EUROPEAN JOURNAL OF CANCER 47 (2011) 1837–1845

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejconline.com

Loss of lamin A/C expression in stage II and III colon cancer is associated with disease recurrence

E.J.Th. Belt [a], R.J.A. Fijneman [b,*], E.G. van den Berg [b], H. Bril [c], P.M. Delis-van Diemen [b], M. Tijssen [b], H.F. van Essen [b], E.S.M. de Lange-de Klerk [d], J.A.M. Beliën [b], H.B.A.C. Stockmann [e], S. Meijer [a], G.A. Meijer [b]

[a] Department of Surgery, VU University Medical Center, De Boelelaan 1117, 1081HV Amsterdam, The Netherlands
[b] Department of Pathology, VU University Medical Center, De Boelelaan 1117, 1081HV Amsterdam, The Netherlands
[c] Department of Pathology, Kennemer Gasthuis, Boerhaavelaan 22, 2035 RC Haarlem, The Netherlands
[d] Department of Epidemiology and Biostatistics, VU University Medical Center, De Boelelaan 1117, 1081HV Amsterdam, The Netherlands
[e] Department of Surgery, Kennemer Gasthuis, Boerhaavelaan 22, 2035 RC Haarlem, The Netherlands

ARTICLE INFO

Article history:
Available online 27 May 2011

Keywords:
Colon cancer
Disease recurrence
Lamin A/C
Prognosis
Biomarker

ABSTRACT

Aim of the study: Loss of the nuclear lamina protein lamin A/C (LMNA) has been observed in several human malignancies. The present study aimed to investigate associations between LMNA expression and clinical outcome in colon cancer patients.

Patients and methods: Clinicopathological data and formalin-fixed paraffin embedded tissues were collected from 370 stage II and III colon cancer patients. Tissue microarrays were constructed, stained for lamin A/C and evaluated microscopically. Microsatellite instability status was determined for 318 tumours.

Results: Low levels of LMNA expression were observed in 17.8% of colon tumours, with disease recurrence occurring in 45.5% of stage II and III colon cancer patients with LMNA-low expressing tumours compared to 29.6% of patients with LMNA-high expressing tumours ($p = 0.01$). For stage II patients, disease recurrence was observed for 35.7% of LMNA-low compared to 20.3% of LMNA-high expressing tumours ($p = 0.03$). Microsatellite stable (MSS) tumours exhibited more frequently low LMNA expression than microsatellite instable (MSI) tumours (21% versus 9.8%; $p = 0.05$). Interestingly, disease recurrence among LMNA-low and LMNA-high expressing MSS tumours varied significantly for stage III patients who had not received adjuvant chemotherapy (100% versus 37.8%; $p < 0.01$) while no such difference was observed for patients who received adjuvant chemotherapy (46.7% versus 46.0%; $p = 0.96$).

Conclusion: These data indicate that low expression of LMNA is associated with an increased disease recurrence in stage II and III colon cancer patients, and suggest that these patients in particular may benefit from adjuvant chemotherapy.

* *Corresponding author:* Tel.: +31 20 4442405; fax: +31 20 4443844.
E-mail address: rja.fijneman@vumc.nl (R.J.A. Fijneman).

0959-8049/$ - see front matter
doi:10.1016/j.ejca.2011.04.025

1. Introduction

Worldwide nearly 1 million people are diagnosed with colorectal cancer each year, half of whom die within 5 years.[1] Currently, the primary method for assessing prognosis for individual patients is the Tumour-Node-Metastasis (TNM) staging system.[2] This classification forms the basis for therapeutic decision making, e.g. to decide who benefits from adjuvant chemotherapy. Adjuvant 5-fluorouracil (5-FU)-based chemotherapy increases median 5-year survival of stage III colon cancer patients from 51% to 64%.[3] In contrast, no convincing evidence exists for a beneficial effect of postoperative adjuvant chemotherapy for stage II colon cancer. Nevertheless, 20–30% of these patients will develop recurrent disease after resection of the primary tumour.[4,5] Therefore, there is an evident need to identify stage II CRC patients at high risk of relapse. Prognostic biomarkers aim to identify such patient subgroups, to indicate who will benefit from adjuvant therapy.

Genomic instability is a common feature in colorectal cancer pathogenesis. Chromosomal instability is observed in approximately 85% of colorectal cancers, and is characterised by gains and losses of relatively large chromosomal segments.[6,7] Microsatellite instability (MSI) is observed in about 15% of sporadic colorectal cancers, and is caused by inactivation of the DNA mismatch repair system. Importantly, MSI tumours have a favourable outcome, with less lymph node involvement and reduced occurrence of metastasis compared to microsatellite stable (MSS) tumours.[8,9] As such, MSI status of primary colon cancers can be used as a prognostic marker for therapy selection.

The nuclear lamina protein lamin A/C (LMNA) has been described as another potential prognostic marker for colorectal cancer.[10] The nuclear lamina is composed of proteins termed lamins that are members of the intermediate filament family. In humans, one A-type and two B-type lamin genes have been identified. The lamin A/C gene encodes two isoforms that are formed by alternative RNA splicing, the A-type lamins A and C. *LMNA* germline mutations are involved in a range of human disorders collectively referred to as laminopathies, including several forms of muscular dystrophy, Hutchinson–Gilford progeria syndrome and Werner's syndrome.[11] Functionally, in addition to maintaining lamina stability, lamin proteins have also been associated with cellular mechanisms such as nuclear pore positioning and function, heterochromatin organisation, gene expression, cell cycle progression and apoptosis, stem cell differentiation, DNA replication and repair and cellular migration.[12–18] Signal transduction pathways influenced by lamins include Wnt- and TGFβ-signalling, which are of particular relevance to colon carcinogenesis.[19] Although lamins are commonly expressed by most differentiated somatic cells, LMNA expression may be reduced in transformed cells as has been demonstrated for several tumour (sub)types, including colorectal neoplasms.[10,20–25] The aim of the present study was to evaluate the protein expression of LMNA in stage II and III colon cancer tissue samples to investigate its potential as a prognostic biomarker that may aid therapy selection.

