Histol Histopathol (2001) 16: 707-712

http://www.ehu.es/histol-histopathol

Histology and Histopathology

Cellular and Molecular Biology

Development and progression of malignancy in human colon tissues are correlated with expression of specific Ca^{2+}-binding S100 proteins

Y. Bronckart[1], C. Decaestecker[1], N. Nagy[2], L. Harper[1], B.W. Schäfer[3], I. Salmon[2], R. Pochet[1], R. Kiss[1] and C.W. Heizman[3]

[1]Laboratory of Histopathology, Faculty of Medicine and [2]Department of Pathology, Eramus University Hospital, Free University of Brussels (ULB); Brussels, Belgium and [3]Department of Pediatrics, University of Zürich, Zürich, Switzerland

Summary. The expression levels of seven different S100 proteins (S100A1, S100A2, S100A3, S100A4, S100A5, S100A6, and S100B) were characterized by immunohistochemistry in the epithelial versus connective tissues of a series of 35 colon specimens, including 6 normal samples, 5 adenomas with low-grade dysplasia, 5 adenomas with high-grade dysplasia, and 19 cancers. The results showed that S100A2, S100A3, and S100B proteins could not (or only marginally) be detected in colon tissues. On the other hand, the expression of S100A6 increased in epithelial tissues directly proportional to the increase of malignancy. The percentage of epithelial (or connective tissue) cells expressing S100A4 significantly decreased as the malignancy grade increased. The expression level of S100A1 proteins was somewhat higher in the connective tissues of normal cases and adenomas with low-grade dysplasia than in adenomas with high-grade dysplasia and cancers. This pattern of expression was not observed in epithelial tissues. While the node-positive cancers did not express S100A1, about half of the node-negative specimens did. The expression levels of S100A5 were similar in different epithelial tissues. However, in the connective tissues the expression levels decreased inversely proportional to the increase in pathological grading of the specimens. Therefore, the present study implicates several S100 proteins as useful tools for histochemical typing of colon cancer malignancy development.

Key words: Colon cancer, S100 proteins, Connective tissue, Epithelial tissue, Diagnostic

Offprint requests to: Robert Kiss, PH.D., Laboratory of Histopathology, Faculty of Medicine - Université Libre de Bruxelles, 808 route de Lennik 1070 Brussels, Belgium. Fax: 322 555 62 85. email: rkiss@med.ulb.ac.be

Introduction

The S100 proteins belong to the superfamily of proteins containing the EF-hand Ca^{2+}-binding motif (Nakayama and Kretsinger, 1994; Schäfer and Heizmann, 1996; Heizmann and Cox, 1998). In contrast to calmodulin, which is ubiquitously expressed, S100 proteins are found in specific cell types such as nerve, glial, or epithelial cells. Recently, a large subset of new S100 proteins was identified, and a cluster of 13 genes encoding S100 proteins was identified on human chromosome 1q21 (Schäfer et al., 1995; Schäfer and Heizmann, 1996; Wicki et al., 1996a,b; Heizmann and Cox, 1998), which is evolutionarily conserved on mouse chromosome 3 (Ridinger et al., 1998). Involvement of S100 proteins in biological functions such as cell proliferation, apoptosis, motility, exocytosis or cytoskeletal organization has already been demonstrated (Schäfer and Heizmann, 1996; Heizmann and Cox, 1998). Although their role in cancer pathogenesis still remains to be elucidated, recent reports consistently show that specific S100 proteins, especially S100A4, modify the biological behavior of several tumor cells (Grigorian et al., 1996; Lloyd et al., 1998). Furthermore, Keirsebilck et al. (1998) reported that S100A4 is inversely regulated with respect to E-cadherin, which is an invasion suppressor molecule. In addition, S100A2 is a possible candidate for tumor suppression in breast cancer (Wicki et al., 1997). Moreover, we demonstrated that the expression levels of several S100 proteins (S100A3, S100A4, S100A6, S100B) significantly varied with respect to the increasing level of malignancy in brain tumors deriving from the astroglial lineage, while S100A2 was never expressed in such tumors (Camby et al., 1999). Several studies already report the differential expression of S100A4 and S100A6 proteins in colon tissues, in relation to malignancy development (Takenaga et al., 1997; Komatsu et al., 2000; Stulik et

