The Korean Journal of Pathology 2010; 44: 477-82
DOI: 10.4132/KoreanJPathol.2010.44.5.477

Clinicopathological Significance of S100A4 Expression in Non-small Cell Lung Carcinomas

Eun Ah Jung · Hyun Deuk Cho
Ji-Hye Lee · Mee-Hye Oh

Department of Pathology,
Soonchunhyang University College of Medicine, Cheonan, Korea

Received : December 2, 2009
Accepted : May 31, 2010

Corresponding Author
Mee-Hye Oh, M.D.
Department of Pathology, Soonchunhyang University College of Medicine, 23-20 Bongmyeong-dong, Cheonan 330-721, Korea
Tel: +82-41-570-3582
Fax: +82-41-570-2455
E-mail: mhoh0212@hanmail.net

Background : S100A4 has been implicated in invasion and metastasis of various malignant tumors. The aim of this study was to investigate whether or not S100A4 plays an important role in non-small cell lung carcinomas (NSCLCs). **Methods :** Sixty-seven patients with NSCLC including 37 with squamous cell carcinomas (SCCs) and 30 with adenocarcinomas (ADs) who had undergone surgical resection were analyzed. S100A4 expression was analyzed by immunohistochemistry using tissue microarray blocks. **Results :** S100A4 expression was positive in 56 (83.6%) of 67 NSCLC cases. ADs were more frequently S100A4 positive than SCCs (p = 0.017). However, no significant correlation was observed between S100A4 expression and age, gender, pT, pN or tumor, node and metastasis (TNM) stage. Two distant metastatic cases revealed an S100A4 positive reaction. Kaplan-Meier survival curves with the log-rank test showed no correlation with 3-year survival (p = 0.782) or 5-year survival (p = 0.227) in either group of patients according to S100A4 expression. **Conclusions :** S100A4 expression was not correlated with age, gender, pT, pN or TNM stage or survival in patients with NSCLCs. Therefore, S100A4 expression may not be useful as a prognostic marker for NSCLCs. However, S100A4 expression showed a higher positivity in ADs than in SCCs.

Key Words : S100A4 protein, human; Carcinoma, non-small cell lung; Prognosis

Lung cancer is the leading cause of cancer-related death among men and women worldwide. Despite recent advances in chemotherapy, radiation therapy, and surgery, the overall survival rate of lung cancer is still less than 15%.[1] In Korea, the incidence of lung cancer is also growing rapidly in both men and women. The incidental rate of lung cancer is the second in men and the fifth in women, and the rate of death from lung cancer is the first in men and the second in women.[2] These results are particularly related to the large percentage of the population in advanced stage lung cancer at the time of diagnosis and the poor effect of treatment associated with metastatic disease.[3] Therefore, an investigation of biomarkers affecting tumor progression, metastasis, and overall survival is needed.

The S100A4 protein is a member of the S100 family of calcium-binding proteins, and has been suggested as a metastasis-associated molecule. S100A4 is expressed not only in primary and metastatic tumors, but also in normal cells, such as macrophages, neutrophils, T lymphocytes, and smooth muscle of blood vessels. S100A4 plays a role in regulating the cell cycle and cell motility, and modulates intercellular adhesion.[4-8] Up-regulation of S100A4, which has been shown in a variety of human carcinomas, including bladder cancer,[9,10] breast cancer,[11,12] thyroid cancer,[13] gastric carcinoma,[14] pancreatic cancer,[15] and non-small cell lung carcinoma (NSCLC),[16-19] has been associated with disease progression, metastasis, and decreased patient survival. Despite the potential importance of the S100A4 proteins in various types of cancer, only a few comprehensive S100A4 protein expression analyses in NSCLCs have been performed to date. Therefore, we performed our study to investigate the correlation between S100A4 expression and clinicopathological factors, and to evaluate the prognostic significance of S100A4 expression in NSCLCs including squamous cell carcinoma (SCC) and adenocarcinoma (AD).

