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# Nuclear Mechanics in Cancer

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## Abstract

Despite decades of research, cancer metastasis remains an incompletely understood process that is as complex as it is devastating. In recent years, there has been an increasing push to investigate the biomechanical aspects of tumorigenesis, complementing the research on genetic and biochemical changes. In contrast to the high genetic variability encountered in cancer cells, almost all metastatic cells are subject to the same physical constraints as they leave the primary tumor, invade surrounding tissues, transit through the circulatory system, and finally infiltrate new tissues. Advances in live cell imaging and other biophysical techniques, including measurements of subcellular mechanics, have yielded stunning new insights into the physics of cancer cells. While much of this research has been focused on the mechanics of the cytoskeleton and the cellular microenvironment, it is now emerging that the mechanical properties of the cell nucleus and its connection to the cytoskeleton may play a major role in cancer metastasis, as deformation of the large and stiff nucleus presents a substantial obstacle during the passage through the dense interstitial space and narrow capillaries. Here, we present an overview of the molecular components that govern the mechanical properties of the nucleus and we discuss how changes in nuclear structure and composition observed in many cancers can modulate nuclear mechanics and promote metastatic processes. Improved insights into this interplay between nuclear mechanics and metastatic progression may have powerful implications in cancer diagnostics and therapy and may reveal novel therapeutic targets for pharmacological inhibition of cancer cell invasion.

## Introduction

The cell nucleus was the first organelle discovered in the 17th century. In the oldest preserved depictions of the nucleus, Antonie van Leeuwenhoek described a central "clear area" in salmon blood cells that is now commonly acknowledged as the nucleus [1]. A more detailed description of the nucleus was subsequently provided by the botanist Robert Brown, who first articulated the concept of the nucleated cell as a structural unit in plants [1]. Today, the nucleus is recognized as the site of numerous essential functions in eukaryotes, including storage and organization of the genetic material, DNA synthesis, DNA transcription, transcriptional regulation, and RNA processing. In cancer biology, much of the research has traditionally been focused on this "DNA-centric view", starting with the identification of oncogenes and tumor-suppressor genes to the establishment of the multiple

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"hits" (*i.e.*, mutations) concept now commonly accepted as a requirement for cancer initiation and progression [2]. Recently, however, it has become apparent that in addition to these genetic components, it is necessary to take the physical, *i.e.*, biomechanical, factors of tumor cells and their microenvironment into consideration. Research conducted within the last 10 years has revealed that cancer cells have reduced stiffness [3–7], generate increased contractile forces [8], and are strongly influenced by their biomechanical environment [9,10]. Furthermore, not only can cancer cells be mechanically distinguished from non-tumorigenic cells, but physical measurements also allow telling apart highly invasive cells from less invasive cells, for example, by their increased cell deformability [4] and increased traction forces [8], yielding the promise of future diagnostic and prognostic applications. Here, we focus on a particular aspect of cellular mechanics that has traditionally received less attention in cancer cell biology: the role of nuclear structure and mechanics in cancer progression.

Despite many advances in understanding the biology of cancer and its associated molecular changes, the most common and reliable diagnosis of cancer cells in tissue biopsies by pathologists still relies on the presence of morphological changes in nuclear structure, *i.e.*, increased size, irregular shape and organization [11]. Nonetheless, the functional consequences of these characteristic changes have yet to be determined; thus, it remains unclear whether the observed morphological changes merely correlate with other, more difficult to observe cellular defects, or whether they can directly contribute to the disease progression.

In recent years, a growing number of studies have reported altered nuclear envelope composition in various cancers [12,13]. The structure and composition of the nucleus, particularly the nuclear envelope, play an important role in cellular mechanics and function, ranging from determining nuclear deformability and fragility [14–17] to participating in mechanotransduction signaling, *i.e.*, the sensing of biomechanical factors and the corresponding signaling response [18,15]. One potential mechanism by which changes in nuclear envelope composition could contribute to cancer progression is that softer and more lobulated nuclei facilitate cancer cell invasion through dense tissues, where cells often have to pass through constrictions smaller than the nuclear diameter [19,20]. Furthermore, the physical coupling between the nucleus and the cytoskeleton is critical for cytoskeletal organization and cell polarization [21–24], which could further affect cancer cell migration. In the following, we provide a brief review of normal nuclear structure and mechanics, highlight changes that occur during oncogenic transformation, and discuss recent findings suggesting an important role of nuclear mechanics and nucleo-cytoskeletal coupling in cancer progression.

## Normal nuclear compartmentalization and structure

The nucleus is a highly compartmentalized organelle that can be roughly subdivided into the nuclear envelope and the nuclear interior (Fig. 1), the latter representing most of the chromatin in diverse states of organization [25], the nucleolus, and diverse smaller subnuclear structures such as Cajal bodies and nuclear speckles [26–28]. In addition, the nuclear interior contains a still incompletely defined structural network (*i.e.*, the

nucleoskeleton or nuclear matrix), which may provide additional mechanical support and also act as scaffold for transcriptional complexes and other nuclear processes. The nuclear envelope forms the physical barrier between the nucleus and the cytoplasm. It consists of two phospholipid bilayers, the inner and the outer membranes, and the underlying nuclear lamina, a dense protein meshwork mostly comprised of lamins. The inner and outer nuclear membranes are connected at the sites of nuclear pore complexes (NPCs) and encapsulate the perinuclear space or lumen.

