

Differential expression of nuclear lamins in normal and cancerous prostate tissues

ROSELLA CORADEGHINI¹, PAOLA BARBORO¹, ALESSANDRA RUBAGOTTI^{1,2}, FRANCESCO BOCCARDO^{1,2}, SILVIO PARODI^{1,2}, GIORGIO CARMIGNANI³, CRISTINA D'ARRIGO⁴, ELIGIO PATRONE⁴ and CECILIA BALBI¹

¹Istituto Nazionale per la Ricerca sul Cancro; ²Dipartimento di Oncologia, Biologia e Genetica,

³Dipartimento di Urologia, Università di Genova, Largo Rosanna Benzi 10, I-16132 Genova;

⁴Istituto per lo Studio delle Macromolecole, Sezione di Genova, Via De Marini 6, I-16146 Genova, Italy

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Abstract. The process of carcinogenesis is characterized by definite changes in the protein composition of the nuclear matrix. We have recently found that lamins form, in addition to the nuclear lamina, an intranuclear web of thin fibrils. This finding prompted us to address the question of whether changes in the expression of lamins occur in the course of tumor development. In prostate cancer, lamin B undergoes a significant increase; interestingly, its nuclear content strongly correlates with tumor differentiation. Moreover, all the lamins show reproducible alterations in the distribution of the isoelectric variants, suggesting that dephosphorylation events could trigger changes in the pattern of gene expression by inducing structural rearrangements of the nuclear scaffold.

Introduction

The nuclear matrix (NM), the protein scaffold which directs the folding of chromatin inside the interphase nucleus, plays a general role in the spatial and temporal coordination of gene activation events; therefore, changes in the gene expression pattern occurring in the course of carcinogenesis are expected to depend on the impairment of its regulatory functions (1,2). As a fact, in all the tumor studied the NM shows specific structural and protein changes; the evolution toward the malignant phenotype is associated with the appearance of some new proteins and with the cessation of the expression of others (3). We have recently shown that the development of human prostate cancer (PCa) is characterized by an increase in the complexity of the protein pattern of the NM. Poorly differentiated tumors express proteins that are not detected in more differentiated tumors (4,5); moreover, a few newly expressed proteins are significantly correlated with the risk of biochemical progression (6).

Until now, the lack of knowledge of the organization and protein composition of the scaffold has seriously hampered the identification of the functional consequences, if any, of the large nuclear and chromatin alterations occurring in transformed cells (1,2,7). The number of copies per nucleus of the protein showing significant changes in PCa roughly corresponds to that of the scaffold associated regions of DNA (5), suggesting that these species represent fine modulating factors of gene activity. Thus, the question of whether structural components of the NM are involved in the control of cancer development (1,8) remains unsolved. In 1994, Zeng *et al* (9) suggested that the nuclear mitotic apparatus protein (NuMA) is a major structural component of the nuclear skeleton; in 1995, Hozák *et al* (10) reported the first evidence of the presence of lamins in the internal NM. Only in the last couple of years, however, have both the composition and structure of the internal NM been definitively characterized by high resolution electron and immunoelectron microscopy in our laboratory. Lamins are organized into a three-dimensional network of thin fibers which extend throughout the nucleus, whereas NuMA forms small islands anchored to the network; the stability of the internal NM strongly depends on the integrity of heterogeneous nuclear RNA (8,11). These findings prompted us to characterize the changes in the structural proteins of the NM in PCa by quantifying first the expression of lamins. The results presented here demonstrate that, in PCa, the expression of lamin B undergoes a significant increase correlated to the level of differentiation.

Materials and methods

Patients and tissue samples. PCa specimens were obtained from up to 37 patients undergoing radical retropubic prostatectomy for clinically localized prostate cancer. Normal prostate tissue (NT) specimens were obtained from the controlateral lobe to the cancer zone of 17 patients from the group reported above and from 3 additional patients undergoing prostate vesiculo cystectomy for bladder cancer. Tissues were immediately frozen in liquid nitrogen and stored until use, and were histologically confirmed by hematoxylin and eosin staining. The patient characteristics are summarized in Table I. The tumors were classified according to the TNM system and graded according to the criteria of Gleason (12).