al., 2000). In addition, others report different expression levels of S100B in the nerve cells of colon tissues under various pathological conditions (inflammatory bowel disease, ulcerative colitis, acute appendicitis, etc) (Kubota et al., 1992; Waraich et al., 1997; Monforte-Munoz et al., 1998). However, none study compared the level of expression of several S100 proteins in colon tissues, whether these tissues are normal, dysplastic or malignant. The present work thus characterizes the differential expression levels of 7 different S100 proteins in a series of 35 human colon specimens (including normal cases, adenomas with low- versus high-grade dysplasia, and cancers), demonstrating correlation between S100 protein expression and different stages of neoplasia.

Materials and methods

Specimens

The series of 35 colon tissue specimens consisted of 6 normal, 10 adenoma, and 19 cancer samples. The normal specimens came from routine biopsies taken from normal colorectal areas of patients subjected to coloscopy.

Dysplasia grading was carried out as recently detailed (Bronckart et al., 1999). All the gradings were performed by three pathologists simultaneously reviewing each case through a multihead microscope. The adenomas were graded (low versus high-grade dysplasia) in relation to the most advanced focus of dysplasia observed. A consensus classification of each case was obtained by means of the criteria that we have described previously (Bronckart et al., 1999).

Cancer staging was carried out according to the TNM system (Beahrs et al., 1992). The 19 cancer specimens included 4 T1, 5 T2, 5 T3, and 5 T4 cases. Of the 19 samples, 8 exhibited node invasions. None of the 8 node-positive colon cancers exhibited distal metastases. All of the 19 cancer cases were M0 according to the TNM staging system.

Immunohistochemical staining

The tissue specimens were fixed in 4% buffered formaldehyde and embedded in paraffin. Five-μm sections from each specimen were stained with the various antibodies against S100 proteins at 25 ± 1 °C for 60 minutes (Camby et al., 1999). The following dilutions were used: S100A1 - 1:2000, S100A2 - 1:500, S100A3 - 1:2000, S100A4 - 1:2000, S100A5 - 1:500, S100A6 - 1:10000, and S100B - 1:10000. Visualization of immunoreactive protein was achieved with the avidin-biotin-peroxidase complex (ABC) kit reagents (Vector Labs, Burlingame, CA), with diaminobenzidine/H_2O_2 as the chromogenic substrates. The control tissues were incubated with the corresponding pre-immune sera, the second antibody only, or with polyclonal antibodies against S100 proteins preabsorbed with the corresponding antigens. Counterstaining was done with toluidine blue.

Antibodies against human recombinant S100A2, S100A3, and S100B were raised in rabbits, and antibodies against human recombinant S100A1, S100A4, and S100A6 were raised in goats (Föhr et al., 1995; Huang et al., 1996; Ilg et al., 1996).

Evaluation of immunohistochemical staining

The analysis was carried out according to the methodology adapted from Gamallo et al. (1993), showing a correlation between levels of expression and histological tumor typing. We estimated the staining intensity of each S100 protein (Staining Intensity {SI} variable) and the abundance of S100 protein immunoreactive cells (Labeling Index {LI} variable) both in the epithelial and the connective tissues. The LI variable was graded from 0 to 2 by counting the percentage of positive cells (for epithelial tissue) or of positive areas (for connective tissue) in at least 20 fields (x400) per specimen. The LI scores were 0 in the absence of staining, 1 when 1-30% of cells or tissue area were reactive, and 2 when more than 30% of cells or tissue area were reactive. The SI variable was graded 0 in the absence of staining (or staining equivalent to background staining in the negative control), 1 where staining was weak to moderate, and 2 where staining was intense. The final LI/SI score for a given colon specimen corresponded to the most frequent of the 20 LI/SI scores assessed for each specimen.

The scores were examined independently by two investigators. In case of disagreement, a third investigator examined the scores.