### The outer nuclear membrane

The outer nuclear membrane is continuous with the endoplasmic reticulum (ER); like the ER, its surface is scattered with ribosomes. The outer nuclear membrane exhibits a high degree of similarity to the ER membrane in terms of protein, enzyme and lipid composition [29]. Nonetheless, recent studies have suggested that the outer nuclear membrane displays a certain degree of specialization [30] and participates in protein synthesis and processing [31]. The specialized protein composition of the outer nuclear membrane likely results from retention of specific proteins by direct interaction with inner nuclear membrane proteins across the lumen, thereby enriching them compared to the ER fraction [32,33]. In mammals, one particularly important family of outer nuclear membrane proteins are the nesprins [34], which play a central role in connecting the nucleus to the cytoskeleton [35–39].

### The nuclear lumen and nuclear pore complexes

The nuclear lumen, also commonly termed the perinuclear space, is a 30 to 50 nm wide aqueous space separating the inner from the outer nuclear membrane that is continuous with the ER lumen [40]. It accommodates the luminal domains of integral nuclear membrane proteins [41]. The inner and outer nuclear membranes come together at sites of NPC insertion [42]. NPCs act as the main gateway for molecules between the cytoplasm and the nuclear interior (and also proteins of the inner nuclear membrane). Small molecules can diffuse freely through the NPC, while the exchange of macromolecules larger than ~40 kDa is mediated by a tightly controlled import and export mechanism requiring nuclear import and export signals and interaction with specific transport molecules [43–45].

### The inner nuclear membrane

The inner nuclear membrane contains at least 70 to 100 unique membrane-associated and integral membrane proteins that are retained at the inner nuclear membrane through interaction with nucleoplasmic proteins (*e.g.*, lamins) and chromatin [13]. Most of these proteins have only been identified in recent proteomic studies [46–50], and the function of several of the nuclear envelope transmembrane proteins remains unclear. Some well-characterized inner nuclear membrane proteins include lamin B receptor (LBR), lamina-associated polypeptides (LAPs) [30], emerin, MAN1, nurim, nesprins and Sad1p/UNC-84 (SUN) proteins [13]. Mislocalization or loss of these proteins due to mutations in nuclear envelope proteins causes a spectrum of diseases collectively known as laminopathies that include certain types of muscular dystrophies (*e.g.*, Emery-Dreifuss muscular dystrophy and limb-girdle muscular dystrophy), dilated cardiomyopathy, and the premature aging disease Hutchinson-Gilford progeria syndrome [51].

### The nuclear lamina

The lamina corresponds to a dense meshwork of proteins mainly composed of lamins underlying the inner nuclear membrane [52]. Lamins are type V intermediate filaments [53,54] and display the characteristic tripartite molecular organization of all intermediate filaments, which consists of a central α-helical rod domain flanked by a short non-helical N-terminal 'head' and a C-terminal 'tail' domain that includes an Ig-like fold [55].

In vertebrates, lamins are classified into two major classes, A-and B-type lamins, depending on their sequence, expression pattern, and biochemical properties [56,57]. A-type lamins, including lamins A, C, A 10 and C2, result from alternative splicing of the *LMNA* gene on chromosome 1. These proteins are expressed in a tissue-specific manner later in differentiation [58,59], have neutral isoelectric points, and are dispersed upon phosphorylation of lamins during mitosis [60]. Lamin A and C can be distinguished by their unique C-terminal tail and processing: the C-terminus of prelamin A contains a CaaX motif, which is subject to a series of post-translational modifications, including isoprenylation and proteolytic cleavage, to give rise to mature lamin A [61,62]. In contrast, the shorter lamin C has a unique C-terminus that lacks the CaaX motif and does not require post-translational processing. In addition to their localization at the nuclear lamina, A-type lamins are also present in the nuclear interior, where they form stable structures [63].

Unlike A-type lamins, B-type lamins are encoded by two separate genes: *LMNB1* for lamin B1 [64,65] and *LMNB2* for lamin B2 and B3 [66,67]. Only lamins B1 and B2 are found in somatic cells; expression of lamin B3 is restricted to germ cells. Unlike A-type lamins, at least one B-type lamin is expressed in all cells, including embryonic stem cells; B-type lamins are acidic and remain associated with membranes during mitosis [68]. The C-terminus of B-type lamins is also isoprenylated but, unlike prelamin A, does not undergo proteolytic cleavage. Consequently, B-type lamins remain permanently farnesylated, facilitating their attachment to the inner nuclear membrane.