Correspondence to: Dr Cecilia Balbi, National Cancer Research Institute, Largo Rosanna Benzi 10, I-16132 Genoa, Italy
E-mail: cecilia.balbi@istge.it

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Table I. Main characteristics of prostate cancer patients.

Variable	Lamin A/C n (%)	Lamin B n (%)
No. of patients	32	37
Median age in years (range)	64 (48-74)	64 (58-73)
Median pre-treatment PSA level in ng/ml (range)	11.6 (5-50.1)	10.6 (5.0-37.7)
Gleason score ≥ 7	21 (65.6)	23 (62.2)
Depth of invasion (T)		
T2	18 (56.3)	23 (62.2)
T3-4	14 (43.7)	14 (37.8)
Lymph nodes (N)		
N0	18 (56.3)	22 (59.5)
N+	5 (15.6)	6 (16.2)
Nx	9 (28.1)	9 (24.3)
Surgical margins involved	13 (40.6)	14 (37.8)
Seminal vesicles involved	8 (25.0)	8 (21.6)
Neoadjuvant treatment with hormones	5 (15.6)	4 (10.8)

Purification of the NM-Intermediate Filament Complex. The NM-Intermediate Filament (NM-IF) complex was isolated according to Alberti *et al* (5). Protein concentration was determined using the Bio-Rad protein microassay (Bio-Rad, München, Germany) with bovine serum albumin as the standard.

Gel electrophoresis. One-dimensional SDS-polyacrylamide gel electrophoresis (PAGE) was carried out according to Laemmli (13). Twenty μg of NM-IF proteins were loaded on two 10% PAGE and separated at 5 mA/gel for 16 h at a constant temperature of 12°C. One gel was stained with Coomassie brilliant blue R-250, the other used in the Western blot experiments. High resolution two-dimensional electrophoretic analysis was carried out as already described (14). In order to characterize changes in the isoforms of lamins, two-dimensional gel electrophoresis was carried out using the Pharmacia Multiphor II system in the first dimension.

Immunodetection of lamins. Immunodetection of lamins was carried out as already described (5,8) using the affinity-purified goat polyclonal antibodies, lamin A/C (N-18) diluted 1:200 and lamin B (C-20) diluted 1:150 (Santa Cruz Biotechnology Inc.). These antibodies recognize peptides mapping at the amino terminus of human lamins A and C and at the carboxy terminus of human lamin B1 and, to a lesser extent, lamin B2 and B3, respectively.

Determination of the relative amounts of lamins. Since the major components of the NM-IF complex undergo appreciable