MATERIALS AND METHODS

Patients and clinicopathological data

A retrospective analysis was performed using clinical data and tissue samples from 75 patients with NSCLCs (37 SCCs, 30 ADs, three sarcomatoid carcinomas, two large cell carcinomas, two large cell neuroendocrine carcinomas, and one adenosquamous carcinoma) who underwent curative resection from

January 1990 to December 2004. Other histological types, except for SCCs and ADs, were excluded from the study population due to their histological heterogeneity and small numbers. Patients had undergone wedge resection, lobectomy, bilobectomy, and pneumonectomy at Soonchunhyang University Cheonan Hospital. The study population included 67 patients (53 men and 14 women) with tumor, node and metastasis (TNM) stage I-IV NSCLC as follows: I, 31 (46.3%); II, 17 (25.4%); III, 15 (22.4%); and IV, 4 (6.0%). The pT distribution was as follows: pT1, 18 (26.9%); pT2, 37 (55.2%); pT3, 10 (14.9%); and pT4, 2 (3.0%). The pN distribution was as follows: pN0, 37 (56.1%); pN1, 14 (21.2%); pN2, 15 (22.7%); and pN3, 0 (0%), and NX was found in one patient, and distant metastases were found in two (3.0%) patients. Patients ranged in age from 39 to 76 years (mean ± standard deviation, 61.8 ± 8.2 years). Tumor size ranged from 1.0 to 17.0 cm (4.1 ± 2.5 cm). The distribution of histological types was as follows: SCC, 37 (55.2%) and AD, 30 (44.8%). Based on International Association for the Study of Lung Cancer Staging Committee recommendations, the two pathologists reviewed the histological sections according to the 7th edition of the TNM classification.[20] Clinical data were obtained from patient medical records.

Tissue microarray (TMA) construction

The most representative tumor area was carefully marked on hematoxylin and eosin (H&E) stained slides of sample tissue cores (2 mm in diameter) from formalin-fixed, paraffin-embedded tissue blocks. Representative cores were arranged in a new TMA block. A core from each NSCLC specimen and a core from non-neoplastic lung parenchymas were obtained for the TMAs. One section from the block was stained with H&E for tissue confirmation.

Immunohistochemistry

Sections from the TMA blocks were transferred to poly-L-lysine-coated glass slides and air-dried overnight at 37℃. The sections were dewaxed in xylene (three changes), rehydrated in a graded series of decreasing ethanol concentrations, and rinsed in Tris-buffered saline (TBS, pH 7.4). Endogenous peroxidase activity was inactivated with 5% hydrogen peroxide in methanol for 15 minutes at 37℃. Antigen retrieval was performed using a 15-minute microwave treatment in TBS. The tissue was then incubated with primary polyclonal antibody against S100A4 (1 : 400, DakoCytomation, Carpinteria, CA, USA) in a humidified chamber at 4℃ for 16 hours. The secondary antibody was applied using the Envision Detection kit (Dako, Glostrup, Denmark). Diaminobenzidine was used as the chromogen, and the sections were counterstained with Mayer's hematoxylin.

Immunohistochemical scoring

S100A4 expression was cytoplasmic and/or nuclear. S100A4 immunoreactivity was characterized by cytoplasmic or nuclear staining in tumor cells, as well as in vascular wall smooth muscle, lymphocytes, myofibroblasts, and macrophages in the surrounding stromal tissue. Tumor tissue sections were scored using light microscopy by considering for the intensity and proportion of the tumor cells showing immunoreactivity. The scoring procedure was implemented by two pathologists. Nuclear and cytoplasmic staining intensity was subclassified as follows: 0, none; 1, weak; 2, moderate; and 3, strong. The number of positive cells was expressed as the percentage of the total number of tumor cells and assigned to one of the following four categories: 0, none; 1, < 10%; 2, 10-49%; and 3, 50-100%. By multiplying the two scores, the product was designated as the immunoreactive score for each tumor specimen. Scores of 0 and 1 were considered negative. Scores ≥ 2 were considered positive (Fig. 1).