### The nuclear interior

In addition to DNA and histones, the nucleoplasm contains distinct structural and functional elements such as nucleoli [69], Cajal bodies [70], the Gemini of coiled bodies or gems [71], promyelocytic leukemia (PML) bodies [72], and splicing speckles [73]. The growing interest to decipher the detailed structure and composition of the nuclear interior has led to the recent discoveries that the nuclear interior contains actin [74,75], myosin [76,77], spectrin [78] and even titin [79]. It is now well established that actin oligomers or short polymers are present in the nucleus [80–82] and that all isoforms of actin contain nuclear export sequences [83], which may help prevent spontaneous assembly of actin filaments inside the nucleus. To date, many aspects of nuclear actin remain incompletely understood, including its precise structural organization [84]. Nonetheless, nuclear actin has been implicated in a number of functions highly relevant to tumorigenesis, including DNA organization, stabilization, and orientation during replication, determination of nuclear morphology, organization of gene regulatory complexes, and RNA synthesis [85]. The existence and function of the "nuclear matrix" or nucleoskeleton, typically defined as the insoluble structure remaining after nuclease, detergent and high salt treatment of isolated nuclei [86], remains a matter of lively

debate, but given the plethora of structural proteins present in the nucleus and their often low diffusional mobility, it is likely that some (possibly local) structural frameworks exist in the nuclear interior.

## Nuclear mechanics and mechanotransduction

In recent years, it has emerged that physical factors, such as the biomechanical properties of the microenvironment and the mechanical forces acting between cells and their environment, play an important role in cellular function [87]. With regards to cancer cells, modulation of cytoskeletal tension by Rho inhibition alone can be sufficient to phenotypically revert epithelial morphogenesis of malignant cells [10]. Rho proteins belong to the family of small signaling G-proteins (GTPases) that can act as "molecular switches" in regulating actin cytoskeleton dynamics, while also playing important roles in cell polarity, migration vesicle trafficking, mitosis, proliferation and apoptosis [88]. Furthermore, recent studies found that aggressive cancer cells can be distinguished from less invasive and non-tumorigenic cancer cells based on their cytoskeletal stiffness [3] and their contractile force generation [8]. What is now becoming apparent is that in addition to cytoskeletal stiffness and force generation, nuclear deformability, as well as the physical coupling between the nucleus and the cytoskeleton, play a critical role in cell motility in three-dimensional (3-D) environments [20,19]. In this section, we discuss the molecular players governing normal nuclear mechanics, *i.e.*, nuclear deformability and nucleo-cytoskeletal coupling, as well as their potential contribution to cellular mechanosensing. Their involvement in cancer progression is then described in the subsequent section.

### Nuclear deformability and stability

Over the years, a variety of experimental techniques has been developed to probe the mechanical properties of the nucleus, particularly its deformability under applied forces. These approaches include micropipette aspiration [89–93], atomic force microscopy [91,94–96], cell stretching [14,97–99], tracking of particles within the nucleoplasm [100], and, most recently, optical stretching [101] and measuring transit times through microfluidic constriction channels [102,103]. These experiments have revealed that the nucleus exhibits both elastic (the nuclear lamina) and viscoelastic (the nuclear interior) behavior and is typically ~2–10 times stiffer than the surrounding cytoplasm [99,104,93,105]. The precise measurements for the apparent Young's modulus, a measure of material elasticity, range from ~0.1 to 10 kPa, depending on the experimental conditions and technique. This broad range of stiffness measures likely reflects a large degree of cell-to-cell variability, as well as different domains and mechanical behavior probed by the diverse experimental methods. For example, tracking of small particles within the nucleoplasm is sensitive to entanglement of the tracked particle within the nucleoskeleton/chromatin; in addition, the resulting measurements exclude contributions to nuclear stiffness from the nuclear envelope [90,91]. In contrast, cell stretch experiments and other techniques that result in large nuclear deformations will yield "bulk" measurements that combine contributions from the nuclear interior and the nuclear envelope, but may also depend on the mechanical properties of the cytoskeleton and its connection to the nucleus [17].

Micropipette aspiration experiments [90–92] and computational modeling [105] indicate that the mechanical deformability of the nucleus is mainly governed by the nuclear lamina and the nuclear interior; the relative contribution of each component depends on diverse factors such as mechanical load (*e.g.*, applied tension vs. compression), the specific cell type, differentiation state, and chromatin configuration. The contribution of the inner and outer nuclear membranes to the deformability of the nucleus is largely negligible [106], as lipid membranes exhibiting relatively low bending stiffness and a 2-dimensional (2-D) liquid-like behavior, *i.e.*, they can flow in response to applied shear stress, with connections to a large membrane reservoir in the form of the ER [106,16].

