EUROPEAN JOURNAL OF CANCER 46 (2010) 2919–2925

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejconline.com

Nuclear S100A4 is a novel prognostic marker in colorectal cancer ☆

Kjetil Boye [a,b,*], *Jahn M. Nesland* [c,d], *Berit Sandstad* [e], *Gunhild M. Mælandsmo* [a,f,h], *Kjersti Flatmark* [a,g,h]

[a] *Department of Tumor Biology, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway*
[b] *Department of Oncology, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway*
[c] *Department of Pathology, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway*
[d] *Medical Faculty, University of Oslo, Oslo, Norway*
[e] *Clinical Research Unit, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway*
[f] *Department of Pharmacy, Faculty of Health Sciences, University of Tromsø, Tromsø, Norway*
[g] *Department of Surgical Oncology, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway*

ARTICLE INFO

Article history:
Received 19 May 2010
Received in revised form 2 July 2010
Accepted 14 July 2010
Available online 16 August 2010

Keywords:
S100A4
Colorectal cancer
Prognostic marker
Nuclear expression

ABSTRACT

Current staging classifications in colorectal cancer are not able to accurately predict patient outcome, and the need for novel prognostic markers is evident. S100A4 is a Ca^{2+}-binding protein which promotes metastasis in several tumour types, and the aim of the present study was to investigate the prognostic impact of S100A4 expression in colorectal cancer. Two hundred and forty two patients with curatively resected adenocarcinoma of the colon or rectum were prospectively included in the study at the time of surgery. S100A4 expression was analysed by immunohistochemistry, and associations with clinicopathological variables and patient outcome were investigated. Nuclear expression of S100A4 was observed in 29% and cytoplasmic expression was observed in 64% of the tumours. In univariate analysis, nuclear S100A4 was a negative predictor of metastasis-free ($P = 0.006$) and overall survival ($P = 0.01$), whereas cytoplasmic S100A4 was not associated with patient outcome. In multivariate analysis, nuclear localisation was inversely associated with metastasis-free ($P = 0.03$) and overall survival ($P = 0.02$). Interestingly, the prognostic impact was largely confined to TNM stage II, and stage II patients with tumours expressing nuclear S100A4 had a similar prognosis as stage III patients. In conclusion, nuclear expression of S100A4 is a novel prognostic marker in colorectal cancer, and the prognostic value in TNM stage II suggests that nuclear S100A4 could be used in the stratification of stage II patients for adjuvant treatment.

☆ This work was supported by the Norwegian Cancer Society, the Research Council of Norway, the Norwegian Foundation for Health and Rehabilitation, the National Program for Functional Genomics in the Research Council of Norway, the Legacy for Cancer Research and Jeanette and Søren Bothner's Legacy.

* *Corresponding author:* Address: Department of Tumor Biology, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo University Hospital, P.O. Box 4953 Nydalen, NO-0424 Oslo, Norway. Tel.: +47 22781852; fax: +47 22781895.
E-mail address: kjetil.boye@rr-research.no (K. Boye).
[h] These authors contributed equally to this work.

0959-8049/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ejca.2010.07.013

1. Introduction

Distant metastasis is the main cause of death in patients with colorectal cancer. At present, clinical staging and histopathological criteria are the only parameters in clinical use to stratify patients according to the risk of developing metastatic disease after curatively intended surgery. However, current staging classifications are not able to accurately predict patient outcome,[1] illustrating the considerable biological heterogeneity of tumours within the separate disease stages. Molecular markers could possibly account for this diversity, and several prognostic factors have been identified.[2] Still, none of these have so far been proven robust enough to be incorporated into routine practice.[3]

Today, clinical and pathological staging is also the basis for selection of patients for adjuvant treatment. Patients with stage III disease generally profit from adjuvant chemotherapy due to an increased risk of subsequent metastatic disease, whereas adjuvant treatment in stage I and II has shown no clear survival benefit.[4] A number of patients in these early disease stages still develop metastases in distant organs. Thus, there is a need for novel prognostic biomarkers to identify subgroups of patients that could benefit from adjuvant treatment, but also to identify subsets with low probability of treatment benefit that should be spared the toxic effects of chemotherapy.

