Expression of protein S100A4 is a predictor of recurrence in colorectal cancer

Jung Myun Kwak, Hyun Joo Lee, Seon Hahn Kim, Han Kyeom Kim, Young Jae Mok, Young Tae Park, Jong Sang Choi, Hong Young Moon

Jung Myun Kwak, Seon Hahn Kim, Department of Surgery, Korea University Anam Hospital, 126-1, 5 ga, Anam-dong, Seongbuk-gu, Seoul 136-705, South Korea

Hyun Joo Lee, Han Kyeom Kim, Jong Sang Choi, Department of Pathology, Korea University Guro Hospital, 80 Guro-Dong, Guro-Gu, Seoul 152-703, South Korea

Young Jae Mok, Hong Young Moon, Department of Surgery, Korea University Guro Hospital, 80 Guro-Dong, Guro-Gu, Seoul 152-703, South Korea

Young Tae Park, Department of Gastroenterology, Korea University Guro Hospital, 80 Guro-Dong, Guro-Gu, Seoul 152-703, South Korea

Author contributions: Kwak JM, Lee HJ and Moon HY made contributions to the study design, data interpretation, and wrote the manuscript; Kim HK and Choi JS performed the majority of experiments and evaluated immunohistochemical staining; Kim SH, Mok YJ and Park YT made contributions to the study design and critically reviewed the manuscript.

Correspondence to: Hong Young Moon, MD, PhD, Department of Surgery, Korea University Guro Hospital, 80 Guro-Dong, Guro-Gu, Seoul 152-703, South Korea. hymoon@korea.ac.kr

RESULTS: In normal colorectal mucosa, protein S100A4 immunoreactivity was clearly absent in both cytoplasm and nucleus. However, positive immunoreactivity of protein S100A4 was detected in 45 (35.4%) of the tumor cases. There was no significant association between positive immunoreactivity of protein S100A4 and clinicopathological parameters such as tumor differentiation or TNM stage, and also no correlation between the reactivity and E-cadherin or p53 expression. However, positive immunoreactivity of protein S100A4 was found to be associated with tumor recurrence ($P = 0.004$), and was also associated with significantly worse overall survival in the Kaplan-Meier survival analysis ($P = 0.044$). After adjustment for tumor differentiation, tumor depth and nodal status, however, it failed to achieve statistical significance ($P = 0.067$).

CONCLUSION: The expression of protein S100A4 is associated with tumor recurrence and poor overall survival in patients with colorectal cancer.

© 2010 Baishideng. All rights reserved.

Key words: S100A4; E-cadherin; p53; Prognostic factor; Colorectal cancer

Peer reviewer: Dr. John B Schofield, MB, BS, MRCP, FRCP, Department of Cellular Pathology, Preston Hall, Maidstone, Kent, ME20 7NH, United Kingdom

Kwak JM, Lee HJ, Kim SH, Kim HK, Mok YJ, Park YT, Choi JS, Moon HY. Expression of protein S100A4 is a predictor of recurrence in colorectal cancer. World J Gastroenterol 2010; 16(31): 3897-3904 Available from: URL: http://www.wjgnet.com
INTRODUCTION

Although the last decade has brought significant improvements in the disease-free and overall survival of colorectal cancer (CRC) patients; achieved largely by more accurate staging of disease, an improved and expanded role of surgery and increased number of available chemotherapeutic options, approximately 20% of advanced CRC patients still die of recurrence of the disease[1]. Invasion and metastasis, which are the most life-threatening properties of malignant tumors, result from the interaction between tumor cells and the surrounding tissues. The invasion and metastasis processes themselves consist of pathogenic sequential steps, such as proliferation and detachment of neoplastic cells, invasion to extracellular matrix, angiogenesis, vascular dissemination, lodging in a distant vascular bed, extravasation into the target organ, and proliferation. The activation of many genes and the expression of their products have been involved in this progression[2]. Current conventional staging has a significant impact on survival of CRC patients. However, there is marked variability in outcome that exists within each stage, and certain populations of patients with early recurrence, resistance to chemotherapy and decreased survival cannot be predicted using conventional histopathologic staging. Thus, the identification of molecular factors that have prognostic significance in CRC is essential to improve treatment and outcome[3].