Statistical analyses

The four histopathological groups were labeled as follows: normal = 0, low-grade dysplasia = 1, high-grade dysplasia = 2, and neoplastic = 3. Cancer stages were given a score of 1 for low stages (T1 and T2) and a score of 2 for high stages (T3 and T4); similarly, node-negative cancers were given a score of 1 and node-positive cancers a score of 2. The Chi-square (for comparing 2 groups) and the Kendall tests (for comparing more than two groups) were used to evaluate the statistical correlations between the histopathological variables and individual S100 protein expression (estimation for epithelial and connective tissues was performed using both the LI and SI variables). All the statistical analyses were carried out using Statistica (Statsoft, Tulsa, OK, USA).

Results

Morphological illustrations of S100 protein expression

We analyzed colon tissue specimens for expression of different S100 proteins. Figure 1 illustrates the

S100A5 expression observed in normal (A), high-grade dysplastic (B) and neoplastic (C) human colon tissue, and similarly for S100A6 (D-F). This figure shows variations in S100A5 expression in connective tissue and those in S100A6 expression in epithelial tissue.

Correlation of S100 protein expression with different grades of neoplasia

We correlated differential expression of S100 proteins with increasing levels of neoplasia, as summarized in Table 1. S100A2, S100A3, and S100B proteins were barely (or not) detectable in colon specimens. In contrast, expression levels of S100A5 and S100A6 varied considerably among the histopathological groups of human colon specimens (as illustrated in Fig. 1). The expression level of S100A5 protein in connective tissue significantly decreased (both for the LI and SI variables) from normal through adenomas with low-grade dysplasia, adenomas with high-grade dysplasia, to cancers (Table 1), while no significant variations appeared in epithelial tissue. The reverse phenomenon was observed for S100A6 protein in epithelial tissue (but not in connective tissue), with a marked increase of expression directly proportional to the increase in the level of morphological abnormality (Table 1). However, there was no further increase in expression level of S100A6 (in epithelial tissue) from adenomas with high-grade dysplasia to cancers. The percentage of S100A4-positive connective tissue area significantly decreased as the malignancy level increased, while S100A4 staining intensity did not change in relation to the histopathological diagnosis (Table 1). Lastly, the percentage of S100A1-positive cells in connective tissue (but not in epithelial cells) was significantly higher in normal cases and in adenomas with low-grade dysplasia than in adenomas with high-grade dysplasia and in cancers, while staining intensity remained similar (Table 1).

Correlation of S100 protein expression with tumor prognosis

With respect to prognostic variables (stage, node-negative versus node-positive cancers), only S100A1 expression exhibited significant changes. Indeed, the proportion of S100A1-positive samples was 6/11 in the node-negative group versus 0/8 in the node-positive group ($P = 0.01$). Such variance in S100A1 expression occurred only in epithelial tissue.

