Narrative review

Insights from laminopathies:
towards lamin-mediated mechanosignaling in cancer?

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Abstract
Lamins are intermediate filament proteins forming a meshwork underneath the inner nuclear membrane. A-type lamins are one of the two major lamin subtypes. Mutant A-type lamins cause laminopathies, a series of rare genetic diseases. A-type lamins have been shown to be up- or downregulated in different cancers. This review applies three major models explaining pathogenesis in laminopathies to tumorigenesis and metastasis: the “mechanical stress hypothesis” focusing on disrupted nuclear mechanics, the “gene expression hypothesis” referring to altered signaling pathways, and the “mechanosignaling hypothesis” combining the first two models. In contrast to cancer research on A-type lamins in nuclear mechanics, studies on lamin-mediated signaling and mechanosignaling in tumors are sparse. This review uses MAPK signaling and the mechanosensitive genes iex-1 and egr-1 to show how research in laminopathies may be a stepping stone for cancer studies in the field of lamin-associated mechanosignaling.

Introduction
Laminopathies are rare diseases due to lamin mutations and include various pathologies, among which muscular dystrophy, lipodystrophy, neuropathy, and progeroid syndromes (1). Lamins are the primary components of the nuclear lamina, a network of intermediate filament proteins underneath the inner nuclear membrane (2). Less abundant elements of the nuclear lamina comprise diverse nuclear envelope proteins (NET’s) that bind lamins (2). The inner nuclear membrane forms together with the outer nuclear membrane the nuclear envelope that separates the cell’s nucleus from the cytoplasm (3).

Small amounts of lamins are also present in the nucleoplasm. Lamin proteins are classified as either A-type or B-type depending on the genes that code for them (4). A-type lamins are alternative splicing products of the LMNA gene with the isoforms LA and LC being the most prominent ones (5). B-type lamins are encoded by two different genes, LMNB1 and LMNB2 (2). The expression pattern of A-type lamins differs significantly from that of the B-type. A-type lamins are prominent in differentiated cells (6), among which mainly stiff tissue. Conversely, B-type lamins are highly expressed in undifferentiated cells and soft tissue. Most laminopathies are caused by LMNA mutations in A-type
lamins within stiff tissues (1) since mutations in B-type lamins have been shown to be lethal during embryogenesis (7).

Two models have been proposed to explain cellular dysfunction in laminopathies due to lamin mutations (2). According to the “mechanical stress model”, A-type lamins provide nuclear stiffness and stability (8). LMNA mutations may cause reduced nuclear stiffness, decreased resistance to physical strain, and in some cases nuclear envelope rupture (9, 10). LMNA mutations in striated muscle laminopathies have for instance been correlated with less stiff and more fragile nuclei (11). However, the “mechanical stress model” does not apply to all laminopathies. LMNA mutations give rise to increased nuclear stiffness in skin fibroblasts of patients with the Hutchinson-Gilford Progeria Syndrome (HGPS) (12). Independently of the nuclear stiffness, HGPS fibroblast cells show decreased resistance to mechanical stress (12). The “mechanical model” does further not explain why the very same mutation in A-type lamins, present in almost all differentiated cells, leads to tissue-specific diseases (13). The “gene expression hypothesis” complements the mechanical model, suggesting that mutations in A-type lamins and lamin-binding proteins deregulate transcription factors in a tissue-specific way (2). One explanation for this deregulation involves altered signaling pathways due to mutations in lamins and lamin-binding proteins. Many signaling pathways affected in laminopathies, such Wnt or pRb signaling, play a role in cell growth, proliferation and differentiation (2), and are also commonly disturbed in cancer (14).

The structural and gene regulatory role of lamins can be interdependent as described by the concept of mechanosignaling (15). Upon mechanical stimulation, A-type lamins may help translate physical signals into biochemical ones, and thereby activate mechanosensitive signaling pathways (16). A-type lamins are ideal signal transmitters since they physically connect the cytoskeleton to the nucleus by interacting with LINC complex proteins (Link between the nucleoskeleton and cytoskeleton; (15)). LMNA mutations have been shown to impair mechanosensitive signaling such as the NF-κB (17) or Wnt pathway (18), both relevant to cancer.