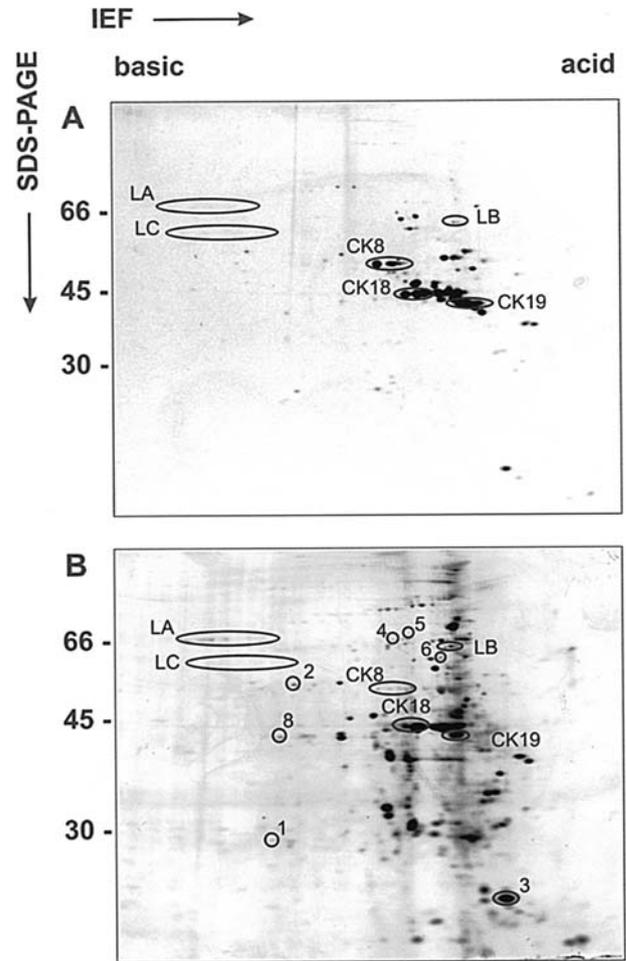


Figure 1. Two-dimensional electrophoretic analysis of the NM-IF complex of NT and PCa specimens from the same patient (A and B, respectively). (A) The thin lines enclose the major components of the cytoskeleton and the nuclear lamina. Ac, actin; LA, LB and LC, lamins A, B and C; V, vimentin; CK8, CK18 and CK19, cytokeratins 8, 18, 19. Tumor-associated NM proteins are marked by circles in B and identified with the same number used in Boccardo *et al* (6). The relative molecular weights of standard proteins in kDa are reported on the left. Silver-stained gels.

changes in expression during PCa development, it was not possible to use an internal control to ensure equal loading on the gels. Therefore, the relative amounts of type A and type B lamins were determined following the quantitative method described by Alberti *et al* (5). The relative intensity of the signal of each protein spot was obtained by normalizing the integrated optical density, obtained by an enhanced chemiluminescence assay kit (ECL), A for the integrated optical density of the corresponding Coomassie stained gel B. In order to reduce differences arising from fluctuations in the experimental conditions of the immunoblot, a fixed (10 μg) amount of rat liver lamins from the same batch were run on the gels and the ratio A/B further normalized by the ECL signal of the lamin standard. This method allowed us to obtain quantitative results.

Statistical analysis. The correlation of the variables undergoing study (lamin relative amount, pre-operative PSA serum level, Gleason score, tumor size, nodal status surgical margin involved, seminal vesicles involved and neoadjuvant

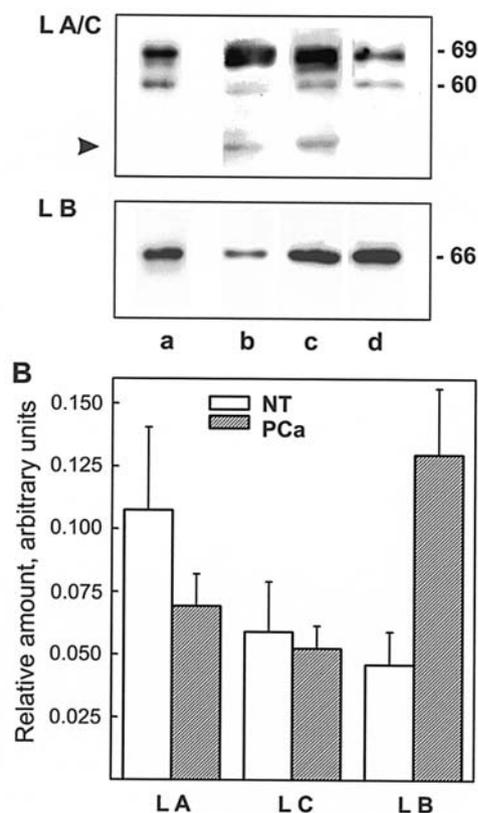


Figure 2. (A) Western blot analysis of the NM-IF complex isolated from NT (lane b) and two PCa specimens with Gleason score 6 and 8 (lanes c and d). Lamins isolated from rat hepatocytes are reported for comparison in lane a. A major proteolytic fragment of lamin A/C is marked by an arrowhead. The relative molecular weights of lamins in kDa are reported on the right. (B) The comparison among the relative amounts of LA, LC and LB in NT and PCa, respectively. The ordinates represent the mean \pm SE.