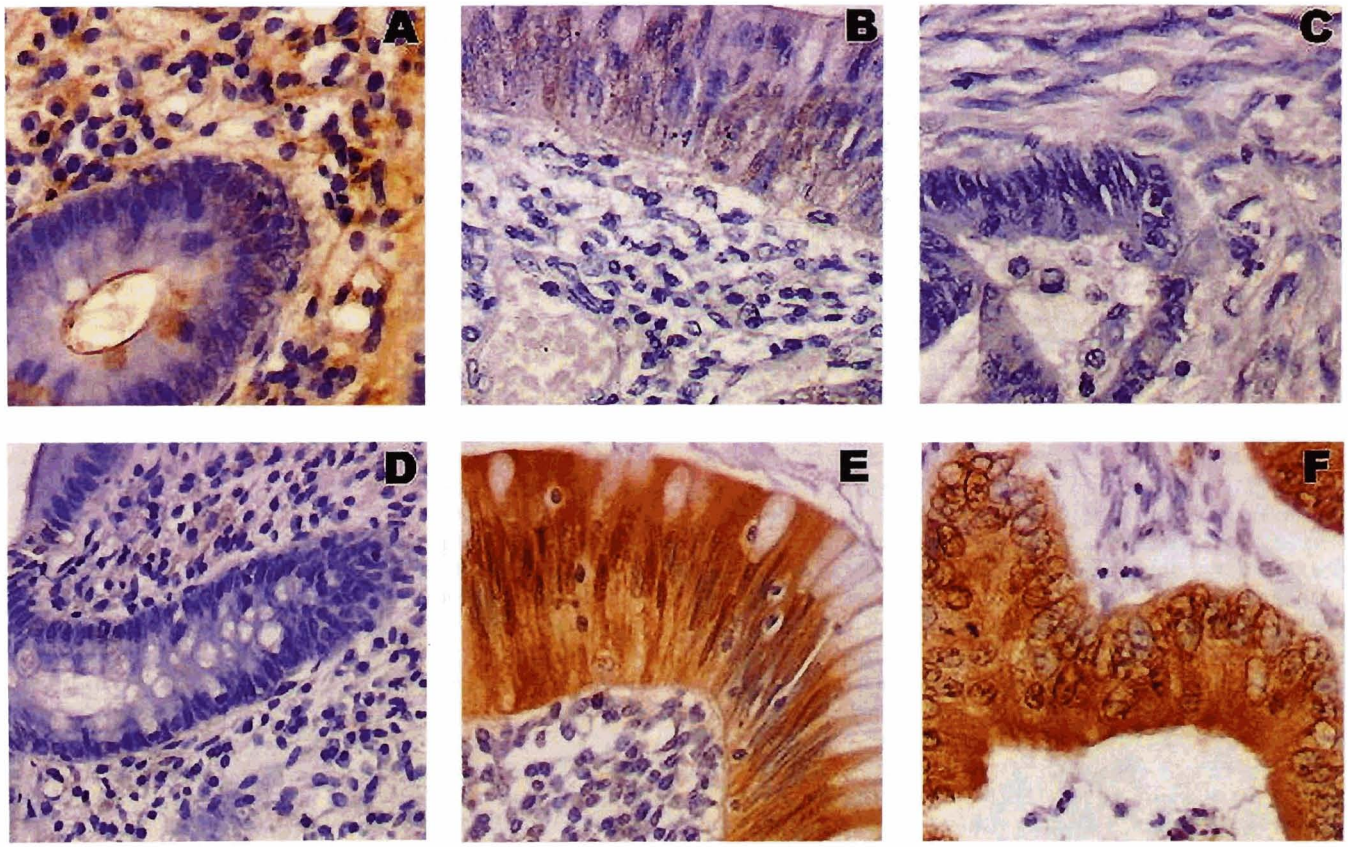

Fig. 1. Illustration of the variation in S100A5 staining in connective tissue between normal **(A)**, high-grade dysplasia **(B)** and neoplastic **(C)** human colon tissue (x 200). Illustration of the variation in S100A6 staining in epithelial tissue between normal **(D)**, high-grade dysplasia **(E)** and neoplastic (**F**) human colon tissue (x 200).

Table 1. Correlations between S100 protein expression and histopathological diagnoses. Proportions of positive cases for each S100 protein.

HISTOPATHOLOGICAL DIAGNOSES	TISSUE TYPE	S100A1	S100A2	S100A3	S100A4	S100A5	S100A6	S100B
normal	connective	3/6	0/6	2/6	6/6	5/6	2/6	0/6
(n = 6)	epithelial	2/6	0/6	0/6	2/6	3/6	0/6	0/6
low-grade dysplasia	connective	5/5	0/5	2/5	4/5	3/5	1/5	0/5
(n = 5)	epithelial	3/5	0/5	0/5	4/5	3/5	2/5	0/5
high-grade dysplasia	connective	1/5	0/5	0/5	3/5	1/5	0/5	0/5
(n = 5)	epithelial	3/5	0/5	0/5	4/5	3/5	4/5	0/5
cancer	connective	2/19	2/19	2/19	11/19	1/19	5/19	0/19
(n = 19)	epithelial	6/19	0/19	0/19	10/19	10/19	14/19	0/19
P level of statistical	connective: LI variable	- 0.006	n.s.	n.s.	- 0.003	- 0.000001	n.s.	n.s.
significance	connective: SI variable	n.s.	n.s.	n.s.	n.s.	- 0.000001	n.s.	n.s.
(n.s. = not significant	epithelial: LI variable	n.s.	n.s.	n.s.	+ 0.009	n.s.	+ 0.00006	n.s.
{P > 0.05})	epithelial: SI variable	n.s.	n.s.	n.s.	n.s.	n.s.	+ 0.00004	n.s.