One potential prognostic factor is S100A4, a small, calcium-binding protein belonging to the S100 protein family.[5] S100A4 promotes metastasis in experimental animal models from a number of tumour types, and is involved in several steps of the metastatic cascade, including cell motility, invasion and angiogenesis.[6–9] The protein is localised in the nucleus, cytoplasm and the extracellular space, and the expression of S100A4 has been shown to predict patient outcome in several tumour types.[6,10,11] However, the prognostic impact of nuclear S100A4 has so far not been assessed. In a prospectively collected panel of tumour specimens from colorectal cancer patients we have previously demonstrated that nuclear expression of S100A4 correlated with tumour stage.[12] In the present study, the association between S100A4 protein expression and patient outcome in this patient cohort was investigated.

2. Patients and methods

2.1. Patient cohort

Between September 1998 and July 2000, 316 patients from five hospitals in the Oslo region were included in the study at the time of primary surgery for assumed or verified colorectal cancer.[13] The study was approved by the Regional Ethics Committee (#S-98080) and informed consent was obtained from the patients. Seventy-four patients were excluded from the present study for the following reasons: not invasive cancer (25), histology other than adenocarcinoma (5), distant metastases at the time of surgery (34), inadequate surgical margins (7), preoperative chemoradiotherapy (2) and unknown stage of disease (1). The study population thus included 242 patients in TNM stages I–III who had undergone curative surgery, and paraffin sections were available from 237 of these patients. Follow-up data were obtained from consecutive reports from physicians at the participating hospitals. Valid observations of the presence or absence of distant metastases required radiological examination. For patients not attending scheduled controls, data were retrieved from the patient records at the hospitals and by contacting the patients' general practitioners. In addition, survival data were obtained from the National Registry of Norway and updated by October 1st 2008. The cause of death was registered and classified as death from colorectal cancer, death of other causes or death of unknown cause. For overall survival, median follow-up of patients still alive was 9.1 years (range 8.2–10.0). The primary tumour sections were re-evaluated by the study pathologist (J.M.N.) to ensure consistent staging and grading, and representative sections for each tumour were identified for subsequent immunohistochemical analysis.

2.2. Immunohistochemistry

The immunohistochemical staining procedure has been described previously.[12] Briefly, sections of formalin-fixed, paraffin-embedded tissue were immunostained using the biotin–streptavidin-peroxidase method (Supersensitive Immunodetection System, LP000-UL; Biogenex, San Ramon, CA, USA) and the Optimax Plus Automated Cell Staining System (Biogenex) using rabbit polyclonal anti-S100A4 antibody (DAKO, Glostrup, Denmark). Negative controls included replacement of the primary antibody with normal polyclonal rabbit IgG of the same subclass and concentration and incubation of sections with rabbit polyclonal anti-S100A4 antibody pre-absorbed with 100 μg/ml recombinant human S100A4. Positive controls (sections from colorectal tumour tissue known to express high amounts of S100A4) were included in all series. Cytoplasmic and nuclear staining were recorded as separate variables, and the number of S100A4-positive tumour cells was semi-quantitatively estimated and graded from 0 to 5 (percentage of positive carcinoma cells in parentheses): 0 (0%), 1 (1–4%), 2 (5–9%), 3 (10–14%), 4 (15–49%), and 5 (>50%). For all statistical analyses, tumours in grade 1–5 were grouped as positive. Classifying the borderline positive tumours (1–4% positive tumour cells) as negative did not significantly affect the survival analyses for cytoplasmic S100A4.

2.3. Statistical analysis

Associations between S100A4 staining and clinicopathological variables were tested using two-tailed Fisher's exact test or linear-by-linear association chi-square test. Univariate survival analysis was performed according to the Kaplan–Meier method, and survival was compared using the log rank test. Multivariate analysis was conducted using the Cox proportional hazards regression model with backward, stepwise elimination of variables. Survival was measured from date of surgery until death for overall and disease-specific survival, and from date of surgery until diagnosis of distant metastasis for metastasis-free survival. Data analysis was performed using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). P-values < 0.05 were considered statistically significant.