2. Patients and methods

2.1. Patients

Between 1996 and 2005, 454 patients underwent surgical colon cancer resection at the Kennemer Gasthuis hospital in Haarlem, The Netherlands, that were classified as stage II ($T_{3–4}$, N_0, M_0) or stage III ($T_{1–4}$, $N_{1–2}$, M_0) according to the 4th edition of the TNM-classification system.[2] Clinicopathological patient characteristics were retrieved from clinical and histopathology reports. Disease recurrence was defined as either local tumour recurrence or distant metastasis, diagnosed by computed tomography imaging and/or histopathology. Excluded from this study were patients with a history of colorectal malignancy ($n = 12$), patients with irradical resections of the primary tumour ($n = 9$), and patients who were lost for follow up ($n = 8$) or died within 3 months after surgery ($n = 39$). The remaining study population consisted of 386 stage II and III colon cancer patients with a median follow up period of more than 57 months. Collection, storage and use of tissue and patient data were performed in agreement with the 'Code for Proper Secondary Use of Human Tissue in The Netherlands'.[26]

2.2. Microsatellite instability analysis

DNA was isolated from formalin-fixed paraffin-embedded (FFPE) tissues of the colon cancer samples as previously described.[27] Briefly, haematoxylin–eosin stained sections were used to select areas with a tumour cell contribution of at least 70%, while adjacent 10 μm serial sections were macro dissected for DNA isolation. DNA samples were analysed for microsatellite instability using MSI Analysis System, Version 1.2 according to the manufacturer's instructions (Promega, Madison, USA). This PCR-based assay uses five mononucleotide repeat markers to determine MSI status. PCR products were separated by capillary electrophoresis using ABI 3130 DNA sequencer and output data were analysed using the accompanying package GeneScan 3100 (Applied Biosystems, Foster City, CA, USA). Tumours were classified as microsatellite instable (MSI) when instability was observed for two or more markers, otherwise they were considered to be microsatellite stable (MSS). MSI status could not be determined for 15% of tumour samples due to insufficient quality of the FFPE-derived DNA.

2.3. Tissue Microarrays

Tissue Microarrays (TMAs) were constructed from the 386 stage II and III colon cancer samples.[28] Briefly, FFPE colon cancer resection specimens were used as donor blocks. Six tissue cylinders (core biopsies) with a diameter of 0.6 mm were punched from morphologically representative tissue areas and transferred into recipient TMA paraffin blocks.

2.4. LMNA immunohistochemical staining

Four micrometre sections were deparaffinised in xylene and rehydrated through a graded series of alcohol. Endogenous

peroxidase activity was quenched with 0.3% hydrogen peroxide in methanol. Samples were microwave-heated in 10 mM citrate buffer solution (pH 6.0) for antigen retrieval, and incubated with mouse monoclonal anti-Lamin A and C (clone Jol2, Abcam Inc., Cambridge, UK) diluted in Antibody Diluent (Dako Netherlands BV, Heverlee, Belgium) overnight at 4 °C. Staining was visualised using the Powervision+ kit (Immunologic, Duiven, The Netherlands) and diaminobenzidine as a chromagen, followed by counterstaining with Mayer's Haematoxylin. LMNA staining of non-epithelial cells in the tumour stroma served as an internal positive control. Overall, LMNA expression could be evaluated for 370 stage II and III colon cancer patients.

2.5. *Evaluation of LMNA expression*

Slides were scanned with a digital Mirax slide Scanner system (3DHISTECH Ltd., Budapest, Hungary) equipped with a 20× objective (Carl Zeiss B.V., Sliedrecht, The Netherlands). The intensity of LMNA nuclear protein expression was scored on a scale from 0 to 3: 0 = negative; 1 = weak; 2 = moderate; 3 = strong. Examples of LMNA expression are shown in Fig. 1. Focussing on loss of LMNA expression, the score was based on the weakest observed staining intensity present in at least 20% of tumour cells. Using Receiver Operating Characteristics (ROC) curve analysis to determine the optimal cut-off score,[29] patients with score 0 were assigned as 'LMNA low' and patients with a score $\geqslant 1$ as 'LMNA high'. All samples were examined and scored independently by two investigators (E.J.Th. Belt and E.G. van den Berg) without knowledge of clinicopathological data at the time of assessment. The inter-observer agreement was high (Cohen's weighted kappa value $K_w = 0.79$, with 95% confidence interval (CI): 0.75–0.84), resulting in undisputed classification into 'low' and 'high' LMNA levels for 95% of the patients.

2.6. *Statistical analysis*

Statistical analysis was performed with SPSS 15.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Differences in proportions between groups were examined using Pearson's Chi-square test or Fisher's exact test. Survival rates were displayed using Kaplan–Meier curves and compared using the log rank test. Multivariate analysis was performed using backward stepwise logistic regression. All reported p-values are two-sided with a threshold for significance of 0.05.