n: number of cases. For each S100 protein the semi-quantitative evaluation of the immunohistochemical staining was made in epithelial and connective tissues by means of two variables: the staining intensity (the SI variable) and the relative abundance of S100 protein immunoreactive cells (Labeling Index or LI variable). The SI variable was graded as 0 (equivalent to background staining in the negative control slide), 1 (weak to moderate staining) or 2 (intense staining). The LI variable was graded as 0 = no staining, 1 = between 1 and 30% of immunostained cells or tissue area, 2 = more than 30% of immunostained cells or tissue area. Positive cases included all cases with an LI/SI phenotype different from 0/0. The Kendall test was used to evaluate the statistical correlations between LI (or SI) scores and the histological grade (normal = 0, low-grade dysplasia = 1, high-grade dysplasia = 2, cancer = 3). The sign "-" associated with a given P value indicates a negative correlation, while the sign "+" indicates a positive correlation.

Discussion

Histologically, adenomas are defined as benign neoplasms of gland-forming epithelia. High-grade dysplasia features in adenomas are considered to be immediate precursors of colorectal carcinomas. Considering pathogenesis of colon carcinomas as a development of pre-existing adenomas (Bronckart et al., 1999), high-grade dysplasia may be the link between benign adenomas and invasive cancers (Sugarbaker et al., 1985; Steele et al., 1993). Recently, Allen (1995) reviewed all known molecular events occurring in the transformation of a normal colon mucosa into a neoplastic one, emphasizing the histological standpoint reported above. The molecular abnormalities underlying this progression include early dysfunction in the adenomatous polyposis coli (APC) gene (Allen, 1995).

The aims of the present study were to investigate: i) whether progression towards increasing levels of neoplasia was associated with an altered expression of distinct S100 proteins; and ii) if expression of specific S100 proteins might have prognostic significance.

We chose the S100 family of proteins because of their already described association with tumor progression and their interactions with the p53 tumor suppressor gene (Baudier et al., 1992; Tan et al., 1999).

Some S100 proteins regulate motility of normal cells and the cytoskeleton, particularly by altering the invasiveness of tumor cells (Selinfreud et al., 1990, 1991; Grigorian et al., 1996; Wicki et al., 1997; Keirsebilck et al., 1998; Lloyd et al., 1998; Mandinova et al., 1998; Camby et al., 1999).

Baudier et al. (1992) reported that p53 may be a cellular target for the members of the S100 protein family involved in the control of the cell cycle at the G0-G1/S boundary. The S100A1 and S100B proteins can interact with the p53 tumor suppressor gene (Baudier et al., 1992), which plays a significant role in the development of malignancy in colon tumors (Allen, 1995). The S100B protein interacts in a calcium-dependent manner with the p53 protein, and this interaction inhibits p53 protein phosphorylation by protein kinase C *in vitro* (Baudier et al., 1992). It was also shown (Tan et al., 1999) that p53 positively regulates S100A2 expression. S100B was also found to activate Ndr, a nuclear protein kinase known to be involved in cell division and cell morphology (Milward et al., 1998).

The data obtained in the present study demonstrate that of the seven S100 proteins tested, the expression levels of S100A5 and S100A6 varied considerably relative to the histopathological grades of the tissues; in particular, the expression levels of S100A5 in connective tissues and S100A6 in epithelial tissues (see Table 1). In fact, both staining intensity and percentage of positive tissue areas for S100A5 markedly decreased in the connective tissues inversely proportional to the increase in histopathological grades, while the reverse correlation was observed for S100A6 in epithelial tissues. This is in agreement with a notion that S100A6 is involved in cell proliferation (Calabretta et al., 1986) and exocytosis (Okazaki et al., 1994). Our S100A6-related results also perfectly match those recently reported by Komatsu et al. (2000) which show (by means of western blot and immunohistochemical analyses) high expression levels of S100A6 in epithelial tissue of adenocarcinomas in comparison to normal mucosa and, to a lesser extent, to adenomas. To date, the physiological role of S100A5 has

not yet been identified. It is therefore not possible to determine the putative molecular mechanisms underlying the decrease of S100A5 expression in relation to the increase of malignancy. The possibility remains that the high level of S100A5 expression in the connective tissue of both normal and weakly dysplastic colon tissue reflects the accumulation of S100A5 secreted by epithelial cells, while this secretion decreases as these epithelial cells become highly dysplastic or malignant. It should be also emphasize that we were not able to perform a distinction between tumor-associated connective tissue and regular (non-tumor) mesenchyme.