The importance of the nuclear lamina in providing structural support to the nucleus and controlling nuclear size is now well established [17,12], with the nuclear lamina acting as a load-bearing, elastic shell surrounding a viscoelastic nuclear interior [90,91,107]. Experiments on cells from gene-modified mice lacking specific lamin isoforms [98] and *Xenopus* oocytes ectopically expressing human lamins [95] suggest that lamins A and C are the main contributors to nuclear stiffness, with loss of lamin A or C resulting in softer, more deformable nuclei, while increased expression of lamin A results in stiffer, less deformable nuclei.. Given the structural similarities between A-type and B-type lamins, it may be somewhat surprising that these proteins have distinct roles in affecting nuclear deformability. However, recent findings suggest that A- and B-type lamins—and even lamins A and C—may form distinct but overlapping networks [108,109], and that A-type lamins may form a thicker protein network at the nuclear envelope [110]; however, as imaging the nuclear lamina in intact somatic cells with sufficiently high resolution remains technically extremely challenging, the exact structure and organization of the lamina and the different lamin isoforms at the nuclear envelope remains unclear. Interaction of specific lamin isoforms with other nuclear (envelope) proteins may serve as additional explanation for the distinct roles of the diverse lamins in nuclear mechanics. For example, loss of the inner nuclear membrane protein emerin, which directly interacts with lamins A/C, results in more deformable nuclei, although to a lesser degree than functional loss of lamins A/C [92,97]. In addition, functional loss of lamins due to mutations or (partial) deletion cam also affects chromatin organization [111–114], which could affect nuclear deformability.

Further illustrating the importance of A-type lamins in nuclear mechanics, lamin A/C-deficient cells have more deformable nuclei that are more susceptible to rupture under mechanical stress [115,14]. Of note, mutations in A-type lamins, as well as emerin, cause a spectrum of human diseases (laminopathies) that include Emery-Dreifuss muscular dystrophy, limb-girdle muscular dystrophy, dilated cardiomyopathy, Dunnigan-type familial partial lipodystrophy, and Hutchinson-Gilford Progeria syndrome [51]. In many cases, cells from affected patients show characteristic features such as misshapen nuclei, increased nuclear fragility, and herniations [16]; furthermore, *LMNA* mutations resulting in disease affecting cardiac and skeletal muscle often cause defects in nuclear mechanics [116], providing a potential disease mechanism for the muscular laminopathies.

Importantly, lamins also interact with other inner nuclear membrane proteins (*e.g.*, emerin, LAPs and LBR), nuclear pore components, DNA, chromatin and transcription factors (*e.g.* retinoblastoma protein [Rb], SREBPs, GCL and MOK2), and structural proteins such as

nuclear actin and titin [117]. These interactions could further modulate nuclear stiffness by forming nucleoskeletal structures or affecting chromatin organization and transcriptional regulation. For example, nuclear abnormalities have been observed in cells depleted of large repeat-domain proteins such as titin and αII-spectrin [118,119]. On the other hand, the role of nuclear actin in providing structural support to the nucleus remains unclear [84]. Through their interaction with SUN proteins, nesprins, and Samp1, lamins also play an important role in connecting the nucleus to the surrounding cytoskeleton [120], as discussed in more detail below.

Besides the nuclear lamina, chromatin is an important contributor to nuclear stiffness. Unlike the mostly elastic nuclear lamina, chromatin exhibits more viscoelastic material behavior, *i.e.*, it flows when subjected to forces (Fig. 2) and undergoes plastic deformations [107,106]. Chromatin decondensation during initial lineage commitment of embryonic stem cells is associated with a significant softening of the nucleus [101]. Subsequently, the viscoelastic deformability of the cell nucleus in human embryonic stem cells changes during further cellular differentiation [107], becoming 6-times stiffer and also less fluid-like during terminal differentiation. It remains unclear, however, to what extent this behavior is caused by changes in chromatin organization, *e.g.*, switching from loose euchromatin to more compacted heterochromatin, or results from the increased expression of A-type lamins in differentiated cells.

## Nucleo-cytoskeletal coupling

Over the last 10 years, it has become well established that the nucleus is physically coupled to the surrounding cytoskeleton [120]. Many of the molecular components are highly preserved throughout evolution, being present in unicellular organisms such as yeast all the way to mice and humans [121]. Building on work in yeast and drosophila, several of the molecular details of nucleo-cytoskeletal coupling were first unraveled in *C. elegans*, where UNC84 and ANC-1, in conjunction with Ce-lamin, participate in the actin-dependent anchorage and positioning of the nucleus [122–124,32,125]. Subsequent studies have confirmed that closely related proteins are also responsible for nucleo-cytoskeletal coupling in mammalian cells; this physical connection is now commonly referred to as the Linker of Nucleoskeleton and Cytoskeleton (LINC) complex [126]. In the strictest definition, the LINC complex contains two essential parts: (*i*) a member of the trimeric inner nuclear membrane SUN- [127] domain protein family, which engages with nucleoplasmic proteins such as lamins [128,129,121]; (*ii*), KASH- (Klarsicht, ANC-1, Syne Homology) domain containing nesprins located on the outer nuclear membrane that bind across the perinuclear space to the SUN domain of Sun1/Sun2 trimers [130]. The cytoplasmic ends of nesprins interact directly or indirectly with various components of the cytoskeleton, including actin, intermediate filaments (via plectin)[131], and microtubules (via microtubule-binding motors such as dynein and kinesin), thereby completing the physical connection across the nuclear envelope [121]. In many cases, lamins are considered an extended part of the LINC complex, as they bind to SUN proteins and inner membrane variants of nesprins and help tether these proteins to the nuclear interior [132]. Since the cytoskeleton also connects to focal adhesion and cell-cell junctions, cells contain a continuous mechanical network linking the nuclear interior and the extracellular matrix and neighboring cells, thereby allowing

forces exerted from the cellular environment or the cytoskeleton to be transmitted directly to the nuclear interior [133,120,134,39].