3. Results

3.1. *Clinicopathological features and disease recurrence*

LMNA protein expression was examined for 219 stage II and 151 stage III colon cancer patients. Their clinicopathological characteristics are listed in Table 1. Conform

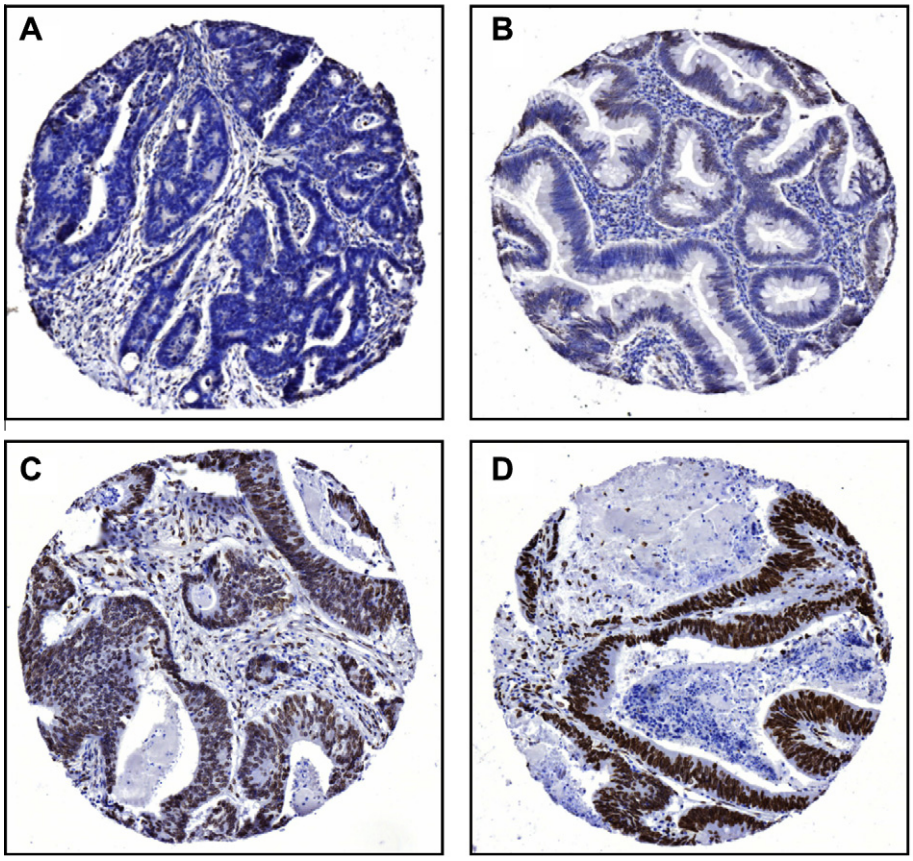

Fig. 1 – Examples of immunohistochemical staining for lamin A/C of colon cancer tissue: (A) negative, (B) weak, (C) moderate, and (D) strong LMNA staining of tumour epithelial cells.

Table 1 – Clinical and pathological characteristics of study population.

	Overall (n = 370)	LMNA-low (n = 66)	LMNA-high (n = 304)	p-Value
Sex				
Male	195 (52.7)	39 (59.1)	156 (51.3)	
Female	175 (47.3)	27 (40.9)	148 (48.7)	NS
Age (years)				
Mean (SD)	71.1 (12.0)	69.2 (11.8)	71.5 (12.0)	
Median (range)	73.2 (28.5–94.0)	69.2 (38.1–90.0)	73.8 (28.5–94.0)	NS
Tumour location				
Right sided	162 (43.8)	26 (39.4)	136 (44.7)	
Left sided	208 (56.2)	40 (60.6)	168 (55.3)	NS
Tumour size (mm)				
Mean (SD)	42.2 (19.3)	40.9 (17.4)	42.5 (19.7)	NS
Tumour stage				
T1	4 (1.1)	1 (1.5)	3 (1.0)	
T2	18 (4.9)	5 (7.6)	13 (4.3)	
T3	314 (84.9)	55 (83.3)	259 (85.2)	
T4	34 (9.2)	5 (7.6)	29 (9.5)	NS
Nodal stage				
N0	219 (59.2)	42 (63.6)	177 (58.2)	
N1	103 (27.8)	18 (27.3)	85 (28.0)	
N2	48 (13.0)	6 (9.1)	42 (13.8)	NS
No. of nodes examined				
Mean (SD)	9.0 (5.2)	8.9 (5.9)	9.0 (5.0)	NS
Microsatellite stability status				
(Failed samples excluded)	(Failed n = 52)	(Failed n = 6)	(Failed n = 46)	
Microsatellite stable (MSS)	257 (80.8)	54 (90.0)	203 (78.7)	
Microsatellite instable (MSI)	61 (19.2)	6 (10.0)	55 (21.3)	0.05
Histological grade				
Well	23 (6.2)	7 (10.6)	16 (5.3)	
Moderate	292 (78.9)	51 (77.3)	241 (79.3)	
Poor	55 (14.9)	8 (12.1)	47 (15.5)	NS
Mucinous differentiation				
Yes	79 (21.4)	8 (12.1)	71 (23.4)	
No	291 (78.6)	58 (87.9)	233 (76.6)	0.04
Ulceration				
Present	284 (76.8)	50 (75.8)	234 (77.0)	
Absent	86 (23.2)	16 (24.2)	70 (23.0)	NS
Angioinvasion				
Present	73 (19.3)	13 (19.7)	60 (19.7)	
Absent	297 (80.3)	53 (80.3)	244 (80.3)	NS
Tumour-Node-Metastasis (TNM) and adjuvant chemotherapy (ACT)				
Stage II	219 (59.2)	42 (63.6)	177 (58.2)	
with ACT	31 (14.2)	3 (7.1)	28 (15.8)	
without ACT	188 (85.8)	39 (92.9)	149 (84.2)	NS
Stage III	151 (40.8)	24 (36.4)	127 (41.8)	
with ACT	81 (53.6)	17 (70.8)	64 (50.4)	
without ACT	70 (46.4)	7 (29.2)	63 (49.6)	0.07
Follow up (months)				
Median (range)	57.3 (3.5–148.6)	59.4 (9.5–125.5)	57.3 (3.5–148.6)	NS

Values in parentheses are percentages unless stated otherwise.
NS: not significant, p-values are all >0.10.

expectations, disease-free survival was significantly worse for stage III patients compared to stage II patients ($p < 0.01$; Fig. 2A). MSI status was determined for 318 cases, and revealed 257 MSS tumours (81%) and 61 MSI tumours (19%). Although not significant in the present dataset, recurrent disease tended to develop more frequently in patients with MSS tumours (35.8%) than in patients with MSI tumours (24.6%; $p = 0.10$). Likewise, disease-free survival tended to be worse for MSS patients than for MSI patients ($p = 0.12$; Fig. 2B).