While cancer is no phenotype of laminopathies (19), cancer cells commonly show nuclear dismorphology and reduced nuclear stiffness (20). Nuclear stiffness slows down metastasis since cancer cell migration through tissues requires nuclear and cellular deformation (21). As in laminopathies, decreased nuclear stiffness is not necessarily correlated with low lamin A expression in different malignant tumors and metastasis (22).

Reflecting on the “mechanical stress model”, this review will discuss lamin A expression in different types of cancer and metastasis. Another focus will be on selected signaling pathways that are deregulated in both cancer and laminopathies. Finally, research on lamin-mediated mechanosignaling in cancer will be explored to see if lamin A expression may be coupled to convergent signaling pathways in specific malignant tumors and stages of metastasis with different levels of physical stress.

Insights from laminopathies may be helpful for cancer treatment in order to target lamin-binding molecules within signaling cascades specific to different tumors and stages of metastasis.

2. Evidence on the structural function of A-type lamins in tumorgenesis and metastasis

Nuclear abnormalities are a common diagnostic marker for cancer, and more particularly for tumor stage (23). Nuclear deformability, enlarged nuclei, nuclear invaginations and protrusions are often observed in cancer cells (23, 24). Whereas various studies on laminopathies show a direct link between nuclear dismorphology and LMNA mutations (25), cancer research on lamin-dependent nuclear alterations exists, but is sparse. Capo-chichi et al. for instance found that in vitro suppression of lamin A/ C in human ovarian surface epithelial (HOSE) cells resulted in large and deformed nuclei (26). Helfand et al. showed that prostate cancer cells in culture and in patient tissues contained numerous nuclear lobulations enriched with lamin A/ C that predicted cell motility and tumor aggressiveness (27).

To apply the “mechanical stress model” of laminopathies to cancer, it would be important to relate lamin expression patterns in tumors extensively to nuclear dismorphology. Yet research in the field mainly focuses on the correlation between A-type lamin and nuclear stiffness with particular regard to cancer metastasis (28, 29). Even though cellular resistance to
physical stress involves nuclear stiffness, the "mechanical stress model" links cellular weakness to nuclear abnormalities in a broader sense (30). A comprehensive analysis of the interplay between A-type lamin and nuclear pleonasms could make lamins a more reliable tumor marker, distinguishing for instance between less invasive cancers and those prone to metastasis.

However, current research puts the usefulness of A-type lamin as cancer biomarker itself into question since there is no homogenous lamin expression pattern in different kinds of tumors, subtypes of cancer and metastasis. It is worthwhile to note that LMNA mutations do not play a significant role in cancerogenesis (5), but that this area has also hardly been researched.

2.1. Differences in lamin patterns due to heterogeneous research designs

Even among specific cancer subtypes differences in A-type lamin expression are observed, and can correlate with inconsistent disease prognoses. Willis et al. for instance found that colorectal cancer patients with high A-type lamin expression were twice as likely to die than patients without A-type lamins in tumor cells (31). The study attributed increased tumor invasiveness to lamin A-triggered downregulation of the cell-adhesion protein E-cadherin via enhanced transcription of T-plastin (31). Interestingly, lamin A and C were found to be differently expressed (31) in different areas of the colonic crypts (= glands of the colon). Whereas stem cells at the bottom of the crypts only showed lamin A expression, proliferating cells did not express any of the isoforms. In the differentiated cells on the top of the crypt, lamin A as well as lamin C were observed (31). Willis et al. hypothesized lamin A to be a stem cell biomarker for more aggressive colorectal tumors (31). This is in line with the "bottom-up" theory according to which cancer cells do not arise from mutations in differentiated but in stem cells (31). Keeping a stem cell-like pool allows cancer cells to self-renew and may promote metastasis (32).