Statistical analysis

The Pearson's chi-square test or Fisher's exact test were used to evaluate of the statistical significance of S100A4 expression as it related to the clinicopathological parameters. Follow-up information was also obtained for a survival analysis. Patient survival was calculated as the time between surgery and death. Patients who were still alive at the time of data collection were censored in the statistical analysis. Survival curves were plotted according to the Kaplan-Meier method. Clinical factors were justified as reasonable by fitting Cox's proportional hazards models. All statistical analyses were performed using the SPSS ver. 14.0 (SPSS Inc., Chicago, IL, USA). Probability values ≤ 0.05 were regarded as significant.

RESULTS

Relationship between S100A4 expression and clinicopathological parameters

S100A4 immunostaining scores ≥ 2 were found in 56 cases

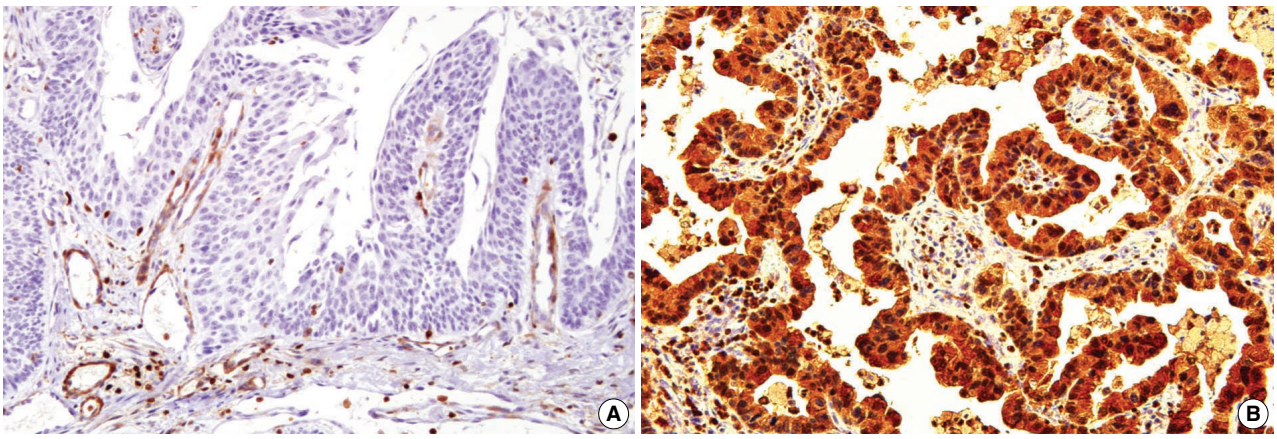

Fig. 1. S100A4 immunohistochemical staining shows negative staining for tumor cells but immunoreactivity to lymphocytes, macrophages, and vascular wall smooth muscle (A) and positive staining in tumor cells (B).

(83.6%). These were categorized as the positive group. Negative S100A4 expression (score of 0 or 1) occurred in 11 cases (16.4%). Histological type was significantly correlated with S100A4 expression. ADs showed positive S100A4 expression more frequently than SCCs (p = 0.017). S100A4 expression was not significantly correlated with age (p = 0.182), gender (p = 0.435), pT (p = 0.580), pN (p = 0.525) or TNM stage (p = 0.952). Two distant metastatic cases revealed positive reactions (Table 1).

