The Role of the Nuclear Lamina in Cancer and Apoptosis

Jos L.V. Broers and Frans C.S. Ramaekers

Abstract Not long after the discovery of lamin proteins, it became clear that not all lamin subtypes are ubiquitously expressed in cells and tissues. Especially, A-type lamins showed an inverse correlation with proliferation and were thus initially called statins. Here we compare the findings of both A- and B-type lamin expression in various normal tissues and their neoplastic counterparts. Based on immunocytochemistry it becomes clear that lamin expression patterns are much more complicated than initially assumed: while normally proliferative cells are devoid of A-type lamin expression, many neoplastic tissues do show prominent A-type lamin expression. Conversely, cells that do not proliferate can be devoid of lamin expression. Yet, within the different types of tissues and tumors, lamins can be used to distinguish between tumor subtypes. The link between the appearance of A-type lamins in differentiation and the appearance of A-type lamins in a tumor likely relates the proliferative capacity of the tumor to its differentiation state.

While lamins are targets for degradation in the apoptotic process, and accordingly are often used as markers for apoptosis, intriguing studies on an active role of lamins in the initiation or the prevention of apoptosis have been published recently and give rise to a renewed interest in the role of lamins in cancer.

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Abbreviations

SCLC	Small cell lung cancer
CIS	Carcinoma in situ
CIN	Cervical intra-epithelial neoplasia
EBV	Epstein–Barr Virus
HPV	Human papillomavirus
HSV	Herpes simplex virus
HIV	Human immunodeficiency virus
GFP	Green fluorescent protein
FTI	Farnesyl transferase inhibitor

Introduction

The number of detailed studies on lamin subtype expression in normal tissues is remarkably low. The first studies on differential A-type lamin expression came from the group of E. Wang [1, 2], who used an antibody against a protein initially called statin. They stated that statin in general is absent in proliferating cells, while non-proliferating cells, induced to senescence, showed a pronounced statin expression [1]. Later studies confirmed that statin was in fact lamin A [3]. The differential expression of A-type lamins in cancer is remarkable. In a variety of epithelia and corresponding carcinomas several attempts have been made to correlate proliferation with the absence of A-type lamins. Also a positive correlation between the degree of differentiation and the presence of A-type lamins has been suggested. A refined insight into differences in protein expression has been obtained by generating antibodies that specifically recognize the main splice variants of the LMNA gene, lamin A or lamin C, as well as antibodies that differentiate between the products of the two different B-type lamin genes, lamin B1 and lamin B2. Older studies did not differentiate between these B-type lamins and just mentioned the expression of lamin B protein. In addition, antibodies that recognize different phosphorylation states of lamins have enabled studying altered lamina associations in the nucleus at the cellular level [4].

In contrast to A-type lamins, there is a general consensus that B-type lamins are ubiquitously expressed in epithelial tissues and carcinomas. Yet, also B-type lamins, and especially lamin B1, are often downregulated in a subset of tumor cells within the same tumor.

In this review we discuss (mainly immunocytochemical) lamin expression studies performed in different normal epithelia and their corresponding carcinomas and discuss the impact of these findings for disease diagnosis.

Programmed cell death or apoptosis is a key mechanism in maintaining a balance between tissue growth and shrinkage. Consequently, suppression of apoptosis is a major process enabling tumor development. However most tumors display extensive apoptosis; especially tumors with high proliferation rates show prominent levels of apoptosis. A main challenge in cancer treatment is shifting the balance in favor of the apoptotic process. Since long it is known that cleavage of lamin proteins by caspases is a necessary step in apoptosis allowing for nuclear membrane degradation to proceed, followed by chromatin condensation [5]. Now, there is mounting evidence that, in turn, abnormal lamina organization can lead to apoptosis. However, knowledge about the exact mechanisms supporting the relationship between the lamina and apoptosis is as yet merely speculative.

Normal Epidermis and Skin Cancer

Epidermis

Comparing the expression of A-type lamins as reported by different groups, one can immediately observe that there is a discrepancy between findings of different research groups. Initial studies by Röber et al. [6] showed a gradual increase in A-type lamin expression in all cell layers of the epidermis of mice starting at the later stages of embryonic development and continuing to increase after birth. Expression of these proteins in all epidermal layers was confirmed in paraffin sections of human epidermis [7]. In contrast, several groups noticed the reduced expression of A-type lamins in a large number of (but not all) basal cells and an increase of expression in suprabasal cells [8–10]. Upon ageing, A-type lamin expression becomes more heterogeneous, when comparing lamin A/C expression in the skin of a young child (1 year) versus old people (>60 years [11]). Using A-type lamin subtype-specific antibodies the absence of lamin A from basal cells was striking. Nuclei were completely devoid of lamin staining. In contrast, lamin C expression was still present in basal cells, however often not forming a clear lamina but rather giving a diffuse intranuclear staining pattern [9]. Basal cells that did not express lamin C appeared to be resting basal cells [10]. These findings are not in line with a recent study [12], showing an increase of A-type lamin expression in basal cells and a decrease in the suprabasal cells. How can these discrepancies be explained? First of all, the research was performed on different species (mouse vs. human). Secondly, different fixation and permeabilization methods were applied (formalin fixation, paraffin embedding, and antigen retrieval vs. unfixed frozen sections or methanol-fixed frozen sections). Thirdly, different A-type lamin antibodies were used. Apparently, these factors can influence the recognition of lamin A/C epitopes.

To further complicate this issue, several studies have stressed the importance of different phosphorylation states of lamins. Several lamin A/C antibodies only recognize certain phosphorylation states of lamins, while others will give a more general labeling [4, 13]. Moreover, epitope masking not due to changes in phosphorylation state can occur, as has been shown for lamin B1 [14]. This problem could be overcome by applying a large panel of antibodies on tissues that were fixed in different ways. Unfortunately, these studies were only performed with a limited type of tissues and few antibodies (e.g., see [4, 14]).