Willis et al. used stage 1 to 4 colorectal adenocarcinomas from 656 patients of the "Netherlands Cohort Study on Diet and Cancer" (31). They further did not make any distinction between patients with and without chemotherapy exposure. This could explain the contradictory findings of Belt et al. who established a link between high colorectal cancer recurrence and reduced A-type lamin expression in 370 colorectal cancer patients (33). Importantly, this study only considered stage 2 and 3 colorectal adenocarcinomas (33). Also Belt et al. discovered for stage 3 adenocarcinomas a 100% disease recurrence for patients without chemotherapy exposure, compared to merely 37.8% for those having undergone chemotherapy (33). An earlier study of Moss et al. also found a relationship between decreased expression of A-type lamin and colon adenocarcinoma (34). However, Moss et al. merely analyzed 17 colon adenocarcinomas. Whereas immunohistochemistry did not stain for A-type lamins, immunoblotting revealed the presence of A-type lamins in cell lysates (34), which makes the results of the study little representative. These inconsistent findings also emphasize that immunohistochemistry is a qualitative approach (35) to detect the presence, but not the quantity of lamin proteins with subtle differences in staining intensity being possibly overseen. Contrary to the other two mentioned studies on colorectal cancer, Belt et al. only used immunohistochemistry, but not immunoblotting. It is also worthwhile to mention that Moss et al. used polyclonal antibodies, compared to Belt et al. and Willis et al. using monoclonal antibodies, which may influence the sensitivity for detecting A-type lamins. Despite their high specificity for a given lamin epitope (36), monoclonal antibodies may fail to detect A-type lamins due to epitope masking. Epitope masking in monoclonal antibodies has been shown to occur as a result of lamin phosphorylation during mitosis (37). Finally, all three studies used paraffin-embedded tissues for immunohistochemistry. Paraffin-embedding has been reported to have a lower sensitivity for antigen detection than frozen sections (38).
Fig. 1 – 3D-reconstruction displaying the nuclear membrane of normal mammary epithelial cells (left) and that of breast cancer cells (right). The breast cancer cells show deep invaginations. Image taken from Bussolati et al. (39)

2.2. Is the “mechanical stress model” applicable to metastasis?

Inconsistent results on lamin expression within the same tumor type challenge the idea of a homogeneous role of A-type lamins in metastasis. The “mechanical stress model” on itself seems contradictory for metastasis in that the protective nuclear stiffness of A-type lamins may benefit and hinder metastasis at the same time. Previous studies showed that A-type lamins correlated positively with nuclear stiffness (28, 40), and that over-expression of A-type lamins impeded cell migration due to this property (28, 41).

On the contrary, Denais et al. found that lamin A prevents nuclear envelope rupture in breast adenocarcinomas and fibrosarcomas during confined cell migration (21). They recorded with fluorescent labeling a decrease of green fluorescent protein-lamin A (GFP-lamin A) at sites of nuclear rupture, suggesting a protective function of lamin A against shear stress (21). Harada et al. confirmed the relevance of lamin-mediated nuclear stiffness for mechanical stress protection, but highlighted a more context-dependent role of nuclear stiffness in metastasis (28). They saw that knockdown of lamin A, and thus decreased nuclear stiffness, had different effects on 3D migration in cells with different ratios of A-to B-type lamins (glioblastoma, lung carcinoma and mesenchymal stem cells). In lung carcinoma cells with an intermediate ratio of A- to B-type lamins, a moderate knockdown of lamin A resulted in a moderate increase in three-dimensional (3D) migration through 3-μm pores. Importantly, 3D migration through 8-μm pores remained unaffected (28), which suggests that nuclear deformability is of greater importance in confined cell migration.

Harada et al. further found that 50% knockdown of lamin A in lung carcinoma cells lead to apoptosis due to mechanical stress, but that the number of migrated cells outweighed the number of apoptotic cells (28). They also found that even within a tumor lamin A levels differ between the tumor center (high lamin A levels) and periphery (low lamin A levels). The research of Harada et al. shows that the extent to which nuclear stiffness is beneficial for mechanical stress resistance or hinders migration depends on cell-type, migration environment and stage of metastasis. Even though Harada et al. rely on 3D cell migration simulating conditions of the living organism, they work in vitro with specific cell lines, as do the aforementioned studies on lamin-mediated nuclear stiffness and mechanical stress resistance. Their level of evidence is therefore low and extrapolation of results difficult.

2.3. Prostate cancer and the “mechanical stress model”

Tumor-specific research on lamins in metastasis is mostly conducted in vitro, and does not explore nuclear stiffness and mechanical stress resistance. Three studies on prostate cancer (PC) demonstrate that differences in research design may further affect predictions about A-type lamins’ metastatic potential.