**A. SUN domain proteins**—The characteristic feature of SUN domain family proteins is a 115–175 amino acid domain that shares homology with the Sad1 protein from *S. pombe* [135] and the UNC84 protein from *C. elegans* [122]. Mammalian cells have five SUN domain proteins, with two of these proteins (SUN1 and SUN2) present on the nuclear envelope in somatic cells (SUN3–5 are testis specific) [136]. SUN1 and SUN2 proteins consist of a helical N-terminal domain that can bind to lamins [137] and nuclear pore complex proteins [138,139], a single pass transmembrane domain to anchor the protein in the inner nuclear membrane [140], a luminal helical domain required for trimerization of SUN proteins [130], and the C-terminal SUN domain, which interacts with the KASH domain of nesprins [126].

**B. Nesprins and other KASH domain proteins**—Mammals have four nesprins (genes *SYNE 1–4*), with nesprins 1–3 having multiple isoforms resulting from alternative splicing, initiation, and termination [121,120,34]. Expression of various nesprin isoforms can be highly tissue-specific [34]. In skeletal muscle, levels of nesprin-1 (first described as Syne-1 for synaptic nuclear envelope protein-1) are highest in synaptic nuclei, suggesting that it might participate in the migration and anchoring of these specialized muscle nuclei [141]. Common to all nesprins is a central region containing multiple spectrin domains, whose number can greatly vary between isoforms [142]; all nesprins (but not all isoforms) contain a ~60 amino acid-long C-terminal KASH domain, consisting of a transmembrane domain and a short, highly conserved luminal domain, which is essential for anchoring nesprins to the nuclear envelop [59,142]. The N-terminal domain of nesprins typically contains specific motifs to interact with different cytoskeletal proteins. For instance, the nesprin-1 and -2 "giant" isoforms (1000 and 800 kDa in size, respectively) contain an actin-binding domain (ABD) composed of two calponin homology domains [143,37,35]; additionally, nesprins-1 and -2 can interact with the microtubule-associated motors dynein/dynactin and kinesin [120]. Nesprin-3 can connect to intermediate filaments via plectin [36]. Nesprin-4 binds the microtubule-associated motor kinesin, and ectopic expression of nesprin-4 induces dramatic changes in centrosome positioning in cells [144]. While localization of larger nesprin isoforms is restricted to the outer nuclear membrane, shorter isoforms can also be present at the inner nuclear membrane, where they can interact with lamins and emerin [145–147,38]. Nesprin isoforms lacking the KASH domain may also be found in other cellular structures. In addition to nesprins 1–4, mammals express at least one additional KASH-domain protein, aptly named KASH5, which is found exclusively in spermatocytes and oocytes, where it plays a critical role in meiosis [148].

**C. Other molecules involved in nucleo-cytoskeletal coupling**—With the growing interest in understanding the mechanics of the nucleus and its connection to the cytoskeleton, several recent studies have focused on identifying additional molecular players involved in nucleo-cytoskeletal coupling. Based on experimental findings in emerin-deficient cells, one study has proposed that emerin binds to microtubules and that a subset of emerin located on the outer nuclear membrane is involved in coupling the centrosome to the

nuclear envelope [149], but it remains unclear whether the emerin-microtubule interaction is direct or mediated through other proteins such as nesprins.

A more recent candidate to be involved in nucleo-cytoskeletal coupling is the inner nuclear membrane protein Samp1 [150], which associates with lamin A/C, emerin, Sun1, and Sun2 [150–152]. During mitosis, Samp1 is associated with the mitotic spindle [150]; during interphase, however, Samp1 is an important component of transmembrane actin-associated nuclear (TAN) lines [152], which promote rearward nuclear movement in polarizing fibroblasts by connecting the nucleus to retrograde actin flow via nesprin-2giant and SUN2 [153]. The involvement of lamins A/C in nucleo-cytoskeletal coupling is further illustrated by the finding that lamin mutants associated with muscular dystrophies can disrupt this retrograde nuclear movement [132] and that lamin A/C is required for retaining Samp1 at the nuclear envelope [152]. Another potential mediator of nucleo-cytoskeletal coupling is the luminal protein torsinA, part of the AAA+ ATPase superfamily. TorsinA interacts with the KASH domains of nesprins 1–3, and loss of torsinA results in mislocalization of nesprin-3 from the nuclear envelope and impaired cell polarization and migration [131]. Given the promiscuous interaction of SUN domain proteins and nesprins [154], it is likely that tissue-specific expression of their isoforms, as well as potential interaction with other nuclear envelope proteins such as Samp1, play an important role in the spatial and temporal control of nucleo-cytoskeletal coupling.