Several groups have investigated the expression of B-type lamins in human epidermal tissues. Most reports mention the presence of lamin B1 throughout the epidermis [7, 9, 10], with a prevalence for decoration of the lower, proliferative layers of the epidermis and the absence of lamin B1 in a subset of the basal cells [8]. Most groups demonstrate the uniform staining of all epidermal layers with lamin B2 antibodies [8–10], whereas sometimes a decrease of lamin B2 expression was found in granular cells [7].

Skin Cancer

In basal cell carcinomas most studies found a reduction of A-type lamins. For instance Oguchi et al. [7] showed a reduction in most of the basal carcinomas examined, using an antibody that did not differentiate between lamin A and lamin C.

Using A-type lamin subtype-specific antibodies, Venables et al. [10] showed a reduced expression of lamin A in the majority of tumors. These tumors appeared to be hyperproliferative based on the expression of the proliferation marker Ki67. Downregulation of lamin C was less common in these carcinomas (5/16 tumors [10]). Using similar antibodies, Tilli et al. [9] found more lamin A-expressing cells than lamin C expressing cells in basal cell carcinomas. Strikingly, both studies showed a nucleolar rather than a nuclear lamina staining in some basal cell carcinomas using a lamin C antibody. Whether this staining corresponds to the intranuclear foci seen in early embryonal development [15] remains to be examined.

In squamous cell carcinomas of the skin, a study by Oguchi et al. [7] showed that most tumors were strongly positive for A-type lamins, with only a minority of cancers showing a reduction. Another study confirmed the expression of both lamin A and lamin C in squamous cell carcinomas of the skin [9]. Most of the tumors with reduced lamin A/C expression were poorly differentiated, confirming the general notion that A-type lamin expression is decreased with loss of differentiation.

Germ-Line Cells and Germ Cell Tumors

Since in general the largest differences in lamin expression can be found upon changes in differentiation, one would expect a large number of studies on lamin expression in development of germ-line cells and in developing embryos. Noticeably,

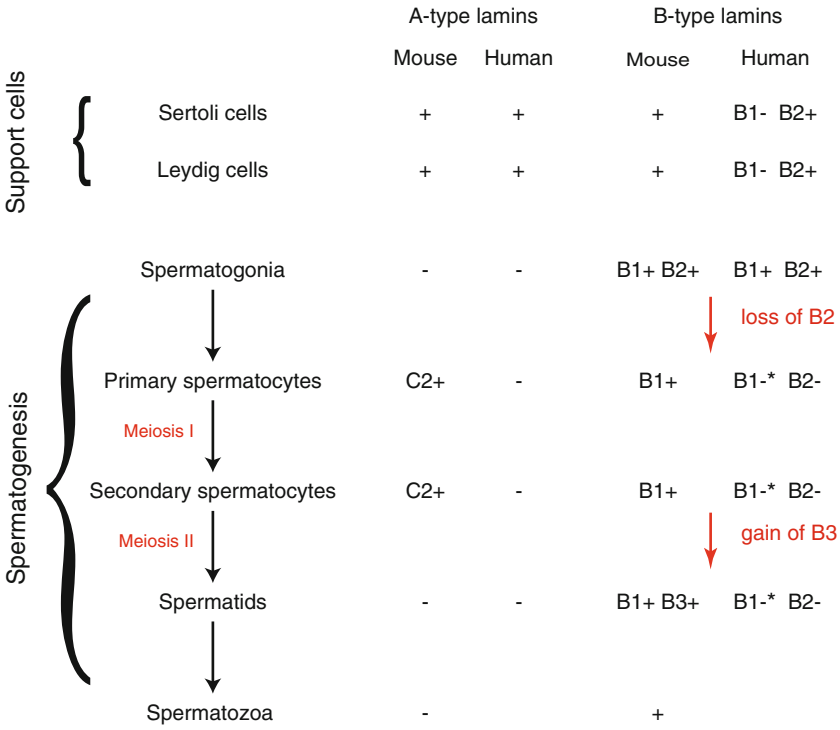

Fig. 1 Overview of reactivity of lamin antibodies in testicular germ cells. Note the prominent changes in lamin expression upon spermatogenesis. *epitope masking? Based on the more extensive studies in mouse tissues, it is likely that lamin B1 is also present in human sperm cell development

this is not the case. To our knowledge only few studies have investigated lamin expression in germ-line tissues and tumors. In fact, only one study has been done on lamin expression in testis and testis tumors using human material [16]. A few more studies have compared lamin expression patterns in normal male and female germ cell types of other species [17–21]. Figure 1 shows an overview of lamin reactivity in male germ-line cells in mouse and human tissue.

Normal Male Germ-Line Cells

Initial studies claimed that no lamins were present during spermatogenesis in chicken [21]; however, subsequent studies in mouse tissues showed that lamins are indeed present in several cells during sperm development [18, 20]. In mouse male gonads, lamin A/C as well as lamin B antibodies reacted with isolated prepuberal Sertoli cells. In addition, anti-lamin B stained the nuclear lamina of all germ-line cell types examined, including primitive spermatogonia, preleptotene, leptotene–zygotene,

and pachytene spermatocytes and spermatids [20]. Later studies showed that lamin B1 and not lamin B2 was expressed in male germ cells [22]. Lamin B3, a splice variant of the lamin B2 gene, was initially suggested to be expressed in spermatocytes during meiosis [23], but a more recent study has challenged this observation and found evidence that lamin B3 is only expressed in spermatids [24]. By contrast, no cells at any stage of spermatogenesis showed expression of lamin A or lamin C [19, 20]. In sperm cell development, however, an alternatively spliced form of lamin C, called lamin C2, has been detected in meiotic stages of spermatogenesis, while no other A-type lamins are expressed during this process [17]. The impact of the absence of this splice variant has been discovered in a study on the development of mouse cells lacking expression of the *Lmna* gene, which showed a failure of prophase I progression and defective sex chromosome pairing in $Lmna^{-/-}$ spermatogenesis [25].