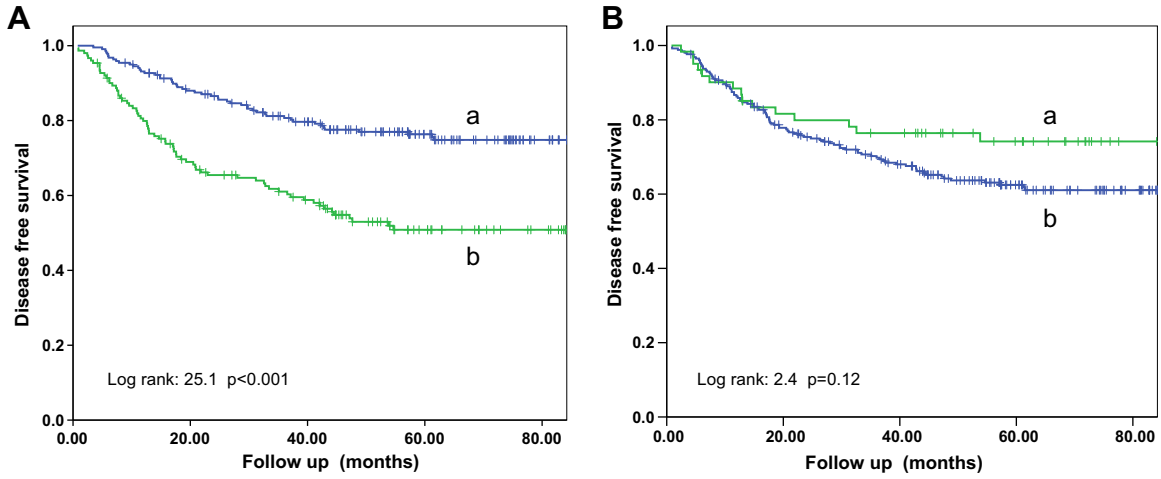

Fig. 2 – Disease free survival curves: (2A) stage II (a; *n* = 219) and stage III (b; *n* = 151) colon cancer patients; and (2B) microsatellite instable (MSI) tumours (a; *n* = 61) and microsatellite stable (MSS) tumours (b; *n* = 257).

3.2. *LMNA expression and disease recurrence*

Immunohistochemical staining revealed 66 tumours with low LMNA expression (17.8%) and 304 tumours with high LMNA expression (82.2%). Disease recurrence occurred more frequently among patients with LMNA-low expressing tumours (45.5%) compared to LMNA-high expressing tumours (29.6%; $p = 0.01$; Table 2). Disease-free survival was significantly worse for patients with LMNA-low expressing tumours ($p = 0.03$; Fig. 3A). Similar percentages of LMNA-low expressing tumours were observed for stage II and III colon cancer patients (19.2% versus 15.9%, respectively). When analysed separately, stage II patients revealed 35.7% recurrence in LMNA-low versus 20.3% recurrence in LMNA-high cases ($p = 0.03$), resulting in a significantly worse disease-free survival for LMNA-low stage II patients ($p = 0.04$; Fig. 3B). A similar trend was observed for stage III colon cancer patients, which revealed 62.5% recurrence in LMNA-low versus 42.5% recurrence in LMNA-high cases ($p = 0.07$; and Fig. 3C). For the subgroup of stage III patients who received chemotherapy, no significant differences in recurrence rates were observed between LMNA-low and -high cases (52.9% and 45.3%, respectively; $p = 0.6$). However, significant differences were observed for the subgroup of stage III patients who did not receive adjuvant chemotherapy, with recurrence occurring in 85.7% of patients with LMNA-low versus 39.7% of patients with LMNA-high expressing tumours ($p = 0.02$; and Fig. 3D).

Table 2 – LMNA expression and disease recurrence in total study population of colon cancer patients.

	Total	LMNA-low	LMNA-high	*p*-Value
All patients (stage II + III, n = 370)				
Recurrence	120 (32.4)	30 (45.5)	90 (29.6)	
No recurrence	250 (67.6)	36 (54.5)	214 (70.4)	0.01
Stage II (n = 219)				
Recurrence	51 (23.3)	15 (35.7)	36 (20.3)	
No recurrence	168 (76.7)	27 (64.3)	141 (79.7)	0.03
- Without ACT (*n* = 188)				
Recurrence	44 (23.4)	13 (33.3)	31 (20.8)	
No recurrence	144 (76.6)	26 (66.7)	118 (79.2)	0.10
- With ACT (*n* = 31)				
Recurrence	7 (22.6)	2 (66.7)	5 (17.9)	
No recurrence	24 (77.4)	1 (33.3)	23 (82.1)	0.12
Stage III (*n* = 151)				
Recurrence	69 (45.7)	15 (62.5)	54 (42.5)	
No recurrence	82 (54.3)	9 (37.5)	73 (57.5)	0.07
- Without ACT (*n* = 70)				
Recurrence	31 (44.3)	6 (85.7)	25 (39.7)	
No recurrence	39 (55.7)	1 (14.3)	38 (60.3)	0.02
- With ACT (*n* = 81)				
Recurrence	38 (46.9)	9 (52.9)	29 (45.3)	
No recurrence	43 (53.1)	8 (47.1)	35 (54.7)	0.58

Values in parentheses are percentages. ACT: adjuvant chemotherapy.