**D. Nucleo-cytoskeletal coupling is critical for many cell functions—**Studies investigating molecules involved in connecting chromatin and cytoskeletal structures have often focused on processes during mitosis and meiosis. For instance, analysis of chromosome condensation during yeast prophase has unraveled a direct interaction between Sad1 (a Sun homologue protein) and meiotic-specific bouquet (Bqt) proteins [155]. Sad1 has also been linked to Kms1 protein [156] and this interaction is known to couple telomeres to microtubules and cytoplasmic dynein [157,158]. Similar results were obtained in C. elegans, where selective inactivation of Sun1 protein or Kdp-1 (KASH domain protein-1) protein delays cell cycle progression [159,160]. In mammalian cells, lamins, SUN proteins, KASH5 and Samp1 have all been implicated in specific roles during mitosis and/or meiosis, [161,148], and loss of A-type lamins causes telomere shortening defects and overall genomic instability [162].

In recent years, research has increasingly focused on the role of LINC complex proteins in interphase cells and consequences of LINC complex disruption. In C. *elegans*, deletion of the nesprin and SUN1 orthologues ANC-1 and UNC-84 result in impaired nuclear positioning and anchoring in muscle cells [122,32]. In mammalian cells, LINC complex disruption causes defects in nuclear positioning, cell polarization, and migration [133] by impairing force transmission between the nucleus and cytoskeleton [24,153]. LINC complex proteins are particularly important during cell migration in 3-D environments, for example, inside collagen matrices or tissues. In particular, lamins A/C, nesprin-2giant, and nesprin3 modulate perinuclear actin organization and actin protrusions; consequently, deletion of lamins A/C or LINC complex disruption results in significantly impaired migration of cells in 3-D collagen matrices [163]. The implications of impaired nucleo-cytoskeletal coupling in cancer progression are discussed in more detail below.

### Nuclear mechanics stiffness and nucleo-cytoskeletal coupling in mechanotransduction

As described above, the cytoskeleton physically connects the nucleus to the cellular microenvironment. Consequently, pulling on integrins on the surface of intact endothelial cells results not only in reorientation of cytoskeletal filaments, but also in distortion of the nucleus and spatial redistribution of subnuclear structures [134]. Similar results, including force-induced dissociation of nuclear protein complexes, were recently obtained in HeLa cells subjected to forces applied via magnetic tweezers [164] and in human umbilical vein endothelial and osteosarcoma cells exposed to fluid shear stress [165]. It has long been speculated that such mechanically induced changes in nuclear structure and chromatin configuration could directly activate specific mechanosensitive genes, for example, by changing accessibility to transcription factors [166,18]. This idea is further supported by studies that have found interactions between applied forces, Rho signaling, cell shape, and histone acetylation [167–169]. Nonetheless, direct evidence for such nuclear mechanosensing remains scarce, and the majority of data are rather correlative, making it difficult to discern whether mechanical forces acting on the nucleus are sufficient to directly induce changes in gene regulation, or whether the observed activation of mechanosensitive genes is the downstream result of signaling cascades originating in the cytoskeleton or the plasma membrane [15]. A recent study [24] addressing this question found that LINC complex disruption had no discernible effect on the mechanically-induced expression of the mechanosensitive genes *Iex-1* and *Egr-1*, whose activation is impaired in lamin A/C-deficient cells [14,170], even though LINC complex disruption resulted in substantially reduced nuclear deformation when the fibroblasts were subjected to substrate strain [24].

At the same time, changes in nuclear envelope composition undoubtedly affect cellular structure and function. For example, LINC complex disruption alters the mechanically induced proliferation of C2C12 myoblasts [171]; LINC complex depletion also causes impaired propagation of intracellular forces and disturbed organization of the perinuclear actin and intermediate filament networks, leading to defects in nuclear positioning and cell orientation [24,171,22]. In the case of impaired expression of mechanosensitive genes in lamin A/C- and emerin-deficient cells, it remains unclear whether this effect is due to direct mechanical defects or a consequence of altered interaction of lamins with specific transcriptional factors. An additional mechanism by which lamins and emerin can affect mechanotransduction signaling was recently identified, revealing that the actin polymerization-promoting activity of emerin at the nuclear envelope can influence nuclear and cytoskeletal actin dynamics, thereby modulating localization and activity of the mechanosensitive transcription factor MKL1 (also known as MRTF-A or MAL), whose localization is dependent on interaction with monomeric G-actin [172].

## Relevance of nuclear mechanics and mechanotransduction in cancer progression

With growing advances in the understanding of the physics of cell motility, the mechanical properties of cancer cells have become an increasing area of interest [3]. As the nucleus is typically the largest and stiffest organelle, often occupying a large fraction of the cell's volume, the properties of the nucleus can dominate the overall cellular mechanical response

when cells are subjected to large deformations [17]. Several lines of evidence suggest that the ability of the nucleus to deform can impose a rate-limiting step in non-proteolytic cell migration in 3-D environments, when cells attempt to squeeze through narrow constrictions imposed by extracellular matrix fibers and other cells (Fig. 3) [20,19]. In this section, we summarize changes in nuclear structure and morphology observed in various cancers and describe the role of nuclear deformability in cell motility. In addition, we discuss the intricate feedback between the mechanics of the cellular microenvironment and intracellular organization and function.