treatment) were investigated by using the Pearson correlation coefficient. A comparison between NT and PCa was made using the independent Student's t-test. All of the P-values reported are two-sided; differences in the lamin content were considered statistically significant for $P \leq 0.05$ (15). The statistical analyses were performed using the SPSS statistical software, version 9.0 (16).

Results and Discussion

Fig. 1 shows representative, high resolution two-dimensional gels of the NM-IF complex isolated from NT (A) and PCa, Gleason score 7 (B); the major cytoplasmic IF proteins and actin are indicated; tumor-specific NM proteins, as identified in previous reports (5,6), are encircled. The large increase in the complexity of the NM composition in PCa can be immediately appreciated on eye inspection of the electrophoretic patterns. Lamins give rise to weakly stained spots as a consequence of the low concentration compared with that of major IF components and of the presence of several isoelectric variants.

Quantitation of the differences in the expression of individual lamins has been obtained by monodimensional immunoblot analysis. The experiments reported in Fig. 2A show that the use of anti-lamin N-18 and C-20 antibodies (see

Table II. Relative amounts of lamins in the NM-IF complex isolated from NT and PCa.

	No. of patients	Mean \pm SE	P-value
Lamin A			
NT	17	0.107 \pm 0.033	0.2
PCa	32	0.069 \pm 0.012	
Lamin C			
NT	17	0.059 \pm 0.020	0.5
PCa	32	0.052 \pm 0.009	
Lamin B			
NT	20	0.048 \pm 0.014	0.03
PCa	37	0.128 \pm 0.026	

Table III. Significance of the relationship between the relative amount of lamin B and patient characteristics.

Patient characteristics	P-value	Pearson's correlation coefficient
Age	0.1	-0.240
PSA	0.3	0.181
Gleason score	0.001	0.521
Depth of invasion	0.5	0.106
Lymph nodes	0.1	0.283
Surgical margins involved	0.4	-0.133
Seminal vesicles involved	0.2	0.230
Neoadjuvant treatment	0.9	0.023

Materials and methods) results in a highly specific recognition of both lamins A/C and B. The arrow indicates a degradation fragment of type A lamin; this peptide has been detected also in NT samples, suggesting that proteolytic cleavage of lamins A and C can be a physiological process and could be associated to apoptotic cell death (17,18). The relative amounts of lamins which remain associated with the NM are shown in Fig. 2B; the results of the statistical analysis are detailed in Table II. The relative amount of lamin B increases significantly ($P=0.03$) in PCa with respect to NT; the opposite trend is observed for both lamins A and C but the differences are not statistically significant. Finally, the data reported in Table III show that the increase in lamin B is strongly correlated with Gleason score. This finding indicates that the expression of this protein is differentiation related, as already noted for the minor components of the NM showing differences between tumor and normal tissue (4-6).

Differences in the expression of lamins between tumor and normal cells have been reported in several studies. As a rule, the expression of lamin A/C was found to decrease in all tumor tissues or cell lines, while the observations on lamin B were limited to verifying that this protein was present (19-23). A major weakness of these studies has been the use of immunohistochemistry techniques, by which only a semi-