3. Results

3.1. Patient characteristics and outcome

Clinical and histological parameters of the study cohort are summarised in Table 1. Mean patient age at the time of surgery was 73 years (range 35–98 years). Thirty patients in TNM stage III (39%) and two patients in stage II (2%) received adjuvant chemotherapy. Postoperative radiotherapy was administered in two cases. All patients included in this study had undergone R0 resections and did not have metastatic disease at the time of diagnosis. Outcome parameters are presented in Table 2. Fifty-seven patients developed distant metastases during follow-up, 31 of which had colon cancer and 26 had rectal cancer. A total of 53 colorectal cancer-related deaths were registered, and 50 of these patients died of metastatic disease, whereas 3 patients died of local recurrence.

Table 1 – Expression of S100A4 and baseline clinicopathological data of the study cohort.

Parameter		Patients	
		Number	Percent
Gender	Female	110	45
	Male	132	55
TNM stage	I	53	22
	II	112	46
	III	77	32
pT	1	8	3
	2	51	21
	3	159	66
	4	24	10
pN	0	165	68
	1	51	21
	2	26	11
Differentiation	Well	7	3
	Intermediate	208	86
	Poor	27	11
Tumour localisation	Colon	163	67
	Rectum	79	33
Lymphocyte infiltration	High	29	12
	Intermediate	156	65
	Low	54	23
	ND	3	
Vascular invasion	Present	47	20
	Absent	194	80
	ND	1	
Perineural invasion	Present	19	8
	Absent	222	92
	ND	1	
Perinodal growth[a]	Present	44	57
	Absent	33	43
Cytoplasmic S100A4[b]	0	86	36
	1	21	9
	2	15	6
	3	29	12
	4	25	11
	5	61	26
	ND	5	
Nuclear S100A4[b]	0	169	71
	1	41	17
	2	18	8
	3	9	4
	ND	5	

ND = not determined.
[a] Perinodal growth was assessed in node-positive patients only.
[b] Data from Flatmark et al.[12]

3.2. S100A4 protein expression and association with clinicopathological parameters

The expression levels and subcellular distribution of the S100A4 protein in this patient cohort have been described previously.[12] Of the 237 tumours analysed for the expression of S100A4, 68 samples (29%) displayed nuclear staining, and cytoplasmic staining was observed in 151 tumours (64%). Nuclear expression of S100A4 was associated with tumour stage at diagnosis as previously reported.[12] In addition, nuclear staining was significantly associated with the presence of lymph node metastasis ($P = 0.03$), reflecting the association with disease stage, and with perineural invasion ($P = 0.03$; Supplementary Table 1). Cytoplasmic expression of S100A4 was associated with vascular invasion ($P = 0.03$), perinodal growth ($P = 0.04$) and tumour localisation in the rectum ($P = 0.04$; Supplementary Table 1). No other significant associations between immunohistochemical staining for S100A4 and clinicopathological variables were observed.

3.3. Association between clinicopathological parameters and patient outcome

The prognostic significance of clinical and pathological variables was investigated by univariate analysis (Table 3). TNM stage, T classification, nodal status, tumour-infiltrating

Table 2 – Outcome parameters of the study cohort.

Outcome parameter[a]		Patients	
		Number	Percent
Distant metastasis	Yes	57	24
	No	185	76
Local recurrence[b]	Yes	11	5
	No	231	95
Death	Yes	116	48
	No	126	52
Cause of death	Colorectal cancer	53	22
	Other	34	14
	Unknown	29	12
Location of metastases[c]	Liver	35	
	Lungs	27	
	Other location	22	

[a] Presence or absence of outcome parameters was registered during the follow-up period as explained in Section 2.
[b] Six patients had isolated local recurrence.
[c] Metastases in more than one location were diagnosed in 24 patients.

Table 3 – Survival analyses of clinicopathological parameters and S100A4.