Kong et al. conducted for three PC cell lines (LNCaP, PC3 and DU145) one transwell migration assay and one scratch migration assay, both with two-dimensional (2D) culture plates (42). Downregulation of lamin A/C slowed down wound healing in the scratch migration assay and delayed migration through the pores of a transwell membrane (42). Over-expression of lamin A/C had the opposite effect. Interestingly, migration through 8-μm pores remained unaffected (42).

These results differ from those of Harada et al. who found that knockdown of lamin A delayed 3D cell migration through 3-μm but not 8-μm pores (Harada et al., 2014). The use of different migration environments (2D by Kong et al.; 3D by Harada et al.) may account for these inconsistencies, as well as the analysis of different cell cultures (Harada et al. used glioblastoma, lung carcinoma and mesenchymal stem cells). Also, Harada et al. analyzed lamin A, whereas Kong et al. investigated both lamin A and C. Kong et al. further did quantify neither the initial amount of lamin A/C in the PC cells nor the amount after up- and downregulation of lamin A/C (42). The experimental design of Kong et al. thus lacks solidity. Research on PC cells by Helfand et al. nevertheless confirmed that A-type lamins speed up cell motility (27), as observed by Kong et al. (42). Helfand et al. compared the motility of a PC cell line (PC-3m) transfected with lamin A with a control using
microscopic live images (27). Just like Kong et al., they worked with a specific cell line, which makes the generalization of results difficult.

A third study by Saarinen et al. evaluated with immunohistochemistry the expression of A- and B-type lamins in cancerous and benign prostate tissue samples of 501 patients about to undergo surgery (43). The correlation between lamin expression and clinicopathological indicators was tested, as well as with biochemical recurrence (BCR) and disease specific survival (DSS). In contrast to the two other studies on prostate cancer (27, 42), this research had the advantage of following a cohort over a long period of time. Low lamin A expression was significantly correlated with lymph node metastasis for the whole cohort, and with poor DSS for high-grade tumors (43). Reduced lamin C levels were significantly correlated with poorer DSS. These findings suggest that a downregulation of lamin A may enhance the metastatic potential in prostate cancer. The research of Saarinen et al. therefore contradicts the results of the studies of Kong et al. and Helfand et al., both associating high expression of A-type lamins with increased cell migration. The studies on lamins in prostate cancer make comparisons of lamin-mediated metastasis difficult due to differences in research design. Further they do not exclusively focus on metastasis nor connect nuclear stiffness and mechanical stress resistance to A-type lamins. Current research does therefore not provide a solid basis to evaluate the “mechanical stress model” for metastasis.

2.4. Cancer research: the limits of the “mechanical stress model”

When reviewing research on the structural role of A-type lamins in cancer, it becomes apparent that studies generally do not relate lamin expression patterns to nuclear abnormalities. Given their aberrant expression throughout different (sub)types of cancer, A-type lamins themselves are inappropriate diagnostic/prognostic markers for tumors. Yet A-type lamins may become useful cancer biomarkers if connected to nuclear dismorphology, and especially with focus on nuclear envelope alterations.

The potential of lamin-dependent nuclear envelope abnormalities for tumor grading was explored by Bussolati et al. for breast cancer cell lines (39). With immunofluorescence staining of B-type lamin and emerin they showed a correlation between nuclear envelope invaginations and breast cancer cells (39). However, the relationship between nuclear envelope invaginations and breast cancer was not very pronounced. Of 273 breast cancer tissues in total, 135 samples showed few folds, whereas 138 samples showed marked nuclear envelope folds (39). Marked nuclear envelope invaginations correlated highly with lymph node metastasis potential (39). Yet the small sample size does not allow to consider this finding as a reliable grading parameter for breast cancer aggressiveness.