Table 1. Relationship between S100A4 expression and clinicopathological parameters in non-small cell lung carcinomas

Parameters	S100A4		n	p-value
	Negative (%)	Positive (%)		
No. of cases	11 (16.4)	56 (83.6)		
Age (yr)				
< 65	5 (45.5)	38 (67.9)	43	0.182
≥ 65	6 (54.5)	18 (32.1)	24	
Gender				
Male	10 (90.9)	43 (76.8)	53	0.435
Female	1 (9.1)	13 (23.2)	14	
Histologic type				
SCC	10 (90.9)	27 (48.2)	37	0.017
AD	1 (9.1)	29 (51.8)	30	
pT				
T1	3 (27.3)	15 (26.8)	18	0.580
T2	5 (45.5)	32 (57.1)	37	
T3	3 (27.3)	7 (12.5)	10	
T4	0 (0)	2 (3.6)	2	
pN				
NX	0 (0)	1 (1.8)	1	0.525
N0	7 (63.6)	30 (53.6)	37	
N1	2 (18.2)	12 (21.4)	14	
N2	2 (18.2)	13 (23.2)	15	
N3	0 (0)	0 (0)	0	
pM				
M0	11 (100)	54 (96.4)	65	1.000
M1	0 (0)	2 (3.6)	2	
TNM stage				
I	5 (45.5)	26 (46.4)	31	0.952
II	3 (27.3)	14 (25.0)	17	
III	2 (18.2)	13 (23.2)	15	
IV	1 (9.1)	3 (5.4)	4	

SCC, squamous cell carcinoma; AD, adenocarcinoma; TNM, tumor, node and metastasis.

Correlation between patient survival and S100A4 expression

Length of survival in months was defined as the interval between surgical resection and either death or the last follow-up. This observation was censored at the last follow-up when patients were alive or had died from something other than cancer. The mean follow-up period was 35.5 months (range, 0 to 140 months). Thirty-nine patients died (39/51, 76.5%) within 5 years after surgery. The 3-, and 5-year survival rates were 32.8% and 23.5%, respectively. Kaplan-Meier survival curves and the log-rank test showed no correlation of S100A4 expression with 3-year survival (p = 0.782) or 5-year survival (p = 0.227) in either group of patients (Fig. 2). Median survival times for negative S100A4 expression and positive S100A4 expression were 25 and 22 months, respectively. Finally, a Cox's proportional hazards model was used to confirm whether or not S100A4 expression was correlated with patient prognosis. Variables were dichotomized. After adjusting for gender, age, and TNM stage, AD (p = 0.012) and TNM stages II-IV (p = 0.025) were independently associated with poor patient survival. No correlation was found between S100A4 expression (p = 0.495) and survival (Table 2).

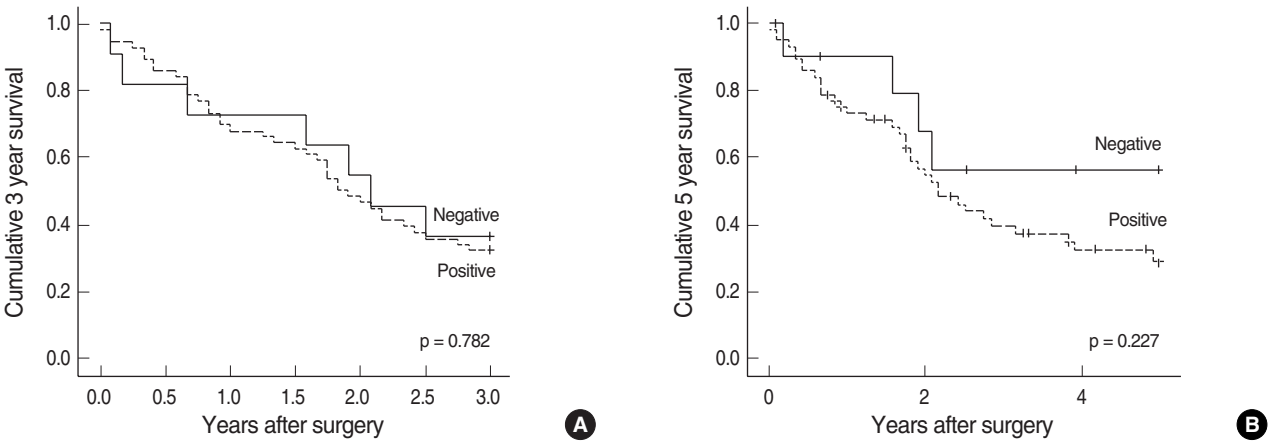

Fig. 2. Kaplan-Meier survival curves demonstrate the relationship between 3-year survival (A) and 5-year survival (B) and S100A4 expression.