	Univariate analysis (P-value, log rank test)			Multivariate Cox regression analysis[b]		
	Metastasis-free survival	Disease-specific survival	Overall survival	P-value	Hazard ratio	95% CI
Gender	NS	NS	NS	NS		
TNM stage	<0.001	<0.001	NS	0.02		
I						
II					1.7	0.6–4.4
III					3.1	1.2–7.7
pT	0.05	0.04	NS			
pN	<0.001	<0.001	0.001			
Differentiation	NS	NS	NS	0.05		
Well						
Intermediate					0.9	0.1–7.1
Poor					2.7	0.3–22.6
Tumour localisation	0.01	NS	NS	0.01		
Colon						
Rectum					2.2	1.2–4.0
Lymphocyte infiltration	0.009	0.006	NS	0.04		
High						
Intermediate					2.7	0.6–11.4
Low					4.7	1.1–20.5
Vascular invasion	0.02	NS	NS	NS		
Perineural invasion	NS	0.03	NS	NS		
Perinodal growth[a]	NS	NS	NS			
S100A4C	NS	NS	NS	NS		
S100A4N	0.006	0.01	0.01	0.03		
0						
1–3					1.8	1.0–3.2

NS = not significant.
[a] Perinodal growth was assessed in node-positive patients only.
[b] Stepwise Cox regression analysis is shown for metastasis-free survival.

lymphocytes, vascular invasion and tumour localisation were associated with the development of metastatic disease. Patients whose tumours were localised in the colon generally had a better prognosis than patients with rectal tumours ($P = 0.01$), whereas there were no significant differences between tumours in the right and left colon (data not shown). The presence of perinodal growth was of course tightly associated with nodal status, but did not contribute any additional prognostic information in the subset of node-positive patients. In accordance with the data for metastasis-free survival, TNM stage, T classification, nodal status and lymphocyte infiltration were associated with disease-specific survival, whereas vascular invasion ($P = 0.06$) and tumour localisation ($P = 0.06$) did not reach statistical significance. Nodal status was the only standard clinicopathological parameter associated with overall survival, whereas a trend was observed for TNM stage ($P = 0.05$).

3.4. *Association between S100A4 expression and patient outcome*

The expression of nuclear S100A4 (S100A4N) was strongly associated with patient outcome (Table 3 and Fig. 1). Patients with S100A4N-positive tumours had a 5-year metastasis-free survival rate of 53%, compared with 76% for patients with S100A4N-negative tumours, and the 8-year overall survival rate was 47% and 61%, respectively. Cytoplasmic S100A4 expression was, however, not associated with prognosis in this patient cohort (Table 3 and Fig. 1). To determine if the relationship between S100A4 expression and patient outcome was independent of other clinical and pathological parameters, multivariate Cox regression analysis was performed. Variables included in the multivariate analysis were S100A4N, S100A4C, age, gender, TNM stage, differentiation, tumour localisation, lymphocyte infiltration, vascular invasion and perineural invasion. Interestingly, S100A4N was independently and significantly associated with metastasis-free survival (Table 3). S100A4N was also an independent prognostic factor for disease-specific survival ($P = 0.01$; hazard ratio 2.3; 95% confidence interval (CI) 1.2–4.4; data not shown) and overall survival ($P = 0.02$; hazard ratio 1.6; 95% CI 1.1–2.4; data not shown).

3.5. *Prognostic impact of S100A4 expression in TNM stage II*

To investigate if S100A4 expression was able to stratify patients into prognostic subgroups within each TNM stage, the patient cohort was divided into the separate disease stages and univariate survival analyses were performed.