Lamin-dependent nuclear envelope dismorphology as a marker for prostate cancer was investigated by Helfand et al. Using immunostaining against A-type lamins, they found a positive correlation between nuclear lobulations and LDMDs (lamin B-deficient microdomains rich in A-type lamins) in cultures of prostate cancer cells (27). Helfand et al. found in clinical samples as well that increased LDMDs correlated with higher Gleason grades (Gleason grading is a rating system for prostate cancer malignancy), which would speak for A-type lamins as diagnostic markers. There is some inconsistency in this study in that Helfand et al. observed over-representation but decreased transcription of aggressive oncogenes when located within LDMDs nearby androgen-response elements (27). Helfand et al. did not elaborate if such decreased oncogene transcription had a rate-limiting effect on prostate cancer progression, but suggested that LDMDs may prevent tumor progression (27). This hypothesis would

Fig. 2 – Example of immunohistochemical staining for lamin A in prostate cancer. Image taken from Saarinen et al. (43)
be in contradiction with the positive correlation between LDMDs and Gleason grading.

Even when considering lamin-dependent nuclear alterations, research on A-type lamin expression in cancer does not fully explain mechanisms underlying tumorgenesis and metastasis.

Are up- or downregulated levels of A-type lamin cause or effect of cancer? The inability of current research to answer this question puts forward the limits of the "mechanical stress model" when applied to cancer. A shortcoming of studies on lamin expression in cancer is their focus on the absence or presence of A-type lamins in tumor and metastasis without considering lamins in relation to other nuclear envelope proteins. However, Capo-chichi et al. showed that levels of the inner nuclear membrane protein emerin can be regulated by lamin A in ovarian cancer (44). They detected with real-time RT-PCR that lamin A increased emerin levels in an ovarian cancer cell line (44). On the contrary, suppression of the transcription factor GATA6 by siRNA was correlated with the loss of emerin in cultured human ovarian surface epithelial (HOSE) cells. Emerin suppression, rather than lamin A loss, lead in most cell lines to nuclear dismorphology and polyploidy, two markers for ovarian cancer (44). This research does not only illustrate the importance of other nuclear envelope proteins for nuclear dismorphology, but also the interplay between A-type lamins and such proteins. Likewise, Matsumoto et al. revealed in vitro a common downregulation of A-type lamins and LINC complex proteins (SUN1, SUN2 and nesprin-2) in breast cancer cell lines (45). In contrast, not all patient samples exhibited a common downregulation of the four proteins. Although in minority, certain patient tissues showed reduced expression of SUN1, but not of SUN2 or A-type lamin. The opposite pattern was also observed (45). These findings contradict co-dependent downregulation of nuclear envelope proteins in breast cancer, but broaden the research perspective beyond A-type lamins. Matsumoto et al. speculated that LINC complex proteins and A-type lamins impact together nuclear stiffness and genomic stability (45).

Signaling pathways may play a central role in the understanding of how A-type lamins and other nuclear envelope proteins interact in cancer. A certain number of nuclear envelop proteins are not only binding partners of A-type lamin, but also key mediators in signaling pathways relevant to cancer.

3. Lamin-linked (mechano)signaling pathways in cancer

Most lamin-associated signaling pathways affect cell growth, proliferation and differentiation (2). Research in laminopathies found that lamin mutations up- or downregulate those pathways either directly or through interaction with other nuclear membrane proteins. The canonical Wnt pathway is for instance involved in cancer stemness, allowing for self-renewal in cancer cells. (46). The transcription of the proto-oncogene Wnt depends on the nuclear translocation of β-catenin (46). β-catenin itself can be repressed by the inner nuclear membrane protein emerin (47) that depends on A-type lamin for its localization in the nuclear envelope. A-type lamin also binds the nucleoplasmic protein LAP2α, which affects the transcription of the retinoblastoma protein (PRb), a tumor suppressor (48). Upon phosphorylation, PRb triggers cell cycle progression via the transcription factor E2F. The lamin-LAP2α complex has been shown to keep PRb hypo-phosphorylated, thereby inhibiting cell-cycle progression (48). There are quite a few other lamin-associated signaling pathways relevant to cancer, but their description would go beyond the scope of this review. Whereas research in laminopathies provides good evidence on lamin-associated signaling, no studies on this topic exist for specific tumors and metastasis. In the next section the MAPK pathway will be discussed as example of how studies in laminopathies could be useful for cancer research.
Fig. 2 – Signaling pathways involved in cancer that are affected by LMNA mutations. Image taken from Bell & Lammerding (5)
3.1. MAPK pathway: upregulation through mutations or deficiency of A-type lamins