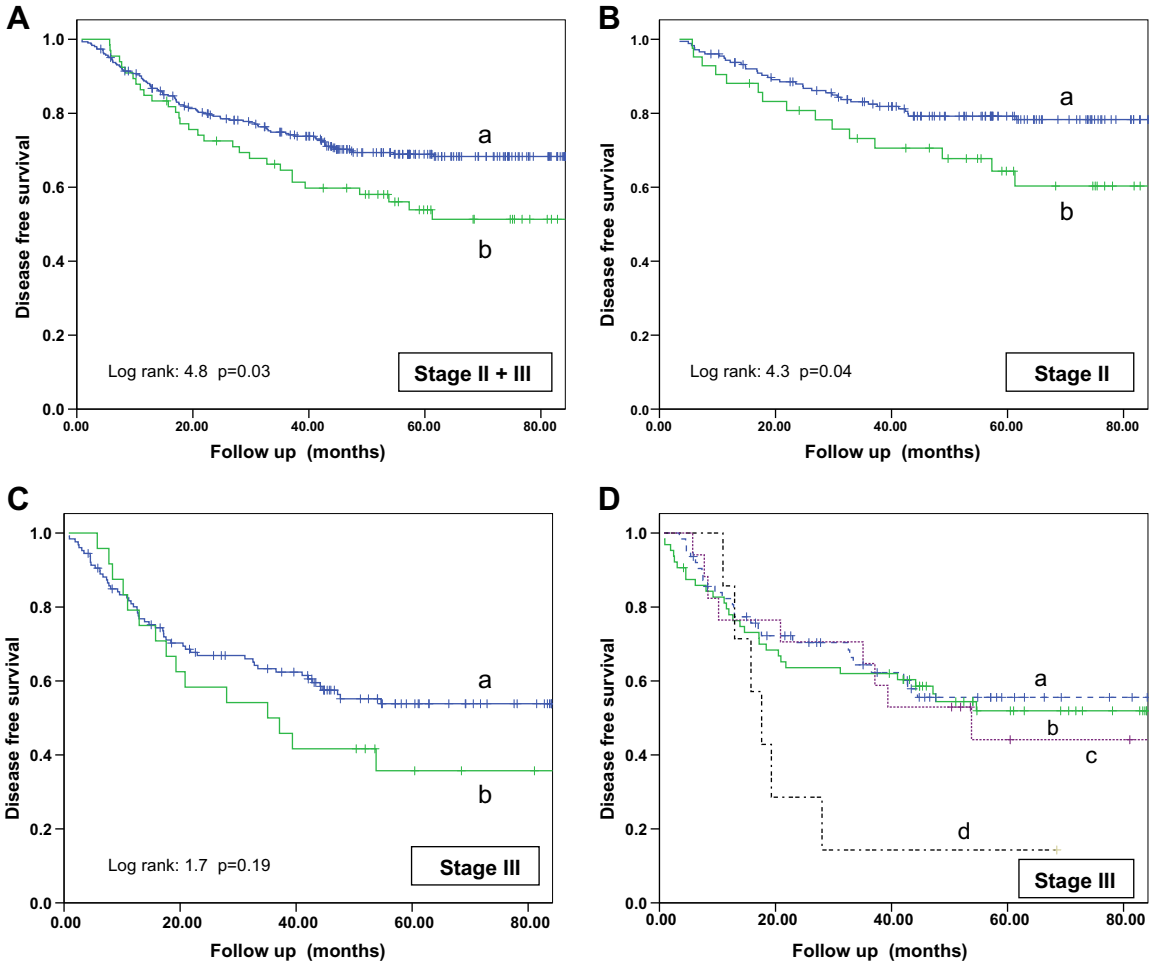

Fig. 3 – Disease free survival curves: (3A) total study population (stage II + III colon cancer patients, *n* = 370) with LMNA-high (a; *n* = 304) and LMNA-low expressing tumours (b; *n* = 66); (3B) stage II colon cancer patients (*n* = 219) with LMNA-high (a; *n* = 177) and LMNA-low expressing tumours (b; *n* = 42); (3C) stage III colon cancer patients (*n* = 151) with LMNA-high (a; *n* = 127) and LMNA-low expressing tumours (b; *n* = 24); and (3D) stage III colon cancer patients stratified for administration of adjuvant chemotherapy (ACT) and LMNA expression, with high LMNA expression without ACT (a; *n* = 63), with high LMNA expression with ACT (b; *n* = 64), with low LMNA expression with ACT (c; *n* = 17), and with low LMNA expression without ACT (d; *n* = 7). Among patients who did not receive adjuvant chemotherapy, there is a significant difference in disease free survival between patients with LMNA-high (a) and LMNA-low (d) expressing tumours (log rank 4.8; *p* = 0.03).

Next, we examined the prognostic effects of LMNA with respect to the effects of two other parameters that are considered to have prognostic value, i.e. MSI-status and treatment with adjuvant chemotherapy. LMNA-low expressing tumours were less frequently observed among MSI tumours (9.8%) than among MSS tumours (21.0%; $p = 0.05$). Considering the generally more favourable prognosis of MSI-positive tumours, the associations of LMNA staining with disease recurrence were re-evaluated on the subgroup of 257 stage II and III MSS tumours. Patients with LMNA-low MSS tumours developed recurrence in 48.1% of cases versus 32.5% of patients with LMNA-high MSS tumours ($p = 0.03$; Table 3). Likewise, the disease-free survival for patients with LMNA-low expressing MSS tumours tended to be worse ($p = 0.06$; Fig. 4A). For stage II patients with MSS tumours, recurrence was observed in 39.4% of LMNA-low cases versus 24.1% of LMNA-high cases ($p = 0.09$). Also, the disease-free survival tended to be worse for stage II LMNA-low expressing MSS tumours ($p = 0.09$; Fig. 4B). For stage III patients with MSS tumours, recurrence was observed in 61.9% of LMNA-low cases versus 42.1% of LMNA-high cases ($p = 0.10$). There was no significant difference in disease-free survival between stage III LMNA-low and -high expressing MSS tumours ($p = 0.18$; Fig. 4C). However, significant differences were observed for the subgroup of stage III patients with MSS tumours who had not received adjuvant chemotherapy, with disease recurrence in all cases with LMNA-low expression (100%) versus in 37.8% of cases with LMNA-high expression ($p < 0.01$; Table 3). Such difference was not observed for stage III patients with MSS tumours who received adjuvant chemotherapy, with disease recurrences in LMNA-low and -high expressing tumours of 46.7% and 46.0%, respectively ($p = 0.96$). The disease-free survival curves for stage III patients with MSS tumours stratified for adjuvant chemotherapy and LMNA expression are depicted in Fig. 4D. The subgroup of patients with MSI

Table 3 – LMNA expression and disease recurrence in colon cancer patients with microsatellite stable (MSS) tumours.