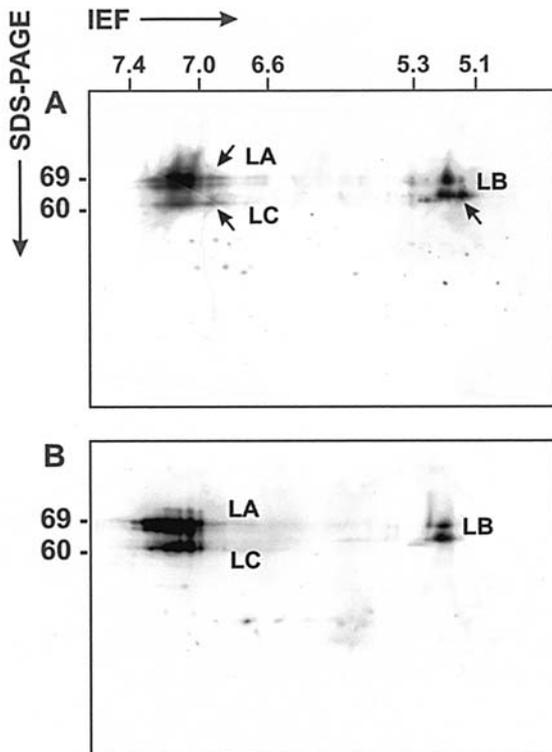


Figure 3. High resolution two-dimensional gel electrophoresis characterization of the isoelectric variants of lamins in NT (A) and PCa (B). Samples resolved by two-dimensional gel electrophoresis were transferred on PVDF membranes and probed with lamin A/C and lamin B antibodies. The arrows in (A) indicate the more acidic variants of lamins which are lost in PCa.

quantitative evaluation of the staining intensity of nuclei in fixed tissue can be obtained. Therefore, it is difficult to compare these previous data with the results of our immunoblot assay. Our work indeed provides clear-cut evidence of a direct link between the expression of lamin B and cell differentiation.

In a recent study, we reported the protein and structural characterization of the internal nuclear matrix and stressed the possible functional role of the fibrillar intranuclear web (8). The latter consists of thin, ~4-nm, lamin fibrils to which NuMA islands are anchored. Furthermore, these observations strongly suggest that RNA might directly mediate the anchoring of NuMA to the lamin scaffold. The lamin web is expected to be a highly dynamic structure involved both in the transition between eu and heterochromatin and in RNA synthesis and processing; fast rearrangements in its organization could be directed by changes in phosphorylation, as it occurs during the transition from late prophase to metaphase at the beginning of cell division (24). We therefore carried out a preliminary characterization of the changes in the phosphorylation of lamins in PCa by combining high resolution two-dimensional electrophoresis with immunoblot analysis. A representative electrophoretic pattern obtained for a PCa sample with Gleason score 6 is reported in Fig. 3B. In PCa, the more acidic isoforms of lamins undergo a significant decrease with respect to NT (Fig. 3A), directly showing the occurrence of a decrease in phosphorylation. Since proliferation should result in an increase in phosphorylation, the observed changes in the isoform distribution might actually reflect mechanistic features of cellular transformation.

Cytokeratins undergo large and definite changes with tumor progression both in the resistant hepatocyte model of hepatocarcinogenesis (14) and in PCa development (4,5). In a previous paper (14), we speculated that tumor associated chromatin rearrangement could arise from changes in the NM induced by cytoskeletal alterations, following the hypothesis of Ingberg and Folkman (25). Spencer and his colleagues (26) have shown that, in a hormone-dependent and estrogen receptor-positive human breast cancer cell line, cytokeratins 8, 18 and 19 are associated with DNA, in confirmation of Ingberg's model. Alternatively, the three-dimensional architecture of both the lamina and the internal nuclear web could directly undergo rearrangements which could, in turn, affect the overall organization of the bulk of chromatin. Spann *et al* (27) used a dominant negative lamin mutant lacking the NH₂-terminal domain to disrupt the normal organization of nuclear lamin; inhibition of RNA polymerase II activity was induced in both mammalian cells and transcriptionally active embryonic nuclei from *Xenopus laevis*. The present study, for the first time, establishes a direct link between structural changes in the nuclear scaffold and transcription, and stimulates future work on the molecular and structural changes lamins undergo in different tumor tissues.

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