

*Original Article*

## Localization of S100C Immunoreactivity in Various Human Tissues

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Using 2-dimensional gel electrophoresis, we previously demonstrated that the S100C protein remarkably decreased after immortalization of normal human fibroblasts, and that this protein caused growth inhibition of human tumor cells when forcibly expressed in these cells, suggesting that S100C plays a significant role in tumor suppression. The present study was carried out to determine what type of human tissues express S100C protein, and, subsequently, whether the S100C content in these tissues changes after normal cells have been transformed into cancer cells. We found that ductal cells in various tissues were positively stained with the S100C protein. In comparison, epithelial cells in digestive organs such as the stomach, small intestine, and colon were not stained as strongly. When 14 pairs of human normal and cancerous tissues were stained with the antibody, decreases in the staining levels of S100C were observed in 6 kinds of cancerous tissues — from the bronchus, mammary duct, renal tubule, prostate, uterus, and testis — in comparison with staining in their normal counterparts. These results suggest that S100C is a new tumor marker protein, the expression of which significantly decreases after malignant transformation of human tissues.

**Key words:** S100C-antibody, human tissues, immunostaining

**S**100C, an EF-hand type Ca<sup>2+</sup>-binding protein belonging to the S100 family, was named after the solubilization of the first isolated protein in 100% saturated ammonium sulfate. This protein family regulates a number of biological activities via interaction with target proteins such as annexins [1], cytosolic phospholipase A<sub>2</sub> [2], the Ca<sup>2+</sup> release channel of the sarcoplasmic reticulum [3], and myosin [4]. In addition, it is currently thought that S100 proteins are involved in the regulation of many cellular processes such as cell cycle

progression and differentiation [5, 6].

At least 13 S100 genes are found to be clustered on human chromosome 1q21 [7]. Interestingly, this chromosomal region is frequently rearranged in tumors, possibly exerting direct or indirect influences on the differential expression of S100 proteins in tumor cells. By 2-dimensional polyacrylamide gel electrophoresis (2-D PAGE), we previously found that S100C was down-regulated in immortalized human cells, and that it caused growth inhibition of human cancer cells when it was forcibly expressed in these cells [8, 9]. The present study was carried out to determine what type of cells in human tissues including various cancers could express S100C protein.

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## Materials and Methods

Surgically resected normal and cancerous tissue pairs studied in the present study included 8 stomach, 1 small intestine, 8 colon, 3 skin, 2 thyroid, 2 lung, 3 breast, 2 liver, 2 kidney, 2 bladder, 2 prostate, 3 uterus, 1 testis, 1 ovary, and 5 brain tissue specimens.

Immunohistochemical staining was performed using 10% phosphate-buffered, formalin-fixed and paraffin-embedded tissues according to the standard protocol. The samples were incubated with a rabbit anti-human S100C antibody (diluted at 1:1000) that was raised in our laboratory as described previously [8], anti-rabbit biotinylated IgG (diluted at 1:100, Vector Lab., CA, USA), and streptavidine-horseradish peroxidase (diluted at 1:200), respectively. Peroxidase activity was visualized using 3-amino-9-ethylcarbazole.