	Total	LMNA-low	LMNA-high	p-Value
All patients MSS (stage II + III, n = 257)				
Recurrence	92 (35.8)	26 (48.1)	66 (32.5)	
No recurrence	165 (64.2)	28 (51.9)	137 (67.5)	0.03
Stage II MSS (n = 141)				
Recurrence	39 (27.7	13 (39.4)	26 (24.2)	
No recurrence	102 (72.3)	20 (60.6)	82 (75.9)	0.09
- Without adjuvant CT (n = 119)				
Recurrence	33 (27.7)	11 (36.7)	22 (24.7)	
No recurrence	86 (72.3)	19 (63.3)	67 (75.3)	0.21
- With adjuvant CT (n = 22)				
Recurrence	6 (27.3)	2 (66.7)	4 (21.1)	
No recurrence	16 (72.7)	1 (33.3)	15 (78.9)	0.17
Stage III MSS (n = 116)				
Recurrence	53 (45.7)	13 (61.9)	40 (42.1)	
No recurrence	63 (54.3)	8 (38.1)	55 (57.9)	0.10
- Without adjuvant CT (n = 51)				
Recurrence	23 (45.1)	6 (100)	17 (37.8)	
No recurrence	28 (54.9)	0 (0)	28 (62.6)	<0.01
- With adjuvant CT (n = 65)				
Recurrence	30 (46.2)	7 (46.7)	23 (46.0)	
No recurrence	35 (53.8)	8 (53.3)	27 (54.0)	0.96

Values in parentheses are percentages. CT: chemotherapy.

tumours contained too few LMNA-low tumours ($n = 6$) to allow meaningful subgroup analysis.

Finally, a multivariate analysis was performed in which LMNA protein expression was incorporated as one parameter together with disease stage, age, tumour site, treatment with adjuvant chemotherapy, MSI status, T-stage, differentiation grade, and angioinvasive growth of the primary tumour. This analysis indicated LMNA, disease stage, and angioinvasive growth as independent risk factors for colon cancer recurrence (Table 4).

4. Discussion

The present study evaluated LMNA protein expression in primary cancer tissue samples from a large cohort of stage II and III colon cancer patients, to investigate its potential as a prognostic biomarker. Our data demonstrate that reduced LMNA expression in epithelial cancer cells is associated with an unfavourable outcome in colon cancer patients (Fig. 3A; Table 2), and indicate LMNA as an independent risk factor for disease recurrence. Low expression of LMNA was significantly associated with increased disease recurrence within the group of stage II colon cancer patients, as well as within the group of stage III colon cancer patients that did not receive adjuvant chemotherapy (Fig. 3B and D; Table 2). Because colon cancer patients with MSI tumours are known to have a better prognosis than patients with MSS tumours, data were stratified for MSI status.[8,9] Although not significant, also within this cohort, patients with MSI tumours tended to have longer disease free survival rates than patients with MSS tumours (Fig. 2B). Within the group of stage II and III patients with MSS tumours low levels of LMNA protein expression were associated with increased disease recurrence (Table 3). Again, this effect appeared to be much more prominent within the group of stage III MSS patients who did not receive adjuvant chemotherapy compared to the group that did receive adjuvant chemotherapy (Fig. 4D; Table 3). However, the size of some of the subgroups used for these analyses became relatively small, emphasising the need to confirm our findings in an independent patient cohort. Together, these data suggest that LMNA protein expression can be used as a prognostic factor to predict whether stage II and III colon cancer patients may benefit from adjuvant chemotherapy.

Previously, one study reported that reduced expression of LMNA was associated with a more favourable prognosis for colorectal cancer patients[10], a result that seems to be contradictory to our findings. However, there are important differences between the study cohorts that can explain this difference. In particular, whereas the present study was restricted to a relatively homogeneous population of stage II and III colon cancer patients, this other study population also included stage I patients and rectal cancer patients. Considering the significant differences in LMNA expression that have been observed among cancer subtypes, for instance between small cell lung cancer and non-small cell lung cancer cell lines, it remains to be resolved whether cancers of the colon behave similar to or differently from rectal cancers. Also adjuvant chemotherapy, and in case of rectal cancer neoadjuvant (chemo-) radiotherapy, may be considerable confounding factors. In fact, the present study found a significant association between LMNA expression and survival rates only for stage III MSS patients who did not receive chemotherapy (Fig. 4D).

Within the group of stage III colon cancer patients who did receive adjuvant chemotherapy, LMNA is not a risk factor for disease recurrence (Tables 2 and 3). These data suggest that LMNA-low expressing tumours are sensitive to (fluorouracil-based) adjuvant chemotherapy. This hypothesis is supported by observations that cells with disrupted lamin A function