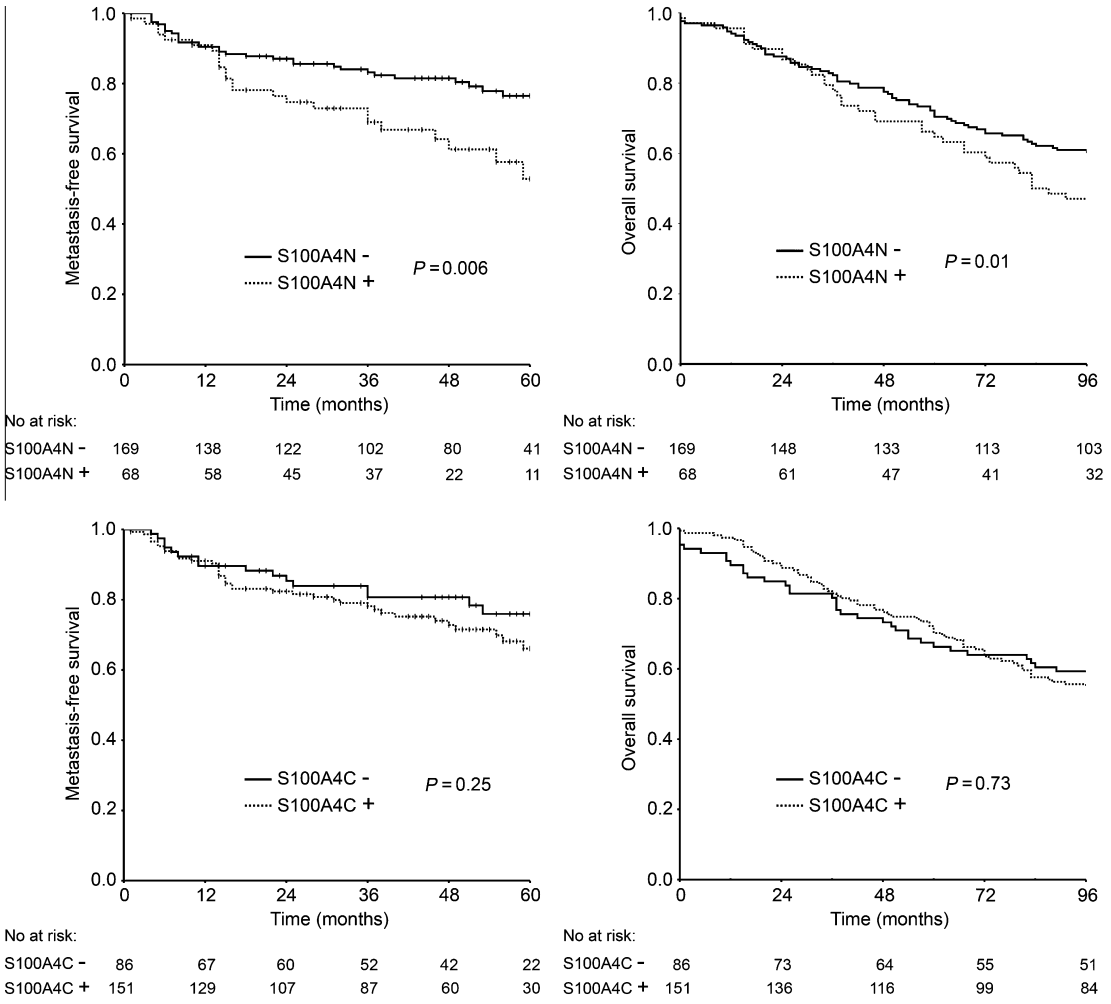

Fig. 1 – Kaplan–Meier survival plots depicting metastasis-free (upper left) and overall survival (upper right) based on nuclear expression of S100A4, and metastasis-free (lower left) and overall survival (lower right) based on cytoplasmic expression of S100A4.

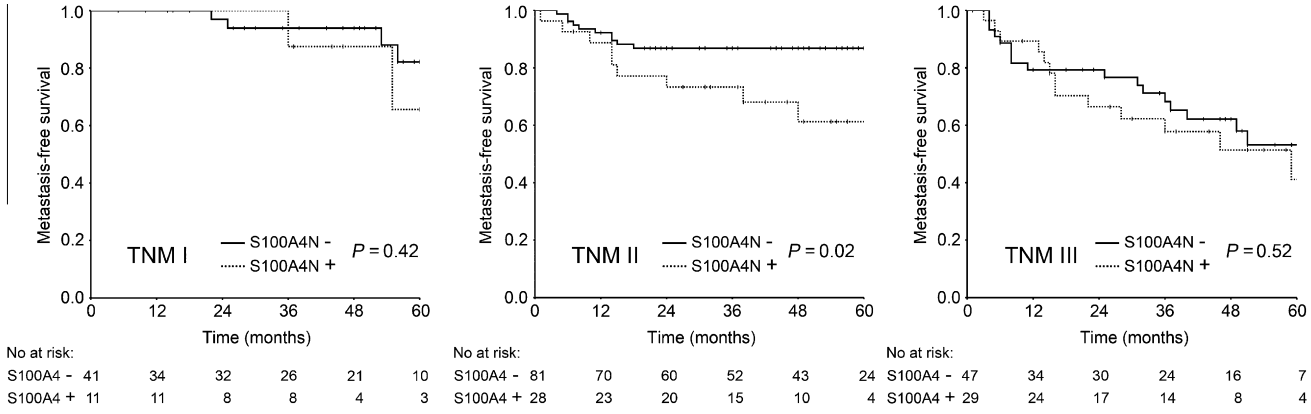

Fig. 2 – Kaplan–Meier survival plots depicting metastasis-free survival stratified according to TNM stage and based on nuclear expression of S100A4.