Table 2. Cox proportional hazards model of non-small cell lung carcinomas

Variables	Relative risk	95% CI	p-value
Sex, male vs female	0.47	0.195-1.177	0.108
Age (yr), < 65 vs ≥ 65	1.32	0.652-2.678	0.438
Histologic type, SCC vs AD	2.53	1.221-5.260	0.012
TNM stage, I vs II-IV	2.18	1.101-4.351	0.025
S100A4	1.46	0.468-4.436	0.495

CI, confidence interval; SCC, squamous cell carcinoma; AD, adenocarcinoma; TNM, tumor, node and metastasis.

DISCUSSION

The S100 protein family is a large family of soluble calcium-binding proteins that were first isolated from bovine brain by Moore[21] in 1965. To date, 25 different proteins have been assigned to the S100 protein family including S100A1-A16, S100B, S100P, S100Z, profilaggrin, trichohyalin, and reperin. Genes encoding most of these proteins are located in a cluster on human chromosome 1q21.[15,22,23] Members of the S100 family are involved in a variety of physiological functions, including cell motility, cell proliferation and differentiation, cell cycle control, regulation of enzyme activity, apoptosis, and calcium-dependent transcriptional regulation.[24-27] Deregulation of S100 expression has been reported in a variety of neoplastic and non-neoplastic diseases including psoriasis, Alzheimer's disease, cystic fibrosis, cardiomyopathy, epilepsy, and amyotrophic lateral sclerosis.[22] Among S100 family members, several S100 proteins, such as S100A2, S100A4, S100A6, S100A7, S100A11, S100P and S100B are postulated to play a role in the progression of human cancer.[26]

The S100A4 protein (also known as Mts1, metastasin, p9Ka, pEL98, CAPL, calvasculin, Fsp-1, and placental calcium binding protein) is a small-molecular-weight (12 kDa) protein with two domains: an S100 domain and a calcium-binding domain (EF domain).[22] This protein has no known enzymatic activity, however, the observation that it binds to several known proteins is indicative of its function. S100A4 binds to actin, non-muscle myosin, and the p53 tumor suppressor protein. This interaction may increase cell motility and modulate p53 function.[28] Parker *et al.*[29] found that when S100A4 expression was upregulated a parallel increase in p53 was detected by immunohistochemistry. This enhanced detection was attributed to p53 stabilization through the formation of an S100A4 and p53 complex. Sequestered in this way, p53 would be effectively dispossessed of its G_1-S checkpoint control and the cell would enter S-phase.

In the present study, S100A4 expression was evaluated in relation to clinicopathological parameters of NSCLCs. Of the 67 patients with NSCLCs, 83.6% were positive for S100A4 expression, which was somewhat higher than previous reports on lung[16-18] and other organs.[9-15,30] However, we used a very wide positive range (scores of 2 to 9). ADs more frequently showed positive S100A4 expression than SCCs (p = 0.017). In contrast to our study, Tsuna *et al.*[18] reported that S100A4 expression showed a higher positivity in SCCs than in ADs. Therefore, we cannot be certain about our results, and additional studies are needed.