The mitogen-activated protein kinase (MAPK) pathway regulates cell proliferation, differentiation, apoptosis, migration and inflammation (49), all potentially relevant to tumorgenesis and metastasis. The subsequent phosphorylation of three protein kinases transmits mitogenic and stress signals from the cell surface to the nucleus, where transcription factors are activated (49). The third protein kinase in the MAPK signaling cascade, the mitogen-activated kinases (MAPK), includes different subgroups (49). Among these subgroups, the mitogen-activated extracellular signal-regulated kinases 1 and 2 (ERK1/2), the stress-activated c-Jun amino-terminal kinases (JNKs) and p38 will be discussed regarding A-type lamins.

Research in laminopathies showed a link between A-type lamins and MAPK upregulation. Muchir et al. detected in cardiomyocytes of mice with LMNA H222P mutation an upregulation of ERK 1/2, JNK (50) and p38 (51). Similarly, in cardiomyocytes of emerin-deficient mice an increased ERK1/2 activation was observed (52). Muchir et al. used real-time RT-PCR to detect downstream genes of the MAPK pathway, and confirmed with immunoblotting over-expression of phosphorylated ERK1/2, JNK and p38 in the hearts of mice (50-52). These techniques used in mice, together with the fact that MAPK upregulation occurred prior to cardiac pathology, are a strong proof for lamin- and emerin-associated MAPK upregulation as disease mechanism. Muchir et al. also found a correlation between knockdown of A-type lamin or emerin, and MAPK overactivation (53). However, they investigated the knockdown of A-type lamins and emerin in cell cultures but not in mice (53). They further did not research a possible interplay between A-type lamins and emerin for MAPK signaling.

Evidence on such interaction was found in vitro by Emerson et al. who observed in fibroblasts of laminopathy patients a lower binding affinity between A-type lamins and emerin due to LMNA mutations (54). They showed with immunoprecipitation that LMNA mutant fibroblasts pulled down less emerin than control cells (54). Emerson et al. further detected in fibroblasts with LMNA mutations an increase in phosphorylated ERK 1/2 after completion of cell spreading (54), which infers MAPK upregulation. Enhanced adhesion onto collagen matrixes, cell proliferation, and cell migration were other key findings (54). Although the research of Emerson et al. was small-scale, it detected lamin-associated MAPK upregulation associated with cell spreading, which is an important step in cancer metastasis. Indeed, MAPK overactivation has been shown involved in colorectal and prostate cancer progression (55, 56). The research on lamin-triggered MAPK upregulation in laminopathies is a solid basis to be further applied to cancer. Further research should also explore the impact of A-type lamin deficiency on emerin in MAPK signaling, which has not been addressed to date by studies on laminopathies.

3.2. Lamin-mediated mechanosignaling as a comprehensive framework for signaling in tumorgenesis and metastasis

The MAPK pathway orchestrates versatile cellular functions such as apoptosis and cell proliferation. Additionally, it has been shown that JNKs may exert antiapoptotic as well as proapoptotic effects in prostate cancer (56). The specificity of signaling pathways therefore needs to be assessed for each type of cancer and tumor stage individually. This also holds for the interplay between different signaling pathways such as MAPK and NF-κB. NF-κB is a protein complex mediating important functions in cancer, among which inflammation, cell proliferation and cell survival (57). Upon extracellular (stress) stimulation NF-κB is activated via phosphorylation of its subunit I Kappa B (IkB). NF-κB then translocates from the cytoplasm into the nucleus where it initiates transcription (57). Research has shown that the MAP kinases JNK and p38 can activate NF-κB (56, 58). There are also cross-talks between NF-κB and other lamin-associated transcription factors such as β-Catenin or Notch (57).