Over the past few years, the S100 family of proteins has emerged as an important group with the capacity to promote invasiveness and metastasis of many human neoplasms. In particular, recent studies have established the mechanisms of action of protein S100A4, and indicate its possible prognostic role in human neoplasia[4-8]. However, studies regarding protein S100A4 have mainly been limited to research laboratories. Moreover, the mechanism of action of protein S100A4 in tumors is not fully understood. Therefore, it would be of great interest to find out whether the detection of protein S100A4 has any predictive value, and also whether it may help select patients who require more extensive diagnostic evaluation to rule out metastatic disease and/or more aggressive treatments.

The aims of this study were to investigate immunohistochemically the prognostic significance of protein S100A4 expression in CRC, compared with clinicopathologic parameters and overall survival, and to investigate the correlation between protein S100A4 expression and E-cadherin and p53, which have been suggested as possible targets of protein S100A4.

MATERIALS AND METHODS

Patients

Formalin-fixed paraffin-embedded specimens were selected from 127 patients with CRC who underwent curative or palliative surgical resection between April 2000 and March 2004 at the Department of Surgery, Korea University Guro Hospital. The 127 patients included 76 males (59.8%) and 51 females (40.2%) with a mean age of 59.3 years (range, 28-88). Clinicopathologic data were based on the histopathologic reports and the clinical records of the patients. Using the American Joint Committee on Cancer (AJCC) TNM system[9], tumors were classified as Stage I in 24 specimens (18.9%), Stage II in 49 (38.6%), Stage III in 49 (38.6%) and Stage IV in 5 (3.9%). The Korea University Medical Center Institutional Review Board granted permission for the study.

Preparation of tissue microarray

Paraffin blocks of formalin-fixed surgical specimens were obtained from the Department of Pathology, Korea University Guro Hospital. Pathological evaluation of all blocks was performed by two pathologists who did not know any information about the patients. In each case, three core biopsies were obtained from representative areas of the corresponding paraffin blocks with a precision instrument. These tissue cores from each specimen with a diameter of 2 mm were punched out and positioned in a recipient paraffin array block. Each case also included three internal controls consisting of non-neoplastic colorectal mucosa. Four-μm sections of these tissue array blocks were then cut and used for immunohistochemical analysis.

Immunohistochemistry

Immunohistochemical staining for protein S100A4, E-cadherin, and p53 was performed using a standard avidin-biotin complex (ABC) method. In brief, all sections were deparaffinized by using a series of xylene baths and then hydrated using a graded alcohol series. They were then placed in citric acid buffer (10 mmol/L) and heated in a microwave oven (700 W) for 12 min to retrieve the antigenicity. The sections were then immersed in methanol, containing 0.3% hydrogen peroxide, for 20 min to block endogenous peroxidase activity. The sections were then washed three times in phosphate-buffered saline (PBS) and incubated in 2.5% normal goat serum for 20 min to reduce nonspecific antibody binding. After washing with PBS, the sections were incubated with primary antibodies for 30 min at room temperature. Rabbit polyclonal antibodies against protein S100A4 (Ab-8, Neomarker, 1:100), monoclonal mouse anti-human E-cadherin (NCH-38, Dako, 1:100), and monoclonal mouse anti-human p53 (DO-7, Dako, 1:100) were used. The reaction products were visualized with diaminobenzidine as a chromogen, and counterstained with commercial hematoxylin.