Surprisingly, nuclear expression of S100A4 was associated with patient outcome only for stage II patients (Fig. 2). However, when stage III patients were stratified according to pN category, S100A4N was significantly associated with metastasis-free survival also for pN1 patients (i.e. 1–3 involved lymph nodes; Supplementary Fig. 1) Interestingly, stage II patients with S100A4N-positive tumours displayed a 5-year metastasis-free survival rate at almost the same level as patients in

stage III (58% and 48%, respectively), and patients with S100A4-negative stage II disease had a similar prognosis as patients in stage I (87% and 79%, respectively).

4. Discussion

In this prospective study, we demonstrate for the first time that the presence of nuclear S100A4 in tumour cells is of prognostic significance in colorectal cancer. Nuclear expression was a significant and robust predictor of metastasis-free and overall survival, and was also associated with patient outcome in multivariate analysis, indicating that the observed results may translate into clinically important differences. The validity of our findings is strengthened by the prospective study design, ensuring an unbiased selection of patients, and the stage distribution and patient survival corresponded well with results from a large population-based study consisting of more than 100,000 patients.[1]

S100A4 protein expression has previously been associated with survival in several tumour types,[6] and in colorectal cancer prognostic significance has been documented in two recent reports.[10,14] In a large patient cohort consisting of 709 cases, Gongoll and colleagues demonstrated that immunohistochemical staining for S100A4 was associated with outcome,[10] and similar results were obtained by Hemandas and colleagues in a relatively small number of patients ($n = 54$).[14] However, nuclear and cytoplasmic expressions were not recorded as individual variables in these reports.

The prognostic significance of nuclear S100A4 in TNM stage II is particularly interesting. Even though stage II patients in general have a good prognosis, the clinical behaviour within this group is heterogeneous, and a significant fraction of patients develop distant metastases. Still, the majority of clinical trials evaluating chemotherapy after surgery in stage II disease have not been able to show a substantial survival benefit, and adjuvant therapy for these patients remains controversial.[4,15,16] Considerable effort has been undertaken to identify risk factors that are able to select patients who would benefit from adjuvant chemotherapy, such as T4 status, tumour perforation, bowel obstruction, perineural invasion, tumour differentiation, vascular invasion, number of lymph nodes examined and microsatellite instability.[17] Based on these criteria, high-risk stage II patients are often offered adjuvant treatment, but the need for markers improving the stratification of patients is evident. The present results suggest that S100A4 is a novel marker of aggressive disease in stage II patients, and interestingly, S100A4N-positive stage II patients had a prognosis similar to stage III patients. No association was detected between nuclear expression of S100A4 and T4 status, tumour differentiation, vascular invasion, perineural invasion or number of lymph nodes examined in stage II patients (data not shown), arguing that the prognostic value of S100A4 is independent of established markers of high-risk disease. Only two of the stage II patients in this study received adjuvant chemotherapy, and no conclusions regarding treatment response could thus be drawn. Based on the present observations, we hypothesise that nuclear S100A4 could be used as a novel marker for the selection of stage II patients for adjuvant treatment, but validation studies are required to verify our findings in separate patient cohorts.

The presence of nuclear S100A4 was associated with cytoplasmic protein expression ($P < 0.001$; Supplementary Table 1), and one might speculate that nuclear staining could be a reflection of increased overall expression of S100A4. However, no association was detected between grade 5 cytoplasmic expression and nuclear staining ($P = 0.63$; data not shown). One possible explanation for the specific localisation of S100A4 in the nuclei of some tumours could be that the nuclear protein differs in charge or size. However, nuclear and cytoplasmic fractions from colorectal cancer cell lines displayed almost identical spot patterns using 2D-PAGE and Western blotting,[18] and further studies aiming to elucidate the molecular mechanisms involved in nuclear translocation of S100A4 are certainly required.