## Altered nuclear structure and morphology in cancer cells

With few exceptions, the nuclei of normal cells have an ellipsoid shape with smooth outlines; in contrast, many cancer cells are easily identifiable by increased nuclear size, irregular nuclear contours, and disturbed chromatin distribution, making nuclear morphology one of the oldest and most commonly used cancer markers [11]. The irregular nuclear outline in cancer cells is mainly the result of grooving, convolutions and invaginations of the nuclear envelope [173]. While the characteristic changes in nuclear morphology in cancer cells are well documented, their cause and consequence remain unclear. Interestingly, the irregular nuclear morphology of cancer cells often bears striking resemblance to the abnormal nuclear shapes observed in cells lacking or expressing mutant nuclear envelope proteins such as lamins A/C, lamin B1/B2, or LBR [174,175], suggesting a possible involvement of dysregulated nuclear envelope proteins [173,176].

This idea is supported by a growing number of publications that report altered expression of lamins in a variety of human tumors, often associated with particularly malignant phenotypes (Table 1). Interestingly, while some cancers frequently show downregulation of lamin A/C [177–179], other cancers have upregulated levels of lamins A/C [177,180,181], and for some cancers, such as colon cancer, both increased [182] and decreased [183] levels of lamin A/C have been reported. Furthermore, even within single tumors and individual cancer cell lines [184], highly heterogeneous expression levels of lamin A/C can be found [185]. Similarly, both high and low levels of lamins A/C have been considered poor prognostic markers for cancer patients, depending on the specific study and cancer subtype. For example, reduced lamin A/C expression is a sign of poor prognosis for patients with gastric carcinoma [186], and patients with stage II and III colon cancer have a significantly increased risk of cancer recurrence when their tumors are marked by loss of lamin A/C expression [183]. At the same time, another study found that patients with increased expression of lamins A/C in colorectal cancer tumors were almost twice as likely to die of the disease than patients with tumors negative for lamin A/C [187], possibly by lamin A/C promoting cell motility [188]. These apparently inconsistent findings point at the multiple roles lamin can play in cancer progression, which will be discussed in more detail below.

In addition to lamins, other nuclear (envelope) proteins have recently been implicated in a variety of cancers. A genome-wide scan in several patients with either breast, colorectal or ovarian cancer revealed genetic alterations in nesprin-1 [189], and another genome-wide study identified mutations in nesprin-1, -2 and lamin A/C in a panel of 100 breast cancer patients [190]. Furthermore, downregulation and mutations in nesprin have been associated

with an increased risk of invasive ovarian cancer [191]. Lastly, several "nuclear matrix" or nucleoskeletal-associated proteins such as NuMA or nucleoporin proteins (NUP 88, NUP 98) have been correlated with aggressive tumor phenotypes [192] and used as prognostic markers of disease [193].

### Implications of altered nuclear envelope composition in cancer

What is the impact of altered nuclear envelope composition on nuclear mechanics? As lamin expression and chromatin organization determine nuclear deformability, it is expected that changes in nuclear architecture will alter the rigidity of the nucleus. In cancer, increased nuclear deformability may benefit metastatic cells that need to pass through narrow interstitial spaces or small capillaries, while defects in nucleo-cytoskeletal coupling may impair migration in 3-D tissues [20]. In addition to these mechanical functions, the nuclear envelope and nuclear interior play important roles in the processing of genetic information [194–196]. Thus, changes in nuclear organization could have consequences on gene expression or DNA stability with important implications in cancer progression.

**A. Nuclear deformability and cell motility—**The abnormal nuclear shapes observed in cancer cells and their resemblance to lamin-deficient or mutant cells, combined with the increasing reports of altered expression of nuclear envelope proteins in various cancers (Table 1), suggests that cancer cells may have altered nuclear mechanics. While direct measurements of nuclear deformability in cancer cells have not yet been reported, studies that measure whole-cell deformability consistently find that cancer cells, particularly highly invasive ones, have increased cellular deformability [4,3,7]. Why should (nuclear) deformability matter in cancer progression? During the metastatic process, cancer cells must undergo modifications and large elastic deformations to invade the tissue surrounding the primary tumor, intravasate blood vessels, survive the physical stresses during circulation in the blood stream, extravasate at new sites in the body, and eventually proliferate in a nutrient-deprived microenvironment [197]. Particularly during invasion and intra- and extravasation, cells penetrate through interstitial spaces and openings ranging in size from 2 to 30 μm [198,199]. Cytoskeletal shape is highly adaptive, owing to the rapid cytoskeletal remodeling and plasma membrane flexibility; consequently, cytoskeletal protrusions can invade spaces of less than 1 $\mu m^2$ in cross-section [200,201]. In contrast, the ability of the nucleus to pass through narrow constrictions is more limited due to its size and stiffness. Transient nuclear deformations, resulting in hourglass- and cigar-shaped nuclei, as well as nuclear protrusions indicative of attempts to pass through narrow constrictions, can be observed (at least transiently) during cancer cell migration *in vivo* [20]. Importantly, a recent report by Friedl, Wolf and colleagues [19] found that deformation of the nucleus poses a rate-limiting step during proteolysis-independent cell migration. They found that in the absence of proteolysis, *e.g.*, during matrix metalloprotease (MMP) inhibition or knockdown, migration of cancer cells through 3-D collagen matrices and polycarbonate filters is limited by the available pore size: cell migration speed and migration efficiency gradually drops with decreasing cross-sectional areas of the constrictions until cell body movement is completely stalled [19]. A similar size-dependent effect was observed by Tong and colleagues [202] when studying cell migration in microchannels with varying width. Indeed, decreasing channel width below 20 μm (at a fixed channel height of 10 μm) resulted in

increasing reduction in migration speed. At the extreme, cells in 3 μm-wide channels had a 70% reduction in migration speed compared to 50 and 20 μm-wide channels. Interestingly, the minimum size requirement for (non-proteolytic) migration through 3-D environments was found to be independent of the shape of the constriction and only depends on the available cross-sectional area [19].

