index (LI) for each case was calculated by counting cells expressing these proteins per 1000 cells. Cases were classified into five categories based on the LI: ++++, 75% or more of cells stained positive; ++, 50-74% of cells stained positive; +, 25-49% of cells stained positive; ±, less than 25% of cells stained positive; –, no positive cells were observed. Cases of ++++ or +++ were classified as the high group and the remaining cases were classified as the low group.

Statistical analyses. The Chi-square test and Fischer’s exact test were employed to analyze the relationship between S100A10 or annexin II expression and various clinicopathological features. A p-value less than 0.05 was regarded as significant.

Results

S100A10 and annexin II expressions were negative in normal follicular cells (Figure 1a). Their immunoreactivity, however, was frequently observed in vascular endothelial cells in peritumoral stroma.

In thyroid neoplasms, immunoreactivity of S100A10 and annexin II could be observed in the cell membrane and their expression levels were significantly linked to each other (Table I). Table II summarizes S100A10 expression in thyroid neoplasms. All of 16 follicular adenomas and 41 of 48 follicular carcinomas (85.4%) stained negatively for S100A10 (Figure 1b). The remaining 7 cases were positive for S100A10, but were classified in the low group. S100A10 was, however, positive in all papillary and anaplastic carcinomas. The S100A10 expression level was significantly higher (p<0.0001) in anaplastic carcinoma than in papillary carcinoma, with 83.3% of anaplastic carcinomas being classified in the high group, while 88.2% of papillary carcinoma were classified in the low group (Figure 1c, 1d).

There was no annexin II expression in follicular adenoma and 79.1% of follicular carcinoma stained negatively for annexin II (not shown). Similar to S100A10, all anaplastic and papillary carcinomas expressed annexin II, but the expression levels significantly differed (p<0.0001) (Figure 1e, 1f) as shown in Table III.

The relationship between the S100A10 expression level and clinicopathological parameters of papillary carcinoma is summarized in Table IV. The S100A10 expression level was not significantly linked to clinicopathological parameters which reflect patient prognosis such as age, tumor size, extrathyroid extension and node metastasis. We also investigated the relationship between annexin II expression and clinicopathological parameters and obtained the same findings (data not shown).
Figure 1. a) Lack of S100A10 expression in normal follicular cells; b) lack of S100A10 expression in follicular carcinoma; c) low expression of S100A10 in papillary carcinoma; d) high expression of S100A10 in anaplastic carcinoma; e) low expression of annexin II in papillary carcinoma; f) high expression of annexin II in anaplastic carcinoma; original magnification: x250.
Discussion

In this study, it was demonstrated that the expression levels of S100A10 and annexin II were significantly related to each other, which is similar to the finding in renal cell carcinoma. This indicates that S100A10 functions as a receptor for plasminogen and stimulates plasminogen activation by forming a complex with annexin II in thyroid neoplasms.

Furthermore, we demonstrated that the incidence of S100A10 and annexin II expressions and their expression levels in thyroid neoplasms varied by histological type. S100A10 and annexin II expression were not found in any follicular adenoma, indicating that the events modulated by their complexes do not contribute to the progression of this disease. Since follicular carcinoma histologically resembles follicular adenoma, follicular adenoma is thought to transform into follicular carcinoma (14). However, the incidence of S100A10 and annexin II expression remained low at 14.6% and 20.1%, respectively, and the expression levels were also low. These findings suggest that S100A10 and annexin II do not play an important role in the progression of follicular carcinoma and malignant transformation from adenoma to carcinoma.

In contrast, S100A10 and annexin II expression were positive in all papillary carcinomas. Since normal follicular cells stained negatively for both, it is suggested that the S100A10 and annexin II complex plays some role in the appearance and/or progression of papillary carcinoma. However, the expression levels were usually low, with 88.2% and 82.8% of cases being classified in the low group for S100A10 and annexin II expression, respectively. Furthermore, expression levels of S100A10 and annexin II did not increase with carcinoma progression (Table IV). Therefore, these proteins may play a constitutive role in papillary carcinoma rather than directly contribute to carcinoma progression. The progression patterns of papillary and follicular carcinomas are different. Follicular carcinoma shows capsular invasion but extrathyroid extension to adjacent organs is less common than in papillary carcinoma. Papillary carcinoma is more likely to show lymph node metastasis but shows distant metastasis less frequently than follicular carcinoma (15). The difference in expression levels of S100A10 and annexin II may reflect the difference in developmental patterns between these two types of carcinoma.

Anaplastic carcinoma usually grows rapidly by extension to adjacent organs, node metastasis, and distant metastasis (9). S100A10 and annexin II were diffusely expressed in 83.3% of anaplastic carcinoma, indicating that they play a role in the aggressive characteristics of this carcinoma.

Conclusion

We demonstrated that S100A10 and annexin II may contribute to the aggressive characteristics of anaplastic carcinoma. Furthermore, they may play a constitutive role in papillary carcinoma but not in follicular carcinoma and adenoma. Further studies regarding the mechanism of their contribution to thyroid carcinoma progression will provide novel information that may be useful in improving therapy for thyroid carcinoma, especially anaplastic carcinoma.
References


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