Two other S100 proteins (S100A1 and S100A4) showed modified expression in relation to the histopathological grades. A negative correlation was obtained with the LI variable evaluated in connective tissue for both S100A1 and S100A4, and a positive correlation in epithelial tissue for S100A4 only (see Table 1). S100A2, S100A3, and S100B immunohistochemistry yielded no diagnostic information.

Expression of S100A1 in node-negative cancer and its lack in node-positive cancers suggests that loss of S100A1 could play a role in the early steps of colon cancer invasion. No significant difference in the expression of the remaining six S100 proteins was observed between stages T1 and T2, or T3 and T4.

In conclusion, the present study suggests that several members of the S100 family of proteins, such as S100A1, S100A4, S100A5, and S100A6, might be involved in the development and progression of malignancy in human colon tissues. While S100A4, S100A5, and S100A6 appear to be important for the development of malignancy, S100A1 may be a regulator during tumor progression. We are now in the process of investigating the possible roles of S100 proteins on cell kinetics and cell motility in tumor cells by means of a video cell tracking system recently developed in our laboratory (De Hauwer et al., 1997, 1998).

Acknowledgments. We thank M. Killen for help with the preparation and Dr. G. Davey for critical reading of the manuscript. The present work has been carried out on the basis of grants awarded by the Fonds de la Recherche Scientifique Médicale (Belgium) and by the Swiss National Science Foundation (grant no. 31-50510.97 to C.W.H) and BIOMED-2 (EU grant no. BMH4-CT96-0319/BBW grant no. 95.0215-1, Switzerland). C.D. is a Research Associate and R.K. a Senior research Associate with the Fonds National de la Recherche Scientifique (FNRS; Belgium).

References

Allen J.I. (1995). Molecular biology of colon polyps and colon cancer. Sem. Surg. Oncol. 11, 399-405.

Baudier J., Delphin C., Grunwald D., Khochbin S. and Lawrence J.J. (1992). Characterization of the tumor suppressor protein p53 as a protein kinase C substrate and a S100B-binding protein. Proc. Natl. Acad. Sci. USA 89, 11627-11631.

Beahrs O.H., Henson D.E., Hutter R.V.P. and Kennedy B.S. (1992). American Joint Committee on Cancer. Manual for staging of cancer. Fourth Edition. JB Lippincott. Philadelphia. pp 81-82.

Bronckart Y., Nagy N., Decaestecker C., Bouckaert Y., Remmelink M., Gielen I., Hittelet A., Darro F., Pector J.C., Yeaton P., Danguy A., Kiss R. and Salmon I. (1999). Grading dysplasia in colorectal adenomas by means of the quantitative binding pattern determination of *Arachis hypogaea*, *Dolichos biflorus*, *Amaranthus caudatus*, *Maackia amurensis* and *Sambucus nigra* agglutinins. Hum. Pathol. 30,1178-1191

Calabretta B., Battini R., Kaczmarek L., de Riel J.K. and Baserga R. (1986). Molecular cloning of the cDNA for a growth factor-inducible gene with strong homology to S-100, a calcium-binding protein. J. Biol .Chem. 261, 12628-12632.

Camby I., Nagy N., Lopes M.B., Schäfer B.W., Maurage C.A., Ruchoux M.M., Murmann P., Pochet R., Heizmann C., Brotchi J., Salmon I., Kiss R. and Decaestecker C. (1999). Supratentorial Pilocytic astrocytomas, astrocytomas, anaplastic astrocytomas and glioblastomas are characterized by a differential expression of S100 proteins. Brain Pathol. 9, 825-843.