While these studies illustrate the importance of nuclear deformability in cell migration in confined environments, the role of the nuclear lamina and nuclear stiffness in this process remains to be explored [20]. At least in neutrophil-like cells, which normally have extremely low levels of lamins A/C and which can migrate through constrictions only a few micrometers in diameter, overexpression of lamin A results in less deformable nuclei that have reduced efficiency at crossing narrow constrictions and that take significantly longer to transit narrow microfluidic channels mimicking capillaries [103]. Similarly, fibroblasts expressing a mutant form of lamin A (progerin) that is responsible for Hutchinson-Gilford progeria syndrome have difficulties migrating through an array of microfabricated pillars spaced 6 μm apart [203], likely due to the increased nuclear stiffness caused by progerin [204,205], as migration on non-constricted surfaces was comparable to cells from healthy controls [203]. Although these findings suggest an important role of lamins A/C in moderating the ability of cells to pass through narrow constrictions, Wolf and colleagues [19] found that the maximal deformation the nucleus could achieve during passage through narrow constrictions, indicated as the ratio of the nuclear cross-section in the constriction to the undeformed nuclear cross-section, was consistently around 1:10, regardless of the cell type studied. These findings suggest that the size limit for nuclear passage through small constriction may be governed by the maximal compressibility of the nucleus. The theoretically maximal compression depends on the solid fraction of the nucleus, as the chromatin (and other nucleoplasmic proteins) can be no further compressed once all void spaces have been eliminated. This idea is consistent with the observed reduction in nuclear volume by up to 60% during migration of skin fibroblasts through microfabricated constrictions [203] and with micropipette aspiration experiments that revealed that the nuclear volume can be compacted to about 20 to 40% of its original size before reaching a state that resists further compression [92,106].

But what about cancers in which increased, rather than decreased, levels of lamin A/C have been reported, which is expected to result in reduced nuclear deformability [98]? Cancer cells are highly plastic and heterogeneous in their gene expression, so it is likely that different subpopulations of cells with distinct roles in cancer progression exist. Increased lamin levels could help protect cells from mechanical stress caused by the high hydrostatic pressure inside solid tumors. At the same time, lamins are also involved in multiple signaling pathways [117,51], which could modulate functions relevant to cancer progression. For example, increased levels of lamin A/C in prostate cancer cause changes in the PI3K/AKT/PTEN pathway [206], and upregulation of lamin A/C in colorectal cancer induces changes in cytoskeletal organization that promote cell motility [188]. As such, it is likely that different cells and tumors have found different approaches to find the best compromise between increasing nuclear deformability and activation of signaling pathways to increase cell motility and invasiveness.

**B. Nuclear rupture of cancer cells—**As described earlier, the nuclear envelope forms a well-defined compartment that acts as a protective shield for the genetic material. In normal cells, nuclear envelope breakdown and reassembly is limited to mitosis and precisely regulated [207]. Recently, Vargas *et al*. [208] reported that in many cancer cells, the nuclear envelope transiently ruptures and then reseals during interphase, resulting in temporary exchange between the nucleus and cytoplasm and the occasional entrapment of cytoplasmic organelles inside the nucleus. Nuclear envelope rupture was associated with the formation of micronuclei, portions of chromatin exiting the nuclear interior, and mislocalization of nucleoplasmic/cytoplasmic proteins. Importantly, the frequency of nuclear rupture events was increased in cells with small defects in the nuclear lamina [208]. These results are consistent with previous reports of increased nuclear fragility in lamin A/C-deficient mouse embryonic fibroblasts [14] and spontaneous (transient) nuclear rupture in these cells [209]. In our laboratory, we have frequently observed that cancer cells undergo transient nuclear rupture while migrating through narrow ($\sim$2 μm $\times$ 5 μm) microfluidic constrictions, with lamin-deficient cells displaying significantly increased rates of nuclear rupture (unpublished observations). Breakdown of the nuclear compartment during repetitive nuclear rupture could potentially result in increased genomic instability and chromatin rearrangements, which could further contribute to cancer progression, but this idea has not yet been experimentally tested.

**C. Changes in chromatin organization in cancer cells—**Epigenetic changes in chromatin configuration can directly impact nuclear stiffness. Therefore, the chromatin modifications frequently observed in cancer cells, including disturbed heterochromatin organization [11], could be associated with altered nuclear deformability and thereby affect 3-D