In a study on human testes, Sertoli, Leydig, and peritubular cells were shown to express both A-type lamins and lamin B2 [16]. Both Sertoli and Leydig cells did express lamin B2 but in general showed no reaction with a lamin B1 antibody. In contrast, spermatogonia were positive for both lamin B1 and lamin B2. Strikingly, no A-type lamins were detected in these cells, despite their highly specialized commitment to differentiate into spermatocytes. In some cases, reactivity with A-type lamin antibodies was seen, but this reaction was weak and only detectable in some of the spermatocytes. Based on the findings in mouse spermatocytes, it was suggested that this weak staining was due to cross-reaction with lamin C2 [16]. Spermatogonia in normal human testis were only partially and weakly positive for lamin B2, while in parenchyma adjacent to seminomas all spermatogonia were clearly positive. Lamin B2 expression in spermatogonia adjacent to seminomas seems, therefore, slightly increased [16].

Using B-type lamin antibodies, human spermatocytes showed no reactivity. While the absence of lamin B2 was in accordance with findings in mouse spermatocytes [22], the absence of lamin B1 in human spermatocytes was unexpected and may be due to epitope masking that has been shown to occur also with this particular antibody in heart tissues [14].

Male Germ Cell Tumors

A study by Machiels et al. [16] showed that the seminomas examined could be divided into two groups: one group contained a mutation in K- or N-RAS (RAS positive), and the second group of seminomas had no detectable mutation in the RAS genes (RAS negative). RAS is one of a family of small GTPases, many of which have been linked to cancers. Using an antibody to A-type lamins, striking differences were observed between these groups: the RAS-negative seminomas were negative for lamin A, and the RAS-positive seminomas were positive, although sometimes weakly. Interestingly, one case was known to contain a heterogeneous population of tumor cells with and without RAS mutation, and this tumor showed a heterogeneous staining pattern with the lamin A antibody. Most seminomas were

negative with another lamin A/C antibody, with only one RAS-positive seminoma case being positive. The lamin A antibody 133A2 showed partial reactivity with only two RAS-positive seminomas. None of the RAS-negative seminomas gave a staining reaction with the A-type lamin antibodies.

Most non-seminomas were positive with lamin A/C antibodies. Strikingly, embryonal carcinomas were found to be negative for lamin A, but positive for lamin A/C using two different lamin A/C antibodies. Low expression of lamin A together with normal or high expression of lamin C may explain this reactivity pattern, although epitope masking for the lamin A antibody cannot be excluded. Normally lamin A and C proteins are expressed to comparable degrees, but an imbalance in the expression ratio of lamin A over C may occur. To examine this phenomenon further, samples of three embryonal carcinomas were used for immunoblotting. When these blots were stained with the lamin A/C antibodies, it was obvious that the reactivity level of the lamin C bands was much stronger than that of lamin A, which confirmed the immunohistochemical observations. The presence of lamin A in the blots was confirmed by a weak reactivity with the lamin A antibody 133A2, which may even be overrepresented as a result of non-tumor components that are present in the tumor tissue, such as small blood vessels, and express A-type lamins. The very low expression level of lamin A and the imbalanced expression of lamin A and lamin C using two different antibodies argued in favor of the interpretation that embryonal carcinomas indeed did not express lamin A and were not negative due to epitope masking.

Yolk sac tumors, choriocarcinoma, and teratoma could not be distinguished from each other by studying A-type lamin expression. All three histologically distinct tumor types gave similar perinuclear staining with the lamin A antibody as well as the lamin A/C antibodies.

When a carcinoma in situ (CIS) adjacent to non-seminomas (Table 1) was negative for lamin B1, spermatogonia were also negative. When a CIS was positive for lamin B1, spermatogonia were also positive. In addition, the reaction of the Sertoli cells in these sections was always opposite to that of spermatogonia and CIS. In normal testis, Sertoli cells were negative and spermatogonia were positive for lamin B1, being the physiological expression pattern.

Uterine Cervical Tissues and Premalignant Cervical Lesions

An extensive study was performed on expression patterns of lamins in normal cervical epithelium and premalignant epithelium lesions, known as CIN (for cervical intra-epithelial neoplasia) [8]. In normal ectocervical stratified epithelium, lamin B2 is expressed in all cell layers. In contrast to other non-keratinizing stratified squamous cell epithelia, lamin B1 is most strongly expressed both in the basal and in the parabasal epithelial cells, with a reduction of staining in the upper cell layers. Also, in contrast to other stratified epithelia, lamin A/C antibodies as well as a specific lamin A antibody showed prominent staining of the entire epithelium.

Table 1 Comparison of expression of lamin subtypes in normal tissues and their corresponding tumors

Tissue	Lamin A	Lamin C	Lamin A/C	Lamin B1[a]	Lamin B2	References
Skin and skin cancer						
Basal cell	–	±	±	++	++	[8–10]
Suprabasal cells	++	++	++	+	– to ++	[7–10]
Squam. cell carcinomas	++	++	++	++	++	[7, 9]
Basal cell carcinomas	– to +	± to +	– to +	++	++	[7, 9, 10]
Germ cells and tumors (male)						
Sertoli cells	+	+	+	–	+	[16, 18–20]
Leydig cells	+	+	+	–	+	[16, 18–20]
Spermatogonia	–	–	–	+	+	[16, 18–20]
Spermatocytes	–	– (C2 +)	–	–/+[a]	–	[16, 18–20]
Spermatids	–	–	–	–/+[a]	– (B3+[b])	[24]
Seminomas						
RAS positive	– to +	– to +	+	+	++	[16]
RAS negative	–	–	–	+	++	[16]
Non-seminomas						
Embryonal cell ca.	–	+	+	+	++	[16]
Other	+	+	+	+	++	[16]
Uterine Ectocervix and CIN						
Basal cell	+		+	++	++	[8]
Suprabasal cell	++		++	± to +	++	[8]
CIN ($I \rightarrow III$)	++ to ±		+ to ±	±	+	[8]
Lymphoid cells and tumors						
Hematopoietic cells			–	+	+	[35]
Granulocytes			–	+	+	[35, 38]
Early lymphoid cells			–	+	+	[37, 39]
T-cells			–	+	+(react –)	[35, 39]
B-cells			– to + (CD30pos)	+	+(react –)	[35, 39]
Mononuclear cells			+	+	+	[40]
PMN cells			–	+	+	[40]
Malignancies						
Lymphoid cells			–	+	+	[34]
Myeloid cells			– to + (diff)	+	+	[34]
Hodgkin's lymphoma	– to ++		– to ++	+	+	[39]
Lung tissues and cancer						
Bronchial basal cells	– to +		– to +	+	++	[8, 44]
Bronchial columnar cells	++		++	– to +	++	[8, 44]
Alveolar cells	++		++	– to +	++	[8, 44]
SCLC			– to ±	– to ++	++	[44]
Adenocarcinomas			– to ++ (cyt!)	– to ++	++	[44]
Squam. cell carcinomas			++	– to ++	++	[44]