Evaluation of immunohistochemical staining

Evaluation of immunohistochemical staining was performed by two independent pathologists. Any discrepancies in scoring were resolved by simultaneous reassess-
ment by both pathologists. The tumor cells whose cytoplasm was stained brown were classified as positive. The protein S100A4 expression of tumor cells was evaluated according to the proportion of positively stained tumor cells. When more than 10% of tumor cells were positively stained, the tumor was considered as “positive expression”. On the other hand, the tumor was considered as “negative expression” when less than 10% of tumor cells were positively stained (Figure 1). In the case of E-cadherin, when more than 90% of tumor cells were positively stained, the tumor was considered as “preserved expression”. On the other hand, the tumor was considered as “reduced expression” when less than 90% of tumor cells were positively stained (Figure 2). p53 expression was evaluated according to the proportion of tumor cells whose nuclei were positively stained. When more than 10% of tumor cells were positively stained, the tumor was considered as “positive expression”. On the other hand, the tumor was considered as “negative expression” when less than 10% of tumor cells were positively stained (Figure 3).

**Statistical analysis**

Statistical analysis was performed using the SPSS for Windows software package (SPSS, Inc., Chicago, IL, Version 12.0). Correlation between the expression
of protein S100A4, E-cadherin, and p53 and various clinicopathologic parameters was evaluated using the chi-squared test or Fisher’s exact test. Overall survival analysis was done by the Kaplan-Meier method. The difference between the survival curves was analyzed by the log-rank test. Significant variables identified on univariate analysis were subjected to multivariate analysis using the Cox regression model. A P value less than 0.05 was considered statistically significant.

RESULTS

Expression of protein S100A4, E-cadherin and p53 in CRC

A wide range of cell types in normal colorectal tissues was stained with polyclonal antibody against protein S100A4. There was a high level of staining of smooth muscle, of the smooth muscle in the walls of vessels, and of infiltrating lymphocytes and macrophages in the stroma. However, in normal colorectal mucosa of all 127 cases, immunoreactivity of protein S100A4 was clearly absent in both cytoplasm and nucleus. Positive immunoreactivity of protein S100A4 was detected in 45 (35.4%) of the tumor specimens. E-cadherin was expressed in cell membranes of all normal colorectal mucosa, and reduced expression of E-cadherin was observed in 48 (37.8%) of the tumor specimens. All normal colorectal mucosa showed negative expression for p53; however, 71 (55.9%) tumors were stained for p53 in their nuclei.

Correlation of protein S100A4, E-cadherin, and p53 expression with clinicopathological parameters

Positive reactivity for protein S100A4 was found to be associated with tumor recurrence (P = 0.004). However, there was no significant association between the expression of protein S100A4 and other investigated clinicopathological parameters, including tumor location, differentiation or TNM stage. Reduced expression of E-cadherin was significantly correlated with tumor differentiation (P = 0.001). As for p53, there was no significant correlation between expression of p53 and clinicopathological parameters (Table 1).

Correlation between protein S100A4 and E-cadherin/p53 expression

There was no significant correlation in co-expression pattern between protein S100A4 and E-cadherin (Kendall’s Tau-b correlation coefficient = 0.068, P = 0.436, Table 2). Also, there was no significant correlation between protein S100A4 expression and p53 expression (Kendall’s Tau-b correlation coefficient = -0.105, P = 0.239, Table 3).
Survival analysis

The median follow-up period for all patients was 58.7 mo (range, 1.1-101.8). The 5-year overall survival rate for the 127 patients was 79.7%. Kaplan-Meier survival analysis showed that tumor differentiation (5-year survival rate 82.3% vs 50.0%, \( P = 0.001 \)), depth of tumor (97.1% vs 72.9%, \( P = 0.001 \)), lymph node metastasis (88.5% vs 68.0%, \( P = 0.001 \)) and positive immunoreactivity of protein S100A4 (86.1% vs 68.3%, \( P = 0.044 \)) were associated with poor overall survival (Table 4 and Figure 4).

In a multivariate analysis, however, the positive immunoreactivity of protein S100A4 failed to have association with worse overall survival after adjustment for tumor differentiation, tumor depth and nodal status, which were significant parameters in a univariate analysis (hazard ratio, 1.985; 95% confidence interval: 0.953-4.134; \( P = 0.067 \), Table 5).