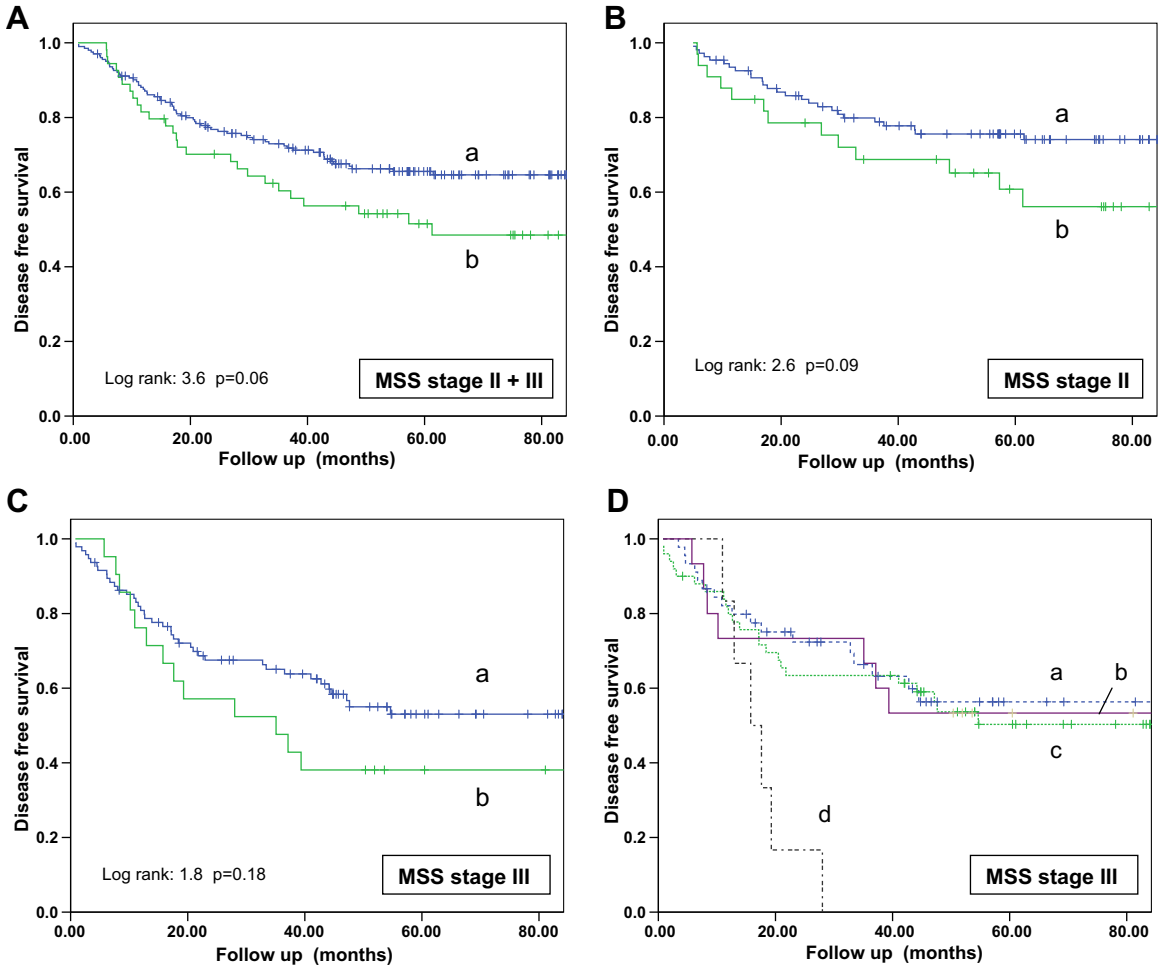

Fig. 4 – Disease free survival curves of patients with microsatellite stable (MSS) tumours: (4A) total study population (stage II + III colon cancer patients, *n* = 257) with LMNA-high (a; *n* = 203) and LMNA-low expressing tumours (b; *n* = 54); (4B) stage II colon cancer patients (*n* = 141) with LMNA-high (a; *n* = 108) and LMNA-low expressing tumours (b; *n* = 33); (4C) stage III colon cancer patients (*n* = 116) with LMNA-high (a; *n* = 95) and LMNA-low expressing tumours (b; *n* = 21); (4D) stage III colon cancer patients stratified for administration of adjuvant chemotherapy (ACT) and LMNA expression, with high LMNA expression without ACT (a; *n* = 45), with low LMNA expression with ACT (b; *n* = 15), with high LMNA expression with ACT (c; *n* = 50), and with low LMNA expression without ACT (d; *n* = 6). Among patients who did not receive adjuvant chemotherapy, there is a significant difference in disease free survival between patients with LMNA-high (a) and LMNA-low (d) expressing tumours (log rank 9.7; $p < 0.01$).

Table 4 – Independent risk factors for colon cancer recurrence (multivariate analysis).

	Odds ratio	95% Confidence interval	*p*-Value
Low LMNA expression	2.6	1.4–4.8	0.003
High disease stage (III)	2.6	1.5–4.5	0.001
Angioinvasive growth	3.6	2.0–6.7	<0.001

have increased sensitivity to DNA damaging agents such as ionising radiation and cytotoxic drugs as etoposide and camptothecin.[30] Recently, loss of A-type lamins was shown to induce changes in nuclear distribution of telomeres and to affect telomere function, thereby inducing chromosomal instability.[14] Interestingly, our data demonstrate that low levels of LMNA expression appear to be more common among MSS (i.e. predominately chromosomal instable) tumours than among MSI tumours ($p = 0.05$), thereby supporting the hypothesis that malfunction of A-type lamins will increase chromosomal instability and stimulate carcinogenesis. Increasing genomic instability may enhance tumour progression, including generation of tumour cell clones that have the capacity to cause disease recurrence. However, neither the correlation between loss of LMNA and increases in chromosomal instability nor the exact molecular mechanisms that induce loss of LMNA were investigated in this study. Hence, additional studies are required to further clarify the effects

of this and other putative functions of LMNA that may contribute to the pathogenesis of colon cancer.

In conclusion, the present study indicates that LMNA protein expression has potential as a prognostic biomarker to aid therapy selection for stage II and III colon cancer patients. In particular, patients with LMNA-low expressing tumours may benefit from adjuvant chemotherapy.

Conflict of interest statement

None declared.

Acknowledgements

This study was supported by the Posthumus Meyjes Foundation (EJThB) and by the AEGON International Scholarship in Oncology (PMDvD).