## Results

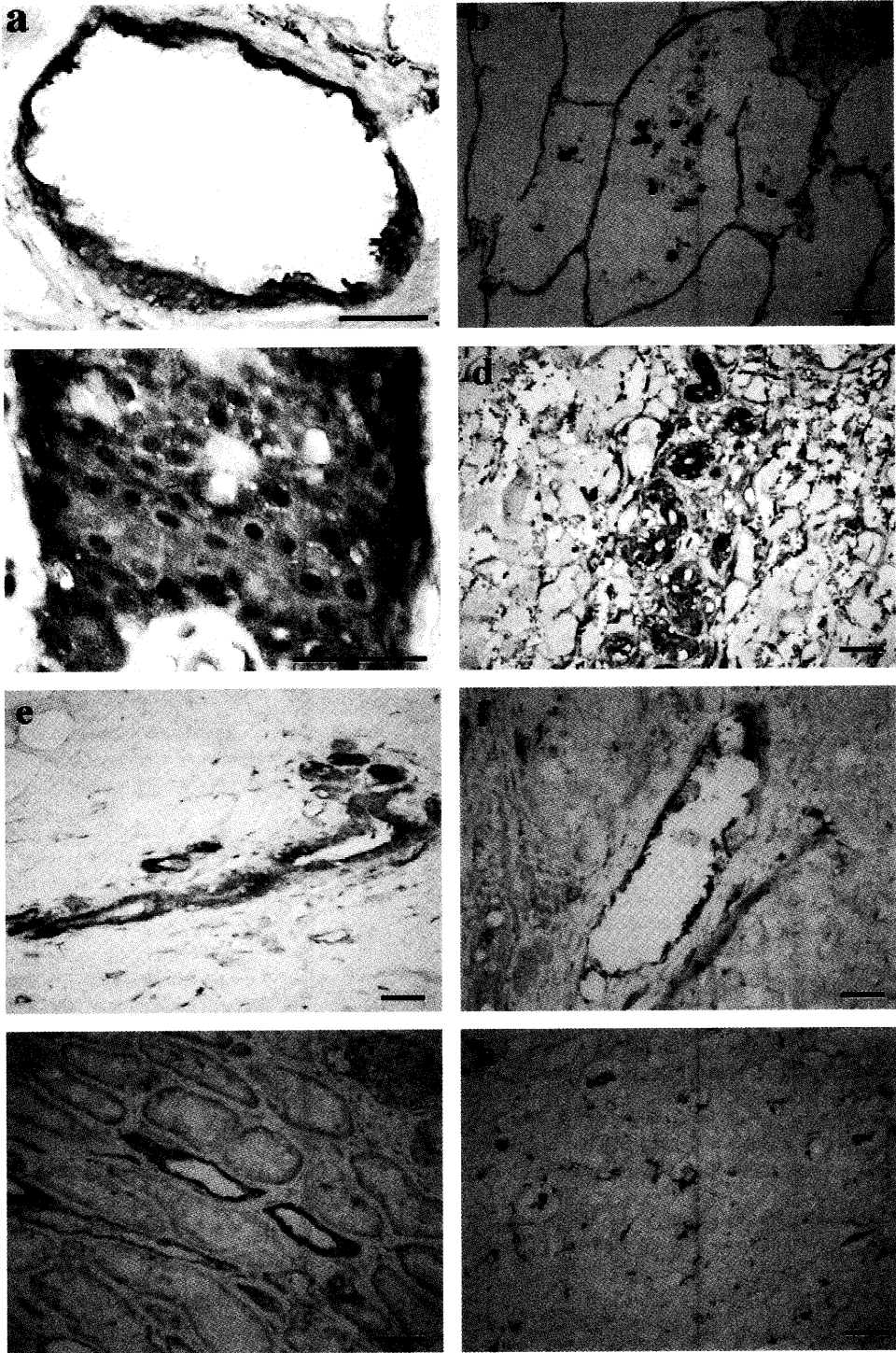
We first performed S100C staining of 19 kinds of human normal tissues, including samples from the stomach, small intestine, colon, skin, adrenal gland, blood vessel, thyroid, lung, mammary gland, liver, bile duct, urinary tubule, bladder, brain, prostate, uterus, testis, and ovary. As shown in Fig. 1a, the cytoplasm of bronchial epithelial cells was positively stained with the S100C antibody, whereas that of lung alveolar cells were not stained (Fig. 1b). Interestingly, the cytoplasm of basal cells of skin showed a positive stain, but once the basal cells left the basement membrane, moving upward, the S100C protein entered the nuclei, leaving the cytoplasm hardly stained (Fig. 1c). In the adrenal gland, the medullary cells revealed a positively stained cytoplasm, though the cortical cells did not (Fig. 1d). The mammary and biliary duct cells were stained positively with the antibody, whereas the mammary gland cells and hepatic cells showed a negative staining (Figs. 1e and 1f). The tubules in the kidney expressed the S100 C protein (Fig. 1g). Although this data was omitted, the cytoplasm of subcutaneous fibroblasts showed positive for S100C staining. In the cerebral cortex, neurons were positively stained but glial cells were not (Fig. 1h). Ductal cells in various organs were stained definitely positive. Other tissues, such as those from stomach, small intestine, colon, thyroid, bladder, testis, and ovary, were weakly stained with the S100C antibody. Hepatocytes were negative for S100C staining.