A correlation of S100A4 expression with disease progression, metastasis and decreased patient survival has been reported in a variety of human carcinomas, however, the results are diverse and controversial. Some reports have shown that S100A4 expres-

sion is a significant predictor of disease progression and cancer-specific survival in bladder cancer,[9] advanced gastric cancer,[14] breast cancer,[11,12] and pancreatic cancer.[15] In contrast, several reports have found no association between S100A4 expression and overall survival in colonic AD[30] and melanoma.[23] Several recently published studies have described a correlation between survival rate and S100A4 expression in pulmonary AD,[17,19] SCC,[18] and NSCLC,[16] respectively. Kimura *et al.*[16] reported a correlation of S100A4 expression with pathological T factor, lymph node metastasis and poor survival in NSCLC. Matsubara *et al.*[17] and Miyazaki *et al.*[19] reported an association of S100A4 expression with vascular invasion and poor prognosis. Tsuna *et al.*[18] revealed on association of S100A4 expression with poor prognosis in SCC, however, this was not correlated with survival in patients with AD. But, we found no statistically significant factor in this study. Our data are based on a retrospective study and a relatively small number of patients from a single institute. Thus, the significance of S100A4 in NSCLCs will require confirmation from additional studies using a larger number of patients, multiple cores of NSCLC specimens and various histological types of NSCLCs.

In summary, our study demonstrated no significant correlation between S100A4 expression and age, gender, pT, pN, TNM stage, or survival in patients with NSCLCs. Therefore, S100A4 expression may not be useful as a prognostic marker for NSCLCs. However, S100A4 expression showed a higher positivity in ADs than in SCCs.

ACKNOWLEDGEMENTS

We would like to thank Prof. Jin Haeng Chung, Department of Pathology, Seoul National University College of Medicine for her valuable technical assistance with the immunohistochemistry.

REFERENCES

1. Kumar V, Abbas AK, Fausto N, Aster JC. Robbins and Cotran: pathologic basis of disease. 8th ed. Philadelphia: Saunders-Elsevier, 2010; 721-31.
2. National Cancer Information Center. Cancer statistics: August 25, 2008 [Internet]. Goyang: National Cancer Information Center; 2008 [cited 2009 Nov 5]. Available from: http://www.cancer.go.kr/cms/statics.
3. Toloza EM, D'Amico TA. Targeted therapy for non-small cell lung cancer. Semin Thorac Cardiovasc Surg 2005; 17: 199-204.
4. Sherbet GV. Metastasis promoter S100A4 is a potentially valuable molecular target for cancer therapy. Cancer Lett 2009; 280: 15-30.
5. Cajone F, Sherbet GV. Stathmin is involved in S100A4-mediated regulation of cell cycle progression. Clin Exp Metastasis 1999; 17: 865-71.
6. Jackson-Grusby LL, Swiergiel J, Linzer DI. A growth-related mRNA in cultured mouse cells encodes a placental calcium binding protein. Nucleic Acids Res 1987; 15: 6677-90.
7. Schmidt-Hansen B, Klingelhöfer J, Grum-Schwensen B, *et al.* Functional significance of metastasis-inducing S100A4(Mts1) in tumor-stroma interplay. J Biol Chem 2004; 279: 24498-504.
8. Shi Y, Zou M, Collison K, *et al.* Ribonucleic acid interference targeting S100A4 (Mts1) suppresses tumor growth and metastasis of anaplastic thyroid carcinoma in a mouse model. J Clin Endocrinol Metab 2006; 91: 2373-9.
9. Matsumoto K, Irie A, Satoh T, *et al.* Expression of S100A2 and S100A4 predicts for disease progression and patient survival in bladder cancer. Urology 2007; 70: 602-7.
10. Yao R, Davidson DD, Lopez-Beltran A, MacLennan GT, Montironi R, Cheng L. The S100 proteins for screening and prognostic grading of bladder cancer. Histol Histopathol 2007; 22: 1025-32.
11. Rudland PS, Platt-Higgins A, Renshaw C, *et al.* Prognostic significance of the metastasis-inducing protein S100A4 (p9Ka) in human breast cancer. Cancer Res 2000; 60: 1595-603.
12. Lee WY, Su WC, Lin PW, Guo HR, Chang TW, Chen HH. Expression of S100A4 and Met: potential predictors for metastasis and survival in early-stage breast cancer. Oncology 2004; 66: 429-38.
13. Zou M, Famulski KS, Parhar RS, *et al.* Microarray analysis of metastasis-associated gene expression profiling in a murine model of thyroid carcinoma pulmonary metastasis: identification of S100A4 (Mts-1) gene overexpression as a poor prognostic marker for thyroid carcinoma. J Clin Endocrinol Metab 2004; 89: 6146-54.
14. Yoon CS, Hyung WJ, Lee JH, *et al.* Expression of S100A4, E-cadherin, alpha- and beta-catenin in gastric adenocarcinoma. Hepatogastroenterology 2008; 55: 1916-20.
15. Ai KX, Lu LY, Huang XY, Chen W, Zhang HZ. Prognostic significance of S100A4 and vascular endothelial growth factor expression in pancreatic cancer. World J Gastroenterol 2008; 14: 1931-5.
16. Kimura K, Endo Y, Yonemura Y, *et al.* Clinical significance of S100A4 and E-cadherin-related adhesion molecules in non-small cell lung cancer. Int J Oncol 2000; 16: 1125-31.
17. Matsubara D, Niki T, Ishikawa S, *et al.* Differential expression of S100A2 and S100A4 in lung adenocarcinomas: clinicopathological significance, relationship to p53 and identification of their target