DISCUSSION

Calcium binding proteins form a large family involved in numerous functions ranging from the control of cell-cycle progression and cell differentiation to enzyme activation and regulation of muscle contraction. The S100 proteins represent one of the largest subfamilies of the calcium binding proteins with at least 19 different members; the degree of homology ranges from 25% to 65%. They were initially characterized as low-molecular weight acidic proteins and named by their solubility in 100% ammonium sulfate (“S100”). S100A4, also known as p9Ka, CAPL, or calvasculin, is a member of the S100 family consisting of 101 amino acids and with a molecular weight of about 11.6 kDa. The corresponding gene, cloned by different groups, is known as \( mts1 \) (metastasin), pEL98, 18A2, 42A, and \( fsP \) (fibroblast-specific protein).

The biologic functions of several S100 proteins in carcinogenesis have not been fully elucidated to date. Recently, however, much interest has focused on S100A4 and some other S100 family members, such as S100A2, S100A6, and S100B, for their potential roles in invasive growth and metastasis of neoplastic diseases. S100A4 or its corresponding mRNA are found at higher levels in

| Table 2  Correlations of protein S100A4 and E-cadherin |
|-----------------------------------------------|
| S100A4 | E-cadherin | n (%) |
| Co-expression pattern | | |
| Negative | Negative | 55 (39.9) |
| Negative | Positive | 34 (24.6) |
| Positive | Negative | 32 (23.2) |
| Positive | Positive | 17 (12.3) |

Kendall’s Tau-b correlation coefficient = -0.035, \( P = 0.681 \).

| Table 3  Correlations of protein S100A4 and p53 |
|-----------------------------------------------|
| S100A4 | p53 | n (%) |
| Co-expression pattern | | |
| Negative | Negative | 35 (25.4) |
| Negative | Positive | 54 (39.1) |
| Positive | Negative | 26 (18.8) |
| Positive | Positive | 23 (16.7) |

Kendall’s Tau-b correlation coefficient = -0.132, \( P = 0.120 \).

| Table 4  Univariate overall survival analysis for seven clinicopathologic parameters |
|--------------------------|-------------|-----------------|-----------------|
| Parameters | Hazard ratio | 95% CI | \( P \) value |
| Gender | | | |
| Male | | 80.9 | 0.817 |
| Female | | 78.0 | 0.817 |
| Differentiation | | | 0.001 |
| Differentiated | | 82.3 | 0.001 |
| Undifferentiated | | 50.0 | 0.001 |
| Depth of tumor | | | 0.001 |
| T1-2 | | 97.1 | 0.001 |
| T3-4 | | 72.9 | 0.001 |
| Lymph node metastasis | | | 0.001 |
| Absent | | 88.5 | 0.001 |
| Present | | 68.0 | 0.001 |
| S100A4 expression | | | 0.044 |
| Negative | | 86.1 | 0.044 |
| Positive | | 68.3 | 0.044 |
| E-cadherin | | | 0.105 |
| Preserved | | 83.1 | 0.105 |
| Reduced | | 74.3 | 0.105 |
| p53 | | | 0.218 |
| Negative | | 83.6 | 0.218 |
| Positive | | 76.6 | 0.218 |

| Table 5  Cox regression analysis on those parameters shown to significantly influence overall survival in a univariate analysis |
|--------------------------|------------------|-----------------|-----------------|
| Parameters | Hazard ratio | 95% CI | \( P \) value |
| Lymph node metastasis | 2.283 | 1.014-5.141 | 0.046 |
| Depth of tumor | 10.374 | 1.391-77.352 | 0.022 |
| Differentiation | 2.748 | 1.059-7.133 | 0.038 |
| S100A4 expression | 1.985 | 0.953-4.134 | 0.067 |