REFERENCES

1. Weitz J, Koch M, Debus J, et al. Colorectal cancer. *Lancet* 2005;**365**(9454):153–65.
2. Sobin LH, Fleming ID. TNM classification of malignant tumors, fifth edition (1997). Union Internationale Contre le Cancer and the American Joint Committee on Cancer. *Cancer* 1997;**80**(9):1803–4.
3. Gill S, Loprinzi CL, Sargent DJ, et al. Pooled analysis of fluorouracil-based adjuvant therapy for stage II and III colon cancer: who benefits and by how much? *J Clin Oncol* 2004;**22**(10):1797–806.
4. International Multicentre Pooled Analysis of B2 Colon Cancer Trials (IMPACT B2) Investigators. Efficacy of adjuvant fluorouracil and folinic acid in B2 colon cancer. J Clin Oncol 1999;17(5):1356-1363.
5. Mamounas E, Wieand S, Wolmark N, et al. Comparative efficacy of adjuvant chemotherapy in patients with Dukes' B versus Dukes' C colon cancer: results from four National Surgical Adjuvant Breast and Bowel Project adjuvant studies (C-01, C-02, C-03, and C-04). *J Clin Oncol* 1999;**17**(5): 1349–55.
6. Hermsen M, Postma C, Baak J, et al. Colorectal adenoma to carcinoma progression follows multiple pathways of chromosomal instability. *Gastroenterology* 2002;**123**(4):1109–19.
7. Pino MS, Chung DC. The chromosomal instability pathway in colon cancer. *Gastroenterology* 2010;**138**(6):2059–72.
8. Boland CR, Goel A. Microsatellite instability in colorectal cancer. *Gastroenterology* 2010;**138**(6):2073–87 [e2073].
9. Soreide K, Janssen EA, Soiland H, Korner H, Baak JP. Microsatellite instability in colorectal cancer. *Br J Surg* 2006;**93**(4):395–406.
10. Willis ND, Cox TR, Rahman-Casans SF, et al. Lamin A/C is a risk biomarker in colorectal cancer. *PLoS One* 2008;**3**(8):e2988.
11. Broers JL, Hutchison CJ, Ramaekers FC. Laminopathies. *J Pathol* 2004;**204**(4):478–88.
12. Capell BC, Collins FS. Human laminopathies: nuclei gone genetically awry. *Nat Rev Genet* 2006;**7**(12):940–52.
13. De Vos WH, Houben F, Hoebe RA, et al. Increased plasticity of the nuclear envelope and hypermobility of telomeres due to the loss of A-type lamins. *Biochim Biophys Acta* 2010;**1800**(4):448–58.
14. Gonzalez-Suarez I, Redwood AB, Perkins SM, et al. Novel roles for A-type lamins in telomere biology and the DNA damage response pathway. *EMBO J* 2009;**28**(16):2414–27.
15. Houben F, Willems CH, Declercq IL, et al. Disturbed nuclear orientation and cellular migration in A-type lamin deficient cells. *Biochim Biophys Acta* 2009;**1793**(2):312–24.
16. Hutchison CJ, Worman HJ. A-type lamins: guardians of the soma? *Nat Cell Biol* 2004;**6**(11):1062–7.
17. Prokocimer M, Davidovich M, Nissim-Rafinia M, et al. Nuclear lamins: key regulators of nuclear structure and activities. *J Cell Mol Med* 2009;**13**(6):1059–85.
18. Worman HJ, Fong LG, Muchir A, Young SG. Laminopathies and the long strange trip from basic cell biology to therapy. *J Clin Invest* 2009;**119**(7):1825–36.
19. Andres V, Gonzalez JM. Role of A-type lamins in signaling, transcription, and chromatin organization. *J Cell Biol* 2009;**187**(7):945–57.
20. Agrelo R, Setien F, Espada J, et al. Inactivation of the lamin A/C gene by CpG island promoter hypermethylation in hematologic malignancies, and its association with poor survival in nodal diffuse large B-cell lymphoma. *J Clin Oncol* 2005;**23**(17):3940–7.
21. Broers JL, Raymond Y, Rot MK, et al. Nuclear A-type lamins are differentially expressed in human lung cancer subtypes. *Am J Pathol* 1993;**143**(1):211–20.
22. Kaufmann SH, Mabry M, Jasti R, Shaper JH. Differential expression of nuclear envelope lamins A and C in human lung cancer cell lines. *Cancer Res* 1991;**51**(2):581–6.
23. Moss SF, Krivosheyev V, de Souza A, et al. Decreased and aberrant nuclear lamin expression in gastrointestinal tract neoplasms. *Gut* 1999;**45**(5):723–9.
24. Roth U, Razawi H, Hommer J, et al. Differential expression proteomics of human colorectal cancer based on a syngeneic cellular model for the progression of adenoma to carcinoma. *Proteomics* 2010;**10**(2):194–202.
25. Stadelmann B, Khandjian E, Hirt A, et al. Repression of nuclear lamin A and C gene expression in human acute lymphoblastic leukemia and non-Hodgkin's lymphoma cells. *Leuk Res* 1990;**14**(9):815–21.
26. Available from: http://www.federa.org/?s=1&m=82&p=9&v=4#866 [accessed 10.10.11].
27. Weiss MM, Hermsen MA, Meijer GA, et al. Comparative genomic hybridisation. *Mol Pathol* 1999;**52**(5):243–51.
28. Simon R, Mirlacher M, Sauter G. Tissue microarrays. *Biotechniques* 2004;**36**(1):98–105.
29. Zlobec I, Steele R, Terracciano L, Jass JR, Lugli A. Selecting immunohistochemical cut-off scores for novel biomarkers of progression and survival in colorectal cancer. *J Clin Pathol* 2007;**60**(10):1112–6.
30. Liu B, Wang J, Chan KM, et al. Genomic instability in laminopathy-based premature aging. *Nat Med* 2005;**11**(7):780–5.