DeHauwer C., Camby I., Darro F., Decaestecker C., Gras T., Salmon I., Kiss R. and VanHam P. (1997). Dynamic characterization of glioblastoma cell motility. Biochem. Biophys. Res. Commun. 232, 267-272.

DeHauwer C., Camby I., Darro F., Migeotte I., Decaestecker C., Verbeek C., Danguy A., Brotchi J., Salmon I., Van Ham P. and Kiss R. (1998). Gastrin inhibits motility, decreases cell death and increases cell proliferation in human glioblastoma cell lines. J. Neurobiol. 37, 373-382.

Föhr U.G., Heizmann C.W., Engelkamp D., Schäfer B.W. and Cox J.A. (1995). Purification and cation binding properties of the recombinant human S100 calcium-binding protein A3, an EF-hand motif protein with high affinity for zinc. J. Biol. Chem. 270, 21056-21061.

Gamallo C., Palacios J., Suarez A., Pizarro A., Navarro P., Quintanilla M. and Cano A. (1993). Correlation of E-cadherin expression with differentiation grade and histological type in breast carcinoma. Am. J. Pathol. 142, 987-993.

Grigorian M., Ambartsumian N., Lykkesfeldt A.E., Bastholm L., Elling F., Georgiev G. and Lukanidin E. (1996). Effect of mts1 (S100A4) expression on the progression of human breast cancer cells. Int. J. Cancer 67, 831-841

Heizmann C.H. and Cox J.A. (1998). New perspectives on S100 proteins: a multi-functional Ca^{2+}-, $Zn2^{+-}$ and Cu^{2+}-binding protein family. BioMetals 11, 383-397.

Huang J.W., Ming Z., Shrestha P., Mori M., Ilg E., Schafer B.W. and Heizmann C.W. (1996). Immunohistochemical evaluation of the Ca^{2+}-binding S-100 proteins S-100A1, S-100A2, S-100A4, S-100A6 and S-100B in salivary gland tumors. J. Oral. Pathol. Med. 25, 547-555.

Ilg E.C., Schäfer B.W. and Heizmann C.W. (1996). Expression pattern of S100 calcium-binding proteins in human tumors. Int. J. Cancer 68, 325-332.

Keirsebilck A., Bonne S., Bruyneel E., Vermassen P., Lukanidin E., Mareel M. and van Roy F. (1998). E-cadherin and metastatin (mts-1/S100A4) expression levels are inversely regulated in two tumor cell families. Cancer Res. 58, 4587-4591.

Komatsu K., Andoh A., Ishiguro S., Suzuki N., Hunai H., Kobune-Fujiwara Y., Kameyama M., Miyoshi J., Akedo H. and Nakamura H. (2000). Increased expression of S100A6 (calcyclin), a calcium-

binding protein of the S100 family, in human colorectal adenocarcinomas. Clin. Cancer Res. 6, 172-177.

Kubota Y., Petras R.E., Ottaway C.A., Tubbs R.R., Farmer R.G. and Fiocchi C. (1992). Colonic vasoactive intestinal peptide nerves in inflammatory bowel disease. Gastroenterology 102, 1242-1251.

Lloyd B.H., Platt-Higgins A., Rudland P.S. and Barraclough R. (1998). Human S100A4 (p9ka) induces the metastatic phenotype upon benign tumor cells. Oncogene 17, 465-473.

Mandinova A., Atar D., Schäfer B.W., Spiess M., Aebi U. and Heizmann C.W. (1998). Distinct subcellular localization of calcium binding S100 proteins in human smooth muscle cells and their relocation in response to rises in intracellular calcium. J. Cell Sci. 111, 2043-2054.

Millward T.A., Heizmann C.W., Schäfer B.W. and Hemmings B.A. (1998). Calcium regulation of Ndr protein kinase mediated by S100 calcium-binding proteins. EMBO J. 17, 5913-5922.

Monforte-Munoz H., Gonzalez-Gomez I., Rowland J.M. and Landing B.H. (1998). Increased submucosal nerve trunk caliber in aganglionosis: a "positive" and objective finding in suction biopsies and segmental resections in Hirschsprung's disease. Arch. Pathol. Lab. Med. 122, 721-725.