Figure 4 Kaplan-Meier survival curves demonstrating statistically significant differences according to the expression of protein S100A4 (log-rank test, \( P = 0.044 \)). Censored observations are shown as tick marks.
metastatic relative to non-metastatic rat[14] and mouse[15] tumor cell lines. Transfection experiments further showed that rodent or human S100A4 can induce a metastatic phenotype in previously non-metastatic rat mammary cells[16,17]. Conversely, antisense S100A4 RNA or anti-S100A4 ribozyme suppressed the metastatic potential of highly metastatic cell lines[18,19]. Moreover, in pilot studies of human colorectal adenocarcinoma specimens, elevated levels of immunohistochemically detected S100A4 are associated with the more malignant carcinomatous regions of the primary tumors and with liver metastases[20]. The tight association between S100A4 expression and metastasis observed in these laboratory analyses has led to a number of studies examining the utility of S100A4 expression as a prognostic marker in human cancers. Protein S100A4 has been shown to be a prognostic marker in a number of human cancers, including breast cancer[21], esophageal-squamous cancer[22], non-small cell lung cancer[23], gastric cancer[24], malignant melanoma[25], prostate cancer[26], and pancreatic cancer[27]. The universality of S100A4 expression in a variety of cancers illustrates the potential use of S100A4 as a marker for tumor metastasis and disease progression.

The purpose of this investigation was to establish clinical significance of the calcium-binding protein, S100A4, in CRC. It was found that 35.4% of CRC specimens were stained strongly by the polyclonal antibodies against protein S100A4, in concordance with earlier reports[27-29]. The staining in specimens is not restricted to only carcinoma cells, because highly expressed levels are also detected in normal tissues, in particular, smooth muscle cells, endothelial cells of both arteries and veins, and some reactive fibroblast-like cells and lymphocytes[30]. However, the present study was undertaken on only carcinoma cells.

In this study, positive expression of protein S100A4 was associated with tumor recurrence, in accordance with previous study[29]. This result suggests that the protein S100A4 may play a role in predicting a patient subgroup which would show more unfavorable outcome, thus leading us to substage-oriented tailored therapy with more intensive treatment and more strict follow-up surveillance.

This study showed that the overall survival of CRC patients who had immunohistochemically detectable levels of protein S100A4 was significantly worse than those CRC patients with negative expression of protein S100A4 according to univariate analysis. Because S100A4 was first discovered as a metastasis-inducing protein in experimental models[14,19], and metastasis is the major event responsible for death in patients of CRC, it is quite possible that protein S100A4 causes earlier deaths by its ability to induce metastasis in human CRC. Although it failed to achieve statistical significance in multivariate analysis, the present result suggests a need for further and larger studies to investigate the role of protein S100A4 expression in CRC.

As a typical member of the S100 family, S100A4 exerts dual functions, both intracellular and extracellular. Intracellularly, it interacts with and functionally modifies the tumor suppressor protein p53, non-muscle myosin II, and liprin[12,13]. S100A4 interacts with the C terminus of p53 and inhibits protein kinase C (PKC) phosphorylation of the tumor suppressor in vitro. Likewise, the interaction between p53 and S100A4 inhibits p53 from binding to its consensus DNA-binding sequence[31]; thus it was expected that S100A4 would be a general inhibitor of p53 function. It has been suggested that a complex of S100A4 with p53 and the sequestration of p53 may result in stimulation of the cells to enter the S phase by abrogating the control functions of p53 at the G1-S checkpoint[32,33]. However, as shown in this study, this possibility was difficult to prove by immunohistochemical analysis of these two proteins in CRC. An examination of p53-regulated genes in S100A4-expressing cells indicates that the expression of several genes are up-regulated (e.g. bax); other genes are down-regulated initially and then later up-regulated (e.g. mdm2), and some genes are inhibited (e.g. p21, thrombospondin-1)[34]. These opposite effects of S100A4 expression on p53-regulated genes could explain why there was no correlation between S100A4 and p53, notwithstanding the potential interaction of these two proteins.