Squam. cell squamous cell, *react* reactive lymph nodes, *PMN cells* polymorphonuclear leukocytes, *diff* differentiated tumors, *cyt!* cytoplasmic reaction

[a]Possibly negative due to epitope masking

[b]Mouse only

In endocervical columnar epithelium, most cells are positive with the antibodies examined except for the reserve cells, which are largely negative for lamin B1. In connective tissue cells of the endocervix all antibodies are positive except for the lamin B1 antibody, which is negative in the fibroblasts but positive with most lymphoid cells.

Metaplastic cervical epithelium showed expression of A-type lamins in all epithelial layers, albeit that not all cells were labeled, similar to normal cervical epithelia. B-type lamins showed a homogenous staining in basal and intermediate layers but a striking decrease in the superficial layers of the tissue. Especially the loss of lamin B1 from these superficial metaplastic cells was striking.

More than 30 cases of CIN were examined for their lamin expression patterns. Strikingly, metaplastic epithelium next to CIN lesions showed an aberrant lamin expression pattern. Expression of A-type lamins was increased in the basal layer and decreased in the suprabasal cell, an inversion of the "normal" A-type lamin expression pattern in stratified squamous cell epithelia. Also, lamin B1 expression was largely decreased in these tissues, while lamin B2 expression was more heterogeneous than in comparable regions of normal epithelia.

From low-grade (CIN I) to high-grade (CIN III) CIN lesions the normal differentiation of the squamous epithelium is increasingly lost. Expression of A-type lamins was highly variable within each layer with strong, weak, and even absence of labeling of nuclei at very close distance from each other. Lamin staining patterns were similar in different layers of either grade/lesion type (CIN I–CIN III) and lamin B2 was present in most epithelial cells, while lamin B1 expression seemed to remain confined to the lower layers of this epithelium. A-type lamins were expressed heterogeneously throughout all layers of the epithelium. However, an overall decrease in the number of cells with lamin staining, as well as an average decrease in intensity of staining, was noticeable in high-grade CIN lesions.

The heterogeneous lamin A/C staining patterns in CIN lesions invite speculation about the correlation between the integration and/or episomal presence of the human papillomavirus (HPV), occurring in nearly all of these tumors [26], and the concurrent loss of A-type lamin expression. Other viruses such as Epstein–Barr virus (EBV), herpes simplex virus (HSV), cytomegalovirus (CMV), and human immunodeficiency virus (HIV) have been shown to disrupt the nuclear lamina after infection, enabling the release of virion particles from the nucleus [27]. This disruption can be due to conformational changes of the lamina, as shown for HSV-1 [28], resulting in increased solubility, but also physical disruption of the nuclear lamina can occur, as seen in lamin-GFP-transfected cells, infected with HSV-1 [29]. In HIV infections the viral protein Vpr induces perforations in the nuclear lamina, leading to dynamic disruptions in the nuclear envelope [30].

Until now, only quantitative tissue studies were performed on the impact of the HPV viral proteins on lamin expression. These studies showed that HPV 16 E5 does cause a downregulation of lamins A/C [31], while HPV 16 E1–4 and HPV 16 E6 do not seem to have a prominent effect on lamin A/C expression [32]. Possibly, HPV integration benefits from a weakened lamina in cases of coinfection with HSV-2 or HIV, since epidemiologic studies indicate an increased risk of developing cervical cancer in these cases [33]. However, direct effects of HPV infections on the lamina structure have not yet been studied at the cellular level.

Lymphoid Cells and Tumors

Normal Blood Cells

Cells, very suitable to study the correlation between lamina expression and proliferation versus differentiation, can be found in the lymphoid system and tumor cells derived from these cells, since the different stages of blood cell development have been very well defined. An extensive review shows an overview of the most important findings in normal blood cells and hematologic malignancies [34].

Initial studies indicated that hematopoietic cells were devoid of A-type lamins, showing that in mouse cells both T and B lymphocytes as well as granulocytes and monocytic cells directly isolated from spleen, thymus, blood, or bone marrow did not express lamin A/C but only lamin B [35]. Comparable studies in rat showed that thymocytes and human pre-B lymphoblasts do not express A-type lamins, while in purified T and B lymphocytes isolated from blood samples A-type lamins could be detected [36, 37]. Human peripheral blood granulocytes express little if any lamin A or lamin C [38]. In a study on human lymph node tissue in patients with Hodgkin's lymphoma it was shown that in most of the reactive lymph nodes investigated A-type lamins were absent [39]. These last findings, combined with those of other animal studies, led us to the suggestion that both B- and T-cells express very low amounts of A-type lamins or no lamins at all. The positive findings by Guilly et al. [37] on immunoblots of isolated B- and T-cells could easily have been caused by contamination with other blood cells that do contain A-type lamins, such as mononuclear neutrophils [40].

Cells expressing CD20 (a marker for B-cell differentiation and present on B-cells but not on plasma cells) do not express A-type lamins, while CD30-positive cells (a marker for activated B-cells) in the paracortex as well as in the medulla in general did show A-type lamin expression. These findings suggest that cells with a higher degree of lymphocyte differentiation do express A-type lamins. Another intriguing observation was the absence of lamin B2 in both centrocytes and centroblasts of the follicle center of the lymph nodes, while the paracortex showed a high expression of lamin B2. A lamin B1 antibody showed reactivity with all cells in all regions of the lymph nodes.