Nakayama S. and Kretsinger R.H. (1994). Evolution of the EF-hand family of proteins. Biomol. Struct. 23, 473-507.

Okazaki K., Niki I., Iino S., Kobayashi S. and Hidaka H. (1994). A role of calcyclin, a Ca(2+)-binding protein, on the Ca(2+)-dependent insulin release from the pancreatic beta cell. J Biol. Chem. 269, 6149-6152.

Ridinger K., Ilg E.C., Niggli F.K., Heizmann C.W. and Schäfer B.W. (1998). Clustered organization of S100 genes in human and mouse. Biochim. Biophys. Acta 1448, 154-163.

Schäfer B.W. and Heizmann C.W. (1996). The S100 family of EF-hand calcium-binding proteins: functions and pathology. Trends Biochem. Sci. 21, 134-140.

Schäfer B.W., Wicki R., Engelkamp D., Mattei M.-G. and Heizmann C.W. (1995). Isolation of a YAC clone covering a cluster of nine S100 genes on human chromosome 1q21: Rationale for a new nomenclature of the S100 calcium-binding protein family. Genomics 25, 638-643.

Selinfreund R.H., Barger S.W., Welsh M.J. and Van Eldik L.J. (1990). Antisense inhibition of glial S100B production results in alterations in cell morphology, cytoskeletal organization, and cell proliferation. J. Cell Biol. 111, 2021-2028.

Selinfreund R.H., Barger S.W., Pledger W.J. and Van Eldik L.J. (1991). Neurotrophic protein S100B stimulates glial cell proliferation. Proc. Natl. Acad. Sci. USA 88, 3554-3558.

Steele G. Jr, Tepper J., Motwani B.T. and Bruckner H.W. (1993). Adenocarcinoma of the colon and rectum. In: Cancer medicine. Holland J.F., Frei E. III, Bast R.C. Jr, Kufe D.W., Morton D.L. and Weichselbaum R.R. (eds). Lea & Febiger. Philadelphia – London. pp 1493-1522.

Stulik J., Osterreicher J., Koupilova K., Knizek J., Bures J., Jandik P., Langr F., Dedic K., Schafer B.W. and Heizmann C.W. (2000). Differential expression of the Ca^{2+} binding S100A6 protein in normal, preneoplastic and neoplastic colon mucosa. Eur. J. Cancer 36, 1050-1049.

Sugarbaker J.P., Gunderson L.L. and Wittes R.E. (1985). Colorectal cancer. In: Cancer: Principles and practices of oncology. DeVita V.T., Hellman S. and Rosenberg S.A. (eds). JB Lippincott. Philadelphia. pp 800-803.

Takenaga K., Nakanishi H., Wada K., Suzuki M., Matsuzaki O., Matsuura A. and Endo H. (1997). Increased expression of S100A4, a metastasis-associated gene, in human colorectal adenocarcinomas. Clin. Cancer Res. 3, 2309-2316.

Tan M., Heizmann C.W., Guan K., Schafer B.W. and Sun Y. (1999). Transcriptional activation of the human S100A2 promoter by wild-type p53. FEBS Lett. 445, 265-268.

Waraich T., Sarsfield P. and Wright D.H. (1997). The accessory cell populations in ulcerative colitis: a comparison between the colon and appendix in colitis and acute appendicitis. Hum. Pathol. 28, 297-303.

Wicki R., Marenholz I., Mischke D., Schäfer B.W. and Heizmann C.W. (1996a). Characterization of the human S100A12 (calgranulin C, p6, CAAF1, CGRP) gene, a new member of the S100 gene cluster on chromosome 1q21. Cell Calcium 20, 459-464.

Wicki R., Schäfer B.W., Erne P. and Heizmann C.W. (1996b). Characterization of the human and mouse cDNAs coding for S100A13, a new member of the S100 protein family. Biochem. Biophys. Res. Commun. 227, 594-599.

Wicki R., Franz C., Scholl F.A., Heizmann C.W. and Schäfer B.W. (1997). Repression of the candidate tumor suppressor gene S100A2 in breast cancer is mediated by site-specific hypermethylation. Cell Calcium 22, 243-254.

Accepted March 13, 2001