Another possible mechanism of action of S100A4 in carcinogenesis is cytoskeletal dysfunction by down-regulation of E-cadherin induced by protein S100A4. E-cadherin is a member of the large cadherin superfamily. It is the predominant intercellular adhesion molecule expressed by intestinal epithelial cells, and functions to mediate epithelial cell-cell adhesion and maintain the integrity of the epithelium[35]. The expressions of E-cadherin and protein S100A4 in two mouse tumor cell lines were found to be inversely regulated, and transfection experiments showed a reciprocal down-regulation of both molecules, suggesting that the invasiveness of tumors expressing protein S100A4 may be at least partially induced by the abrogation of E-cadherin expression[36]. A similar mechanism has also been postulated in humans, on the basis of immunohistochemical analysis of both proteins in a series of non-small cell lung cancer[20] and gastric cancer[28], an inverse correlation of E-cadherin and protein S100A4 expression was demonstrated. In this study, we attempted to immunohistochemically establish an inverse correlation between the expression of protein S100A4 and E-cadherin in CRC; however, data failed to prove the relationship (Kendall’s Tau-b correlation coefficient = -0.035, P = 0.681). Nevertheless, it is quite possible that different antibodies against protein S100A4, different cancer tissue, and small numbers enrolled in this study might have contributed to this difference.

In conclusion, in the present retrospective study, positive immunoreactivity of protein S100A4 is closely associated with cancer recurrence. However, there is no correlation between the expression of protein S100A4 and E-cadherin or p53. The overall survival for patients with CRC expressing immunohistochemically detectable levels of protein S100A4 is significantly worse than for those patients with CRC considered negative for S100A4. Furthermore, protein S100A4 shows borderline tendency toward being a prognostic marker in multivariate regres-
sion analysis. Although these results suggest that protein S100A4 could be a useful biologic predictor of cancer recurrence and poor outcome, subsequent prospective, large-scale studies are required to confirm its importance and relevance in the invasive potential of human CRC.

**COMMENTS**

**Background**

Although current conventional staging has a significant impact on survival of colorectal cancer patients, there is marked variability in outcome within each stage. As the protein S100A4 has been known to promote invasiveness and metastasis of many human neoplasms, the question is raised as to whether this protein represents a useful prognostic marker in clinical practice.

**Research frontiers**

Studies regarding the protein S100A4 have mainly been limited to research laboratories and clinical data are extremely limited. Therefore, it would be of great interest to find out whether the expression of protein S100A4 has any predictive value and may help select patients who require more extensive diagnostic evaluation to rule out metastasis and/or more aggressive treatments.

**Innovations and breakthroughs**

The results of this study showed that positive immunoreactivity of protein S100A4 was associated with tumor recurrence and worse overall survival.

**Applications**

Since there is an association between protein S100A4 expression, tumor recurrence and poor overall survival, this can lead to stage-oriented tailored therapy with more intensive treatment and more strict follow-up surveillance in colorectal cancer patients.

**Terminology**

The protein S100A4 was first discovered as a metastasis-inducing protein in experimental models. It is a polypeptide of 101 amino acids with a molecular mass of 11.5 kDa. The evidence gathered throughout the past few years demonstrates that protein S100A4 is involved in the regulation of invasiveness and metastasis in many human cancers.

**Peer review**

This paper is very well written and has a strong message about expression of protein S100A4 in 127 cases of colorectal cancer, showing a statistically significant association with tumor recurrence and overall survival.