We compared the S100C stainings in 14 pairs of normal and cancerous tissues. As shown in Table 1, there was a significant decrease in S100C staining intensity in cancerous tissues of the bronchus, mammary duct, renal tubule, prostate, uterus, and testis, in comparison with that in their normal counterparts. In other tissues, such as those from brain (glia cells), stomach, small intestine, colon, thyroid, liver (especially hepatocytes), bladder, and ovary, no significant difference between S100C staining in normal and cancerous tissues was found.

## Discussion

By the present immunostaining method, expression of the S100C protein was generally found in the ductal cells and fibroblastic cells in almost all kinds of tissues. The expression levels of S100C were relatively low in epithelial cells of digestive organs such as the stomach, small intestine, and colon, and in the thyroid, bladder, and ovary. Interestingly, no expression was observed in lung alveolar cells, hepatocytes, or glial cells. These findings indicate that the expression of the S100C protein in these ductal cells may be due to the presence of some type of basement membrane, *e.g.*, to the different compositions of various types of collagens. However, further study is needed to determine the relationship between the S100C protein and its functions in the ductal cells.

S100 proteins have been shown to be closely related to neoplastic tissues such as that in breast cancer [7, 10]. In this respect, S100A4 is of particular interest because this protein has been shown to be overexpressed in human breast tumors [11]. When the S100A4 gene was transfected in mouse melanoma cells, the exogenous S100A4-overexpressing cells exhibited a metastatic phenotype [12]. In contrast, S100A2 is down-regulated in breast tumors and a variety of other neoplasms, suggesting that it could be a tumor suppressor [7]. Therefore, the respective expressions of S100A2 and S100A4 may have inverse functions in tumorigenesis. Thus, balance of the expression levels of S100 proteins in the cell may be significant in the regulation of normal cell growth. Like S100A2, S100C is also a growth inhibitor. It is noteworthy that approximately 50% identity exists between S100C and S100A2 at the amino acid level, and that most of the other amino acids are homologous. Thus, S100C and S100A2 may have similar structures and functions. Here we showed that



**Fig. 1** S100C stainings in various human tissues. S100C was immunohistochemically stained by the immunoperoxidase method. a, bronchial duct (the cytoplasm looks dark by the immunostaining, but the nuclei appear pale); b, lung alveolus; c, skin (the left is the basement membrane, and the right is the cornified layer of the skin); d, adrenal gland; e, mammary duct; f, bile duct; g, tubules in the kidney; h, cerebral cortex. Scattered black cells are neurons that stained positively. Bars represent 10  $\mu$ m.

**Table 1** Comparison of S100C staining intensities in 14 pairs of normal and tumor tissues

Tissues (Cases)	Tumor tissues examined	Normal		Cancer	
		Cytoplasm	Nucleus	Cytoplasm	Nucleus
Lung (5)	Adenocarcinoma	++	—	±	—
Mammary duct (3)	Adenocarcinoma	++	—	± or —	—
Renal tubule (2)	Papillary adenocarcinoma	++	—	—	—
Prostate (2)	Adenocarcinoma	++	—	±	—
Uterus (3)	Adenocarcinoma	++	—	±	—
Testis (1)	Germinal tumor	±	—	—	—
Glia cell (5)	Malignant astrocytoma	—	—	—	—
Stomach (8)	Adenocarcinoma	±	—	±	—
Small intestine (1)	Adenocarcinoma	±	—	+	—
Colon (8)	Adenocarcinoma	±	—	±	—
Thyroid (2)	Follicular adenocarcinoma	± and —	—	+ and ±	—
Hepatocyte (2)	Hepatoma	—	—	—	—
Bladder (2)	Transitional cell carcinoma	+ and —	+ and —	±	—
Ovary (1)	Serous adenocarcinoma	±	—	+	—

loss of S100C protein expression was detected in cancer cells of the bronchus, mammary duct, renal tubule, prostate, and uterus, while their normal counterparts were strongly positive for S100C. These results indicate that the expression level of S100C may be related to tumor progression in these tissues, as is the case for expression level of S100A2.

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