genes. Cancer Sci 2005; 96: 844-57.
18. Tsuna M, Kageyama S, Fukuoka J, *et al.* Significance of S100A4 as a prognostic marker of lung squamous cell carcinoma. Anticancer Res 2009; 29: 2547-54.
19. Miyazaki N, Abe Y, Oida Y, *et al.* Poor outcome of patients with pulmonary adenocarcinoma showing decreased E-cadherin combined with increased S100A4 expression. Int J Oncol 2006; 28: 1369-74.
20. Travis WD. Reporting lung cancer pathology specimens: impact of the anticipated 7th Edition TNM classification based on recommendations of the IASLC Staging Committee. Histopathology 2009; 54: 3-11.
21. Moore BW. A soluble protein characteristic of the nervous system. Biochem Biophys Res Commun 1965; 19: 739-44.
22. Tarabykina S, Griffiths TR, Tulchinsky E, Mellon JK, Bronstein IB, Kriajevska M. Metastasis-associated protein S100A4: spotlight on its role in cell migration. Curr Cancer Drug Targets 2007; 7: 217-28.
23. Bolander A, Agnarsdóttir M, Wagenius G, *et al.* Serological and immunohistochemical analysis of S100 and new derivatives as markers for prognosis in patients with malignant melanoma. Melanoma Res 2008; 18: 412-9.
24. Kriajevska M, Bronstein IB, Scott DJ, *et al.* Metastasis-associated protein Mts1 (S100A4) inhibits CK2-mediated phosphorylation and self-assembly of the heavy chain of nonmuscle myosin. Biochim Biophys Acta 2000; 1498: 252-63.
25. Schäfer BW, Heizmann CW. The S100 family of EF-hand calcium-binding proteins: functions and pathology. Trends Biochem Sci 1996; 21: 134-40.
26. Heizmann CW, Fritz G, Schäfer BW. S100 proteins: structure, functions and pathology. Front Biosci 2002; 7: d1356-68.
27. Emberley ED, Murphy LC, Watson PH. S100 proteins and their influence on pro-survival pathways in cancer. Biochem Cell Biol 2004; 82: 508-15.
28. Grigorian M, Andresen S, Tulchinsky E, *et al.* 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: 22699-708.
29. 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: 343-51.
30. Cho YG, Kim CJ, Nam SW, *et al.* Overexpression of S100A4 is closely associated with progression of colorectal cancer. World J Gastroenterol 2005; 11: 4852-6.