**REFERENCES**

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. CA Cancer J Clin 2009; 59: 225-249
2. Liotta LA, Steeg PS, Stetler-Stevenson WG. Cancer metastasis and angiogenesis: an imbalance of positive and negative regulation. Cell 1991; 64: 327-336
3. Willett CG, Tepper JE, Cohen AM, Orlow E, Welch CE. Failure patterns following curative resection of colonic carcinoma. Ann Surg 1984; 200: 685-690
4. Minsky BD, Mies C, Rich TA, Recht A, Chaffey JT. Potentially curative surgery of colon cancer: patterns of failure and survival. J Clin Oncol 1988; 6: 106-118
5. Kahlenberg MS, Sullivan JM, Witmer DD, Petrelli NJ. Molecular prognostics in colorectal cancer. Surg Oncol 2003; 12: 173-186
6. Lukashin EM, Georgiev GP. Metastasis-related mts1 gene. Curr Top Microbiol Immunol 1996; 213 (Pt 2): 171-195
7. Barraclough R. Calcium-binding protein S100A4 in health and disease. Biochim Biophys Acta 1998; 1448: 190-199
8. Sherbet GV, Lakshmi MS. S100A4 (MTSl) calcium binding protein in cancer growth, invasion and metastasis. Anticancer Res 1998; 18: 2415-2421
9. American Joint Committee on Cancer. AJCC cancer staging manual. 6 ed, New York: Springer-Verlag, 2002
10. Schäfer BW, Heizmann CW. The S100 family of EF-hand calcium-binding proteins: functions and pathology. Trends Biochem Sci 1996; 21: 134-140
11. Emberley ED, Murphy LC, Watson PH. S100 proteins and their influence on pro-survival pathways in cancer. Biochem Cell Biol 2004; 82: 508-515
12. Mazzucchelli L. Protein S100A4: too long overlooked by pathologists? Am J Pathol 2002; 160: 7-13
13. Garrett SC, Varney KM, Weber DJ, Bresnick AR. S100A4, a mediator of metastasis. J Biol Chem 2006; 281: 677-680
14. Kawakami K, Yanagisawa K, Watanabe Y, Tominaga S, Nagano K. Different factors bind to the regulatory region of the Na+K(+)-ATPase alpha 1-subunit gene during the cell cycle. FEBS Lett 1993; 335: 251-254
15. Ebralidze A, Tulchinsky E, Grigorian M, Afanasyeva A, Senin V, Revazova E, Lukandin E. Isolation and characterization of a gene specifically expressed in different metastatic cell lines and whose deduced gene product has a high degree of homology to a Ca2+-binding protein family. Genes Dev 1989; 3: 1086-1093
16. Davies BR, Davies MP, Gibbs FE, Barraclough R, Rudland PS. Induction of the metastatic phenotype by transfection of a benign rat mammary epithelial cell line with the gene for p9Ka, a rat calcium-binding protein, but not with the oncogene EJ-ras-1. Oncogene 1993; 8: 999-1008
17. Lloyd BL, Platt-Higgins A, Rudland PS, Barraclough R. Human S100A4 (p9Ka) induces the metastatic phenotype upon benign tumour cells. Oncogene 1998; 17: 465-473
18. Maelandsmo GM, Hovig E, Skrede M, Engebretsen O, Florines VA, Myklebost O, Grigoriev M, Lukandin E, Scallen Knj, Fostad O. Reversal of the in vivo metastatic phenotype of human tumor cells by an anti-CAPL (mts1) ribozyme. Cancer Res 1996; 56: 5490-5498
19. Takenaga K, Nakamura Y, Sakiyama S. Expression of antisense RNA to S100A4 gene encoding an S100-related calcium-binding protein suppresses metastatic potential of high-metastatic Lewis lung carcinoma cells. Oncogene 1997; 14: 331-337
20. Takenaga K, Nakanishi H, Wada K, Suzuki M, Matsuoka O, Matsuura A, Endo H. Increased expression of S100A4, a metastasis-associated gene, in human colorectal adenocarcinomas. Clin Cancer Res 1997; 3: 2309-2316
21. Platt-Higgins AM, Renshaw CA, West CR, Winstanley JH, De Silva Rudland S, Barraclough R, Rudland PS. Comparison of the metastasis-inducing protein S100A4 (p9Ka) with other prognostic markers in human breast cancer. Int J Cancer 2000; 89: 198-208
22. Ninomiya I, Ohta T, Fushida S, Endo Y, Hashimoto T, Yagi M, Fujimura T, Nishimura G, Tani T, Shimizu K, Yonemura Y, Heizmann CW, Schäfer BW, Sasaki T, Miwa K. Increased expression of S100A4 and its prognostic significance in esophageal squamous cell carcinoma. Int J Oncol 2001; 18: 715-720
23. Kimura K, Endo Y, Yonemura Y, Heizmann CW, Schäfer BW, Watanabe Y, Sasaki T. Clinical significance of S100A4 and E-cadherin-related adhesion molecules in non-small cell lung cancer. Int J Oncol 2000; 16: 1125-1131
24. Yonemura Y, Endo Y, Kimura K, Fushida S, Bandou E, Taniguchi K, Kinoshita K, Ninomiya I, Sugiyama K, Heizmann CW, Schäfer BW, Sasaki T. Increased expression of S100A4 and its prognostic significance in esophageal squamous cell carcinoma. Cancer Res 2000; 60: 5498-5501
25. Andersen K, Nesland JM, Holm R, Florines VA, Fostad Ø, Maelandsmo GM. Expression of S100A4 combined with reduced E-cadherin expression predicts patient outcome in malignant melanoma. Melanoma Res 2004; 14: 970-979
26. Saleem M, Adhami VM, Ahmad N, Gupta S, Mukhtar H. Prognostic significance of metastasis-associated protein S100A4 (Mts1) in prostate cancer progression and chemoprevention regimens in an autochthonous mouse model. Cancer Res 2003; 63: 147-153
27. Roest C, Ueki T, Argani P, Jansen M, Yeoh C, Cameron JL, Hruban RH, Goggins M. Overexpression of S100A4 in pan-
creatic ductal adenocarcinomas is associated with poor differ-
entiation and DNA hypomethylation. *Am J Pathol* 2002; 
160: 45-50