Lamin-associated mechanosignaling may be a comprehensive approach to signaling in cancer, relating lamin-modulated response to mechanical stress to specifically activated signaling pathways in certain cancers and tumor stages. Research in laminopathies indeed provides evidence for lamin-dependent activation of mechanosensitive genes and signaling pathways relevant to cancer. Two such mechanosensitive genes are egr-1 and iex-1. Egr-1 has been shown to be an important tumor suppressor gene, and is inhibited in many cancers (59). Increased iex-1 expression has for example been reported in breast
In a mechanical strain experiment, Lammerding et al. detected an impaired transcription of egr-1 and iex-1 for mouse embryo fibroblasts depleted of lamin A/C (17). Although egr-1 is a target gene of NF-κB, Western Blotting did not reveal any impairment of NF-κB activation and nuclear translocation in lamin A/C deficient fibroblasts (17) upon mechanical or cytokine stimulation. Transcription factor binding of NF-κB was functional as well, but transcription itself decreased as assessed by a luciferase reporter assay (17). Lammerding et al. related the downregulated NF-κB transcription to increased apoptosis in lamin A/C deficient mouse embryo fibroblasts upon exertion of mechanical strain (17). DNA cell content was analyzed for apoptotic cells with flow cytometry (17). The study on lamin A/C deficient mouse embryo fibroblasts further revealed a linear increase of nuclear deformation with mechanical strain, and a disrupted nuclear envelope (17). In contrast, a follow-up study on emerin-deficient mouse embryo fibroblasts did neither detect a disrupted nuclear envelope nor nuclear deformation under mechanical strain (61). Nevertheless, mechanical stimulation resulted in a decreased expression of the mechanosensitive egr-1 and iex-1 genes, which was indicated by high cell apoptosis (61). NF-κB signaling was not found to be disrupted (61). Although Lammerding et al. speculated other signaling pathways to be involved in the impaired transcription of egr-1 and iex-1, they did not perform research on this possibility. Interestingly, Lammerding et al. showed that emerin-deficiency decreased the transcription of mechanosensitive genes without inducing nuclear fragility. It would be important to assess if A-types lamins and other nuclear membrane proteins may also affect mechanosignaling without disrupting nuclear mechanics. A particular focus for future research could lay on decreased nuclear stiffness and its implications for mechanosignaling.

In line with the "mechanical stress hypothesis", nuclear dismorphology may explain context-dependent functions of lamin A behind aberrant expression patterns. Harada et al. for instance showed that nuclear stiffness was influenced by cell-type, migration environment and stage of metastasis (28). The ratio of lamin A to lamin B further may explain how strongly A-type lamins correlate with nuclear stiffness (28). Current research on lamin expression in cancer however hardly refers to nuclear dismorphology.

The "gene expression hypothesis", with focus on lamin-associated signaling, may also give insight into how lamins affect different functional pathways across various types of cancer and in metastasis. Research in laminopathies has indeed shown that A-type lamins are involved in the up- and downregulation of numerous signaling pathways altered in cancer (2). The existing research on lamin-associated signaling further puts forward the interplay between lamin A and other
nuclear membrane proteins, such as emerin (54). The "gene expression hypothesis" hereby complements the nuclear mechanics model which considers the effect of lamin expression mainly isolated from other nuclear membrane proteins.

Yet almost no studies have addressed lamin-mediated signaling specifically for tumorgenesis and metastasis. This may be of interest for future research, especially since signaling pathways have various downstream effects, and can be interconnected as for example the MAPK and NF-κB pathways.

Lamin-mediated mechanosignaling in cancer may bridge the gap between the "mechanical stress hypothesis" and the "gene expression model" by linking nuclear dismorphology to the activation of specific signaling pathways at certain tumor stages. Research in laminopathies has revealed lamin-dependent transcription of mechanosensitive genes that are relevant to cancer. Studies on lamin-associated mechanosignaling in tumorgenesis and metastasis however do not exist. Future research should explore if the interplay between A-type lamins and nuclear membrane proteins other than emerin may have an impact on mechanosignaling in cancer. Another question be answered is whether defects in mechanosignaling are solely due to nuclear dismorphology or if A-type lamins alter mechanosignaling pathways in a broader sense.

Finally, future research should use more quantitative methods to gauge lamin expression levels (64). Existing studies heavily rely on qualitative immunohistochemistry in paraffin-embedded cell cultures, which may not detect subtle changes in lamin expression.

Mechanosignaling may provide context-dependent information for targeting A-type lamins or certain of its binding partners in signaling cascades that are crucial for specific cases of tumorgenesis and metastasis. Current cancer research is not yet directed towards lamin-associated mechanosignaling, but insights from laminopathies give a solid basis to do so.

**Literature**