The mechanisms by which nuclear S100A4 promotes metastasis are generally unknown. Biological functions involved in S100A4-induced metastasis include cell motility, invasion and angiogenesis, but in the studies published so far these actions rely on cytoplasmic localisation or extracellular activity of the protein,[6] suggesting that other mechanisms may be associated with nuclear expression. One might speculate that nuclear S100A4 could be involved in transcriptional regulation, either by binding DNA or by interacting with DNA-binding proteins. In support of this theory, S100A4 has been shown to bind p53 in vitro,[19] and other S100 proteins also interact with transcription factors, including p53 and members of the basic helix-loop-helix family, thereby affecting their DNA-binding properties and transcriptional activity.[20–24] In a subset of the tumours in the present study ($n = 40$), no association between the mutational status of TP53 and nuclear S100A4 was detected.[21] In contrast to the suggested hypothesis that S100A4 only exists in cells harbouring mutated p53,[19] S100A4 and wild-type p53 were co-localised in the nuclei of colorectal carcinomas and cell lines.[21] Still, the functional consequence of this co-localisation and whether these proteins actually interact in vivo remain unclear.

Another potential mechanism linking nuclear S100A4 with metastasis in colorectal cancer could be the involvement of S100A4 in epithelial-mesenchymal transition (EMT),[25] as EMT-promoting properties have been suggested to be critical for S100A4-induced metastasis in breast cancer model systems.[26] S100A4 is also overexpressed in cell populations enriched for stem-like cells,[27] consistent with the recent finding that passage through an EMT could confer stem-cell like properties to carcinoma cells.[28] In colorectal cancer, the wnt/APC/β-catenin signalling pathway is associated with both stem-cell features and EMT,[29] and interestingly, S100A4 has been identified as a β-catenin/TCF target gene.[30] Based on our findings, one might speculate that nuclear S100A4 could be associated with a mesenchymal and stem-cell like phenotype in colorectal cancer, resulting in increased metastatic potential, and future investigations aiming to characterise the biological role of nuclear S100A4 are warranted.

In conclusion, this prospectively designed study has identified nuclear expression of the metastasis-promoting protein S100A4 as an independent and robust predictor of adverse prognosis in colorectal cancer patients. The prognostic impact was largely confined to TNM stage II, suggesting that S100A4 is able to identify a subgroup of stage II patients with

more aggressive disease that might benefit from additional treatment. If validation studies are able to confirm our results, S100A4 status should be included as biomarker in future clinical studies investigating the stratification of stage II patients for adjuvant treatment.

Conflict of interest statement

None declared.

Acknowledgements

We acknowledge the contributions of members of the Study Group for Bone Marrow Micrometastases in Colorectal Cancer: Øystein Fodstad, The Norwegian Radium Hospital (Study Group Leader); Hans Olaf Johannessen, Ullevål University Hospital (local study coordinator); Ida Bukholm, Akershus University Hospital (local study coordinator); Rocio Rosales, Asker and Bærum Hospital (local study coordinator); Lisbeth Hårklau, Aker University Hospital (local study coordinator); and Hedin Jacobsen, Diakonhjemmet Hospital (local study coordinator). We also wish to thank Ellen Hellesylt for excellent technical assistance.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejca.2010.07.013.