Hematologic Malignancies

Cell lines derived from different lineages of hematologic malignancies in general showed variable A-type lamin expression. A T-lymphoblast cell line (KE 37) was negative for A-type lamin expression [36], while fully differentiated stages in the B-cell lineage such as represented by the RPMI-6666 line (an EBV-transformed lymphoblastoid B-cell line) and the U266 plasmacytoma line (an IgE-producing human myeloma) showed a strong lamina A/C labeling. In contrast, a previous study

showed that Ig-secreting mouse myeloma cells lack lamin A and C expression [41]. To explain these apparently conflicting findings a detailed study was undertaken by Kaufmann [38], who showed that human myeloid leukaemia cell lines and marrow samples from patients with acute non-lymphocytic leukaemia do express low but detectable levels of A-type lamins. Also, lamins A and C were detected in cell lines of myeloid (KGla), erythroid (HEL), and megakaryocytic (Mo-7e) lineages. Strikingly expression of A-type lamins can be strongly enhanced in the HL-60 human progranulocytic leukaemia cells by inducing differentiation into monocytes using *O*-tetra-decanoylphorbol-13-myristate acetate (TPA) [38]. Samples from patients with chronic myelogenous leukaemia did not show expression of A-type lamins [38]. A study on patients with acute lymphoblastic leukaemia and non-Hodgkin's lymphomas revealed that these tumors do not contain A-type lamins [42].

Jansen et al. [39] have studied lamin expression in nodular sclerosing Hodgkin's disease and noticed a prominent labeling of both Reed–Sternberg cells and Hodgkin cells with A-type lamin antibodies. They also tried to find a correlation between proliferation (using the Ki67 antigen marker) and presence of A-type lamins. While in normal cells Ki67 expression was limited to cells with a reduced A-type lamin expression, this was not the case in neoplastic cells. Both lamin A-positive and lamin A-negative cells expressed Ki67.

Lung Epithelium and Lung Cancer

In normal lung as well as lung cancers the expression of lamins has been investigated using different techniques, including Northern blotting, Western blotting, and immunocytochemistry [8, 43]. From these studies it became clear that both in normal and neoplastic lung tissue a dramatic reduction of A-type lamins can occur. In normal lung lamin A/C expression is only observed in a subset of cells, relating to the differentiation stage of individual cells. A wide range of lamin A/C expression levels is also observed in lung cancers, which may reflect a change in the lamin levels or the differentiation stage of the cell that initiated the tumor or changes in differentiation within these tumors.

Normal Lung Epithelium

Studies on lamin expression in normal bronchial and alveolar cells [8] revealed that A-type lamins were expressed in bronchial columnar cells but showed large differences in expression levels between patient samples in bronchial basal cells. In some samples, no or few basal cells were stained with these antibodies, whereas other samples revealed a uniform positive staining in all bronchial cells. Since these normal lung samples were obtained from (ex-)smokers who developed lung cancers in other regions of the lung, it is tempting to speculate that differences in A-type lamin

expression between samples reflect premalignant stages of the diseases, which are not yet visible upon histological examination of these samples. All alveolar pneumocytes were positive for A-type lamin antibodies.

Lamin B2 was present in virtually all epithelial cells. In contrast, expression of lamin B1 was quite heterogeneous, showing a strong decoration/staining of basal cells of bronchi but not in suprabasal, columnar cells. A similar heterogeneity was revealed in alveolar cells, with only a limited number of cells stained.

Lung Cancer Cell Lines

Kaufmann et al. [43] were the first to describe the prominent decrease of A-type lamins in small cell lung cancer (SCLC) cell lines. They showed that lamin A/C levels were more than 80 % lower in SCLC cell lines compared to non-SCLC lines, as detected by Western and Northern blotting. These findings were confirmed in another study using a different panel of cell lines [44]. In this latter study immunofluorescence confirmed the absence or very weak expression of A-type lamins in SCLC cell lines. From Fig. 2 it becomes clear that at the individual cell level a pronounced variation in lamin C expression can be seen, ranging from complete absence of labeling, via weak diffuse intranuclear labeling, towards cells with a clearly visible nuclear membrane labeling. In contrast, lamin B1 expression appears to be quite homogenous in these cells. In general, nuclei containing a lamina with lamin A and/or lamin C are larger in size than those without A-type lamins (Fig. 2, NCI-H82), and indeed cell lines that differentiate from the classic via the variant SCLC phenotype towards non-SCLC show increased nuclear size along with the appearance of pronounced lamin A/C staining (unpublished). Kaufmann et al. [38] performed an additional and very interesting study on SCLC cell lines. They compared A-type lamin expression in the SCLC cell line NCI-H249 before and after transfection with v-Ha-RAS and found a dramatic increase in lamin A/C expression after transfection. How this v-Ha-RAS transfection, which alters the phenotype of this cell line from SCLC to non-SCLC, impacts on the expression of A-type lamins is not entirely clear yet. A correlation between RAS activation and increased lamin A/C expression has also been found in other studies. As mentioned above, a positive correlation between RAS expression and A-type lamin expression was found in human seminomas [16]. In parallel to these results, a recent study showed that in osteoblast differentiation by FGF3 activation, leading to RAS and ERK activation, expression of lamin A/C is increased [45]. As for SCLC, the mechanism by which increased LMNA expression is achieved upon RAS activation is unclear so far. The recent finding that phospho-ERK, a prominent downstream target of the RAS signaling pathway, is increased in laminopathy cells is intriguing and suggests that not only A-type lamin expression can be induced by RAS signaling but also, conversely, the RAS signaling route can be triggered by defective A-type lamin expression in a feedback loop [46]. Interestingly, both RAS and lamins undergo the same posttranslational modifications, including farnesylation, so a common expression regulation