28 **Gongoll S**, Peters G, Mengel M, Piso P, Klempnauer J, 
Kreipe H, von Wasielewski R. Prognostic significance of 
calcium-binding protein S100A4 in colorectal cancer. *Gastro-
enterology* 2002; 123: 1478-1484

29 **Cho YG**, Kim CJ, Nam SW, Yoon SH, Lee SH, Yoo NJ, Lee 
JY, Park WS. Overexpression of S100A4 is closely associated 
with progression of colorectal cancer. *World J Gastroenterol* 
2005; 11: 4852-4856

30 **Takenaga K**, Nakamura Y, Sakiyama S. Expression of a 
calcium binding protein pEL98 (mts1) during differentiation of 
human promyelocytic leukemia HL-60 cells. *Biochem Biophys 
Res Commun* 1994; 202: 94-101

31 **Grigorian M**, Andreassen S, Tulchinsky E, Kriaevska M, Carl-
berg C, Kruse C, Cohn M, Ambartsumian N, Christensen A, 
Selivanova G, Lukaniidin E. Tumor suppressor p53 protein is 
a new target for the metastasis-associated Mts1/S100A4 
protein: functional consequences of their interaction. *J Biol 
Chem* 2001; 276: 22699-22708

32 **Parker C**, Lakshmi MS, Piura B, Sherbet GV. Metastasis-
associated mts1 gene expression correlates with increased 
p53 detection in the B16 murine melanoma. *DNA Cell Biol* 
1994; 13: 343-351

33 **Jawhari A**, Farthing M, Pignatelli M. The importance of the 
E-cadherin-catenin complex in the maintenance of intestinal 
epithelial homeostasis: more than intercellular glue? *Gut* 
1997; 41: 581-584

34 **Okegawa T**, Li Y, Pong RC, Hsieh JT. Cell adhesion proteins 
as tumor suppressors. *J Urol* 2002; 167: 1836-1843

35 **Angst BD**, Marcozzi C, Magee Al. The cadherin super-
family: diversity in form and function. *J Cell Sci* 2001; 114: 
629-641

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