REFERENCES

1. O'Connell JB, Maggard MA, Ko CY. Colon cancer survival rates with the new American Joint Committee on Cancer sixth edition staging. *J Natl Cancer Inst* 2004;**96**:1420–5.
2. Zlobec I, Lugli A. Prognostic and predictive factors in colorectal cancer. *J Clin Pathol* 2008;**61**:561–9.
3. Edge SB, American Joint Committee on Cancer. *AJCC cancer staging manual*. 7th ed. New York: Springer; 2010.
4. Chau I, Cunningham D. Adjuvant therapy in colon cancer – what, when and how? *Ann Oncol* 2006;**17**:1347–59.
5. Donato R. Intracellular and extracellular roles of S100 proteins. *Microsc Res Technol* 2003;**60**:540–51.
6. Boye K, Maelandsmo GM. S100A4 and metastasis: a small actor playing many roles. *Am J Pathol* 2010;**176**:528–35.
7. 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.
8. Garrett SC, Varney KM, Weber DJ, Bresnick AR. S100A4, a mediator of metastasis. *J Biol Chem* 2006;**281**:677–80.
9. Maelandsmo GM, Hovig E, Skrede M, et al. Reversal of the in vivo metastatic phenotype of human tumor cells by an anti-CAPL (mts1) ribozyme. *Cancer Res* 1996;**56**:5490–8.
10. Gongoll S, Peters G, Mengel M, et al. Prognostic significance of calcium-binding protein S100A4 in colorectal cancer. *Gastroenterology* 2002;**123**:1478–84.
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. Flatmark K, Pedersen KB, Nesland JM, et al. Nuclear localization of the metastasis-related protein S100A4 correlates with tumour stage in colorectal cancer. *J Pathol* 2003;**200**:589–95.
13. Flatmark K, Bjornland K, Johannessen HO, et al. Immunomagnetic detection of micrometastatic cells in bone marrow of colorectal cancer patients. *Clin Cancer Res* 2002;**8**:444–9.
14. Hemandas AK, Salto-Tellez M, Maricar SH, Leong AF, Leow CK. Metastasis-associated protein S100A4 – a potential prognostic marker for colorectal cancer. *J Surg Oncol* 2006;**93**:498–503.
15. Andre T, Afchain P, Barrier A, et al. Current status of adjuvant therapy for colon cancer. *Gastrointest Cancer Res* 2007;**1**:90–7.
16. Segal NH, Saltz LB. Is adjuvant therapy for stage II colon cancer worthwhile, and for whom? *Nat Clin Pract Gastroenterol Hepatol* 2008;**5**:422–3.
17. Benson 3rd AB, Schrag D, Somerfield MR, et al. American Society of Clinical Oncology recommendations on adjuvant chemotherapy for stage II colon cancer. *J Clin Oncol* 2004;**22**:3408–19.
18. Haugen MH, Flatmark K, Mikalsen SO, Malandsmo GM. The metastasis-associated protein S100A4 exists in several charged variants suggesting the presence of posttranslational modifications. *BMC Cancer* 2008;**8**:172.
19. 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.
20. Baudier J, Bergeret E, Bertacchi N, et al. Interactions of myogenic bHLH transcription factors with calcium-binding calmodulin and S100a (alpha alpha) proteins. *Biochemistry* 1995;**34**:7834–46.
21. Berge G, Costea DE, Berg M, et al. Coexpression and nuclear colocalization of metastasis-promoting protein S100A4 and p53 without mutual regulation in colorectal carcinoma. *Amino Acids*, in press.
22. Berge G, Maelandsmo GM. Evaluation of potential interactions between the metastasis-associated protein S100A4 and the tumor suppressor protein p53. *Amino Acids*, in press.
23. Onions J, Hermann S, Grundstrom T. Basic helix–loop–helix protein sequences determining differential inhibition by calmodulin and S-100 proteins. *J Biol Chem* 1997;**272**:23930–7.
24. Santamaria-Kisiel L, Rintala-Dempsey AC, Shaw GS. Calcium-dependent and -independent interactions of the S100 protein family. *Biochem J* 2006;**396**:201–14.
25. Okada H, Danoff TM, Kalluri R, Neilson EG. Early role of Fsp1 in epithelial–mesenchymal transformation. *Am J Physiol* 1997;**273**:F563–74.
26. Xue C, Plieth D, Venkov C, Xu C, Neilson EG. The gatekeeper effect of epithelial–mesenchymal transition regulates the frequency of breast cancer metastasis. *Cancer Res* 2003;**63**:3386–94.
27. Gupta PB, Onder TT, Jiang G, et al. Identification of selective inhibitors of cancer stem cells by high-throughput screening. *Cell* 2009;**138**:645–59.
28. Mani SA, Guo W, Liao MJ, et al. The epithelial–mesenchymal transition generates cells with properties of stem cells. *Cell* 2008;**133**:704–15.
29. Brabletz T, Jung A, Spaderna S, Hlubek F, Kirchner T. Opinion: migrating cancer stem cells – an integrated concept of malignant tumour progression. *Nat Rev Cancer* 2005;**5**:744–9.
30. Stein U, Arlt F, Walther W, et al. The metastasis-associated gene S100A4 is a novel target of beta-catenin/T-cell factor signaling in colon cancer. *Gastroenterology* 2006;**131**:1486–500.