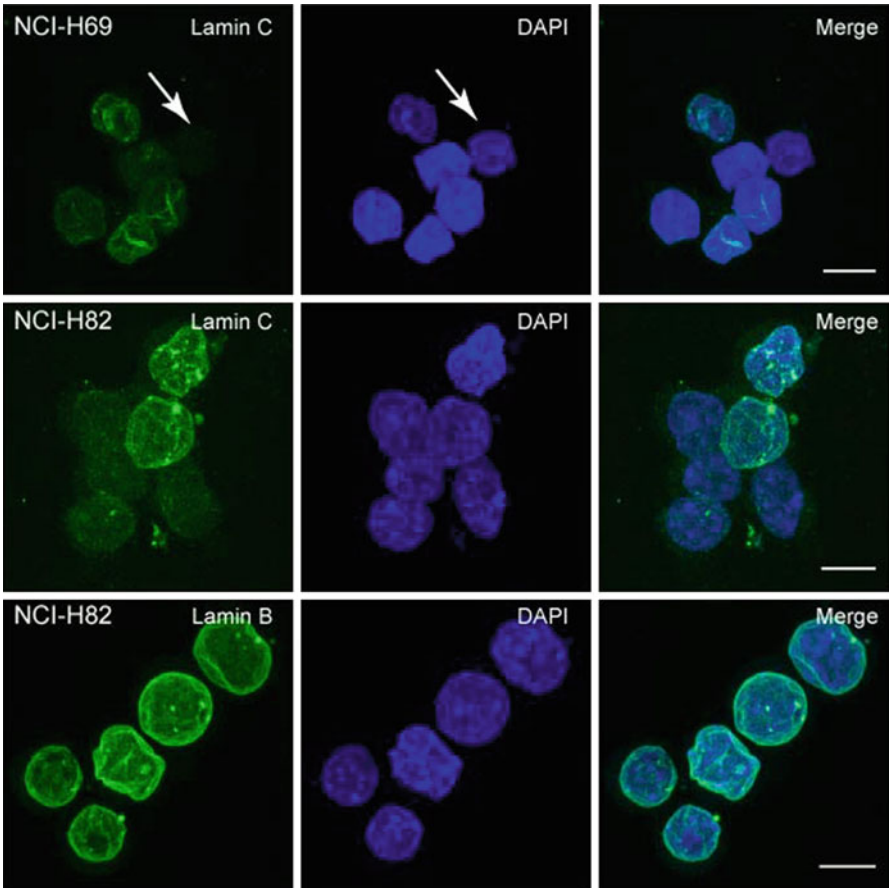

Fig. 2 Confocal z-projections of SCLC cultures immunostained with lamin C or lamin B1 antibodies (*green*). Note the large variation of lamin C immunostaining within a single clump of tumor cells, ranging from invisible (*arrow*) to a clear decoration of the nuclear rim. Note also that nuclei with lamin C in their lamina appear larger than in the neighboring lamin C-negative cells (NCI-H82). Lamin B2 is ubiquitously expressed in all tumor cells. Nuclei were counterstained with DAPI. Scale bars represent 10 μm

pathway should not be excluded. The correlation between RAS activation and increased lamin A/C expression could explain why lamin A/C seems to be a marker for differentiation in some cell systems and a marker for increased proliferation in tumors. It is known that v-Ha-RAS can cause proliferation in some cells and senescence induction in other cells. In the SCLC cell line NCI-H249 it seems that v-Ha-RAS expression leads to a cell phenotype with both increased growth and increased levels of differentiation [47], accompanied by an increase in lamin A/C expression [43], while, as mentioned above, in osteoblasts, enhanced normal RAS expression causes differentiation and senescence [45].

Lung Cancer Tissue Specimens

A detailed study on the differential expression of A- and B-type lamins in both SCLC and non-SCLC revealed that A-type lamin expression is strikingly reduced in most SCLC. In fact, 6 out of 15 cases were scored as having no lamin A/C expression at all [44]. Cells with a higher degree of differentiation, including lung carcinoids (4 out of 6 cases) and non-SCLC (23 out 25 cases), showed a prominent labeling using the A-type lamin antibodies. To our surprise, several non-SCLC tumors showed a pronounced cytoplasmic staining and the absence of nuclear staining. While at the histological section level no cellular or nuclear abnormalities were detected, lamin A/C staining was found in the cytoplasm and not decorating the nuclear rim of these tumors (Fig. 3). These findings urged us to search for lamin abnormalities both at RNA and protein level as well as by mRNA sequencing. In the limited number of tumors examined, no A-type lamin mutations were found (Broers et al., unpublished). Thus, the cause of this cytoplasmic labeling of A-type lamins in lung cancer remains unresolved. Possibly, a disturbed nuclear import mechanism of lamins gives rise to cytoplasmic accumulation of these proteins. A study by Mical and Monteiro [48] showed that the presence of a correct nuclear localization signal is not sufficient for nuclear translocation but that also the CAAX domain and an extra 42 AA central rod domain are needed. Possibly these motifs are not recognized for nuclear import, or alternatively the nuclear import machinery for lamins itself is not functioning in these cells. A similar study in colon adenomas and gastric cancer also revealed cytoplasmic lamin staining [49]. Also, in this latter study no follow-up studies were performed explaining this aberrant lamin localization; however, in our search for lamin mutations in these tumors, we did discover a novel splice variant, lamin AΔ10, that appeared to be present not only in lung cancer cell lines and solid lung cancer but also in other tumors as well as in most normal tissues and cell lines. The expression level, however, was in general much lower than for full-length lamins A and C in the tissues examined, and in these older studies only a nested PCR allowed a reliable detection of the transcript [50]. However, in a recent study in neonatal ductal arterial tissue this transcript could be detected in a single RT-PCR run (35 cycles) [51]. Until now, the number of studies on this splice variant has been very limited. Initial attempts to generate lamin AΔ10-specific antibodies have failed. Lamin AΔ10-tagged GFP localizes normally to the nuclear membrane, forming a network with lamins A and C (Broers et al. unpublished). It is unknown whether lamin AΔ10 needs full-length lamins A and C for incorporation into the nuclear lamina. Since the protein is processed like lamin A, including farnesylation and cleavage of the C-terminus [50], independent incorporation seems likely.

In most lung cancer specimens B-type lamins were expressed in all tumor cells, but in a minority of cases a reduced lamin B2 staining was found [36]. Also, lamin B1 was expressed in all lung cancers examined, albeit with a larger variation of staining intensity in general compared to lamin B2 within tumors (Broers et al. unpublished).

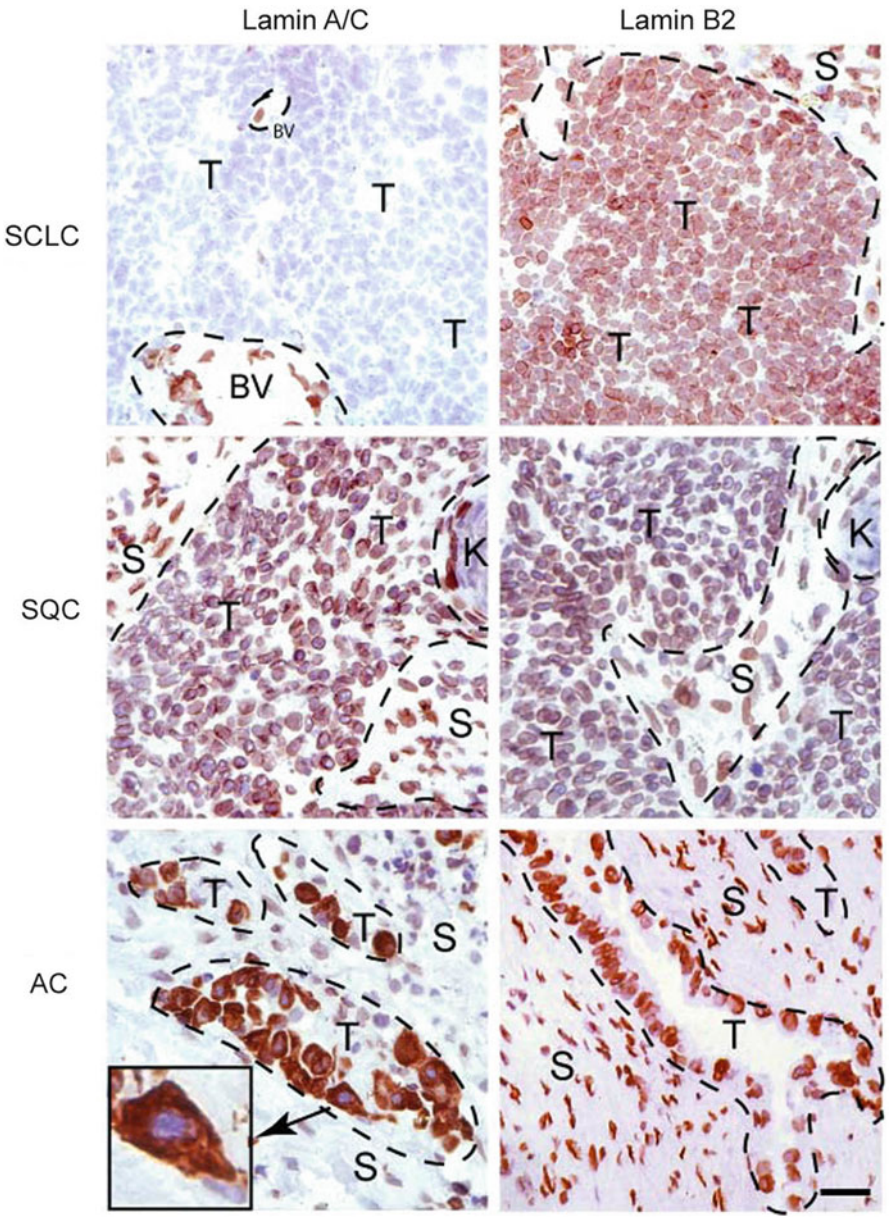

Fig. 3 Immunocytochemical staining of different lung cancer subtypes using antibodies to lamin A/C and lamin B2. Specific immunostaining can be appreciated as a red-brown deposition of aminoethylcarbazole. Nuclei are counterstained with hematoxylin. Tumor areas (T) are indicated, next to surrounding (reactive) stromal areas (S) and blood vessels (BV). Small cell lung cancers (SCLC) in general do not express A-type lamins with a specific decoration of vascular endothelial cells (BV) only. In contrast, a lamin B2 antibody stains all (tumor and non-tumor) cells of SCLC. Squamous cell carcinomas (SQC) show nuclear membrane staining in virtually all tumor cells with a lamin A/C antibody as well as with a lamin B2 antibody. Note the absence of both lamins A/C and B2 in keratinizing areas (K) of the tumor. An adenocarcinoma (AC) shows next to nuclear staining a pronounced cytoplasmic staining in part of the tumor cells (*insert*) using an A-type lamin antibody. Lamin B2 is present in all tumor cells of this adenocarcinoma. Scale bar is 25 μm

Lamins as Markers for Differentiation, Proliferation, and Tumor Progression

As stated in the previous sections, it has become clear that especially A-type lamin expression can be used as a marker for the developmental stage of a tumor. However, in general this only holds true within a certain group of tumors with otherwise similar characteristics. Table 1 summarizes the relationship between normal cells and their derived tumors as well as within tumors. The staining results of all these tissues give rise to a lot of questions regarding their usefulness. How can any conclusions be drawn on general expression patterns, if so many exceptions occur? As stated in the introductory part of this review, the general idea that differential lamin expression can be used for marking differentiation or proliferation is too simple. It is not possible to distinguish whether a decrease in A-type lamin expression in a tumor cell is due to local dedifferentiation of this cell or due to the fact that this tumor cell is derived from a particular normal cell with a lower degree of differentiation. While many factors such as epitope masking and selective recognitions of phosphorylation state of the lamina have obscured the results of these stainings [4, 13, 14], the impact of other factors on lamin expression has been largely overlooked. For instance, to our knowledge, no correlation has been examined in vivo between RAS expression and staining with lamin antibodies. Also, the relative expression of the different A-type lamin isoforms (lamin A, lamin AΔ10, lamin C) has not been addressed thoroughly in most studies.

Lamins in Apoptosis

Lamins as Target Molecules in the Execution of Apoptosis

While the molecular mechanisms by which the unique expression patterns of lamins in tumors remain obscure, the critical importance of apoptosis to the regulation of tumors is well established as are roles of lamins in the apoptotic process. Thus lamin functions in apoptosis could be a link to lamin changes observed in tumors. Numerous studies have been performed on the role of lamins in the execution of apoptosis. In cancer, apoptosis is a common event. Strikingly, most highly proliferative tumors show increased levels of apoptosis, as compared to tumors with a low proliferative capacity. In these former tumors even a small alteration in the percentage of apoptotic cells can lead to a dramatic expansion or shrinkage of tumor size. Lamins appear to be specifically targeted by caspases 3 and 6 that become activated both via the intrinsic and extrinsic pathway of apoptosis. Upon induction of apoptosis cytochrome c release activates procaspases, which cleave target molecules in an amino acid sequence-specific manner. A-type lamins are cleaved at their conserved VEID site, which is located in the non-helical linker region L12 at position 230. Cleavage is mediated specifically by caspase 6 and not by other caspases [52, 53].

It was initially assumed that caspase 6 is also responsible for B-type lamin cleavage at their conserved VEVD site [54]; however, for complete cleavage of lamin B in vivo the presence of caspase 3 seems to be indispensable [55], possibly by forming essential components of the apoptosome complex [56, 57]. Several studies have indicated that an intact lamina can prevent or at least delay the execution of apoptosis, preventing chromatin condensation and fragmentation. For instance, a study by Rao et al. showed that an intact lamina, rendered uncleavable by caspases after mutating the VEVD/VEID lamin cleavage site, could delay the onset of apoptosis for 12 h [58]. In this way the lamina composition could prevent or delay apoptosis in certain tumors, depending on the amount of lamins present and the accessibility of lamins for caspases. Until now, however, no studies have confirmed an altered sensitivity of lamins to degradation in any cancer subtypes. Absence of appropriate (pro-)caspases could be a mechanism to prevent apoptosis indirectly. Indeed, a recent study showed that a caspase-3 gene product (caspase-3s) could counteract on caspase 3 activity, preventing a proper apoptosome assembly [57].

Lamin Mutants Promote the Execution of Apoptosis

From the literature it is not clear whether lamins play an active role in preventing or promoting apoptosis in cancer. As mentioned, an intact lamina is capable of preventing chromatin condensation and fragmentation. Yet, such an intact lamina does not warrant prevention from apoptosis. Lamin phosphorylation can cause depolymerization of the complete lamina network within minutes, as seen in vital imaging studies during mitosis [59, 60]. Also, in the apoptotic process the lamina becomes solubilized very rapidly, even before A-type lamin cleavage has been completed, as seen in CHO cells, transfected with lamin A [54]. In laminopathies, several studies speculate on the direct effects of A-type lamin mutations on the occurrence of apoptosis. Indeed, in cell cultures from laminopathy patients with different lamin mutations increased apoptosis can be found [61]. More pronounced effects could be achieved by exposing patient cells to mechanical strain that elicited an increase in apoptosis in these cells [62]. Yet, studies in laminopathy animal models resulted in conflicting results. Heterozygous *lmna*$^{+/-}$ mice subjected to 6 weeks of moderate or strenuous exercise training did not show induction of apoptosis and even seemed to protect these mice from developing symptoms reflective of laminopathy diseases [63]. In contrast, Lu et al. [64] found a dramatic increase in frequency of apoptosis in the heart of transgenic mice with a human LMNA E82K mutation. They showed that in the heart tissue of these mice both FAS and mitochondrial pathways of apoptosis were activated, leading to increased expression and activation of caspases 8, 9, and 3. Next to mutant A-type lamins (which are not or only rarely found in cancer as far as is currently known), unprocessed lamins, especially progerin, could be responsible for the induction of apoptosis. While a study by McClintock et al. [65] showed that progerin can be expressed by normal cells, and can be associated with the normal ageing process, a recent study, investigating A-type lamin expression

during closure of the ductus arteriosus revealed that progerin is expressed during this process, at which a prominent induction of cell death via apoptosis can be seen in this tissue [51].

How the presence of lamin mutations and/or unprocessed lamins can induce apoptosis is not yet clear. One route to apoptosis could be through mechanical weakening of cells with aberrant lamin expression. Lamin mutations lead to mechanical nuclear weakness [66, 67], which can lead to nuclear and cellular damage and even nuclear ruptures [68]. These events can lead to excessive chromatin damage, which in turn will lead to apoptosis. Indeed, several studies have shown the increase in DNA repair and increased apoptosis and senescence in cell cultures of laminopathy cells [61, 69, 70]. Even in normal cells, overexpression of normal lamin A leads to increased senescence and apoptosis [71]. In cancer, the impact of lamin overexpression may be different since a recent study showed that in prostate cancer cells, transfection of A-type lamins leading to overexpression causes enhanced growth, invasion, and migration by activation of the PI3K/AKT/PTEN pathway [72], while knockdown of lamins in the same cell culture has opposite effects. On the other hand, knockdown of lamin B1 leads to apoptosis rather than necrosis after induction of cell death in the mouse mammary tumor FM3A cell line [73]. In this respect, it is tempting to speculate about the mechanism by which statins, known to block, amongst others, farnesylation of A- and B-type lamins, have a beneficial effect in cancer treatment. Several studies showed that statins can induce apoptosis (e.g., see [74]), while also the more specific lamin-processing inhibitors (farnesyl transferase inhibitors, FTIs) can induce apoptosis in lymphomas [75]. Since these FTIs are also known to inhibit farnesylation of the RAS protein [76], a possible synergistic effect is evoked in these cancers.

Taken together, it can be stated that an active role of lamins in the induction but also the prevention of apoptosis is beginning to emerge, indicating the vital role of these proteins in cell survival. Clearly, the research on the role of lamins in cancer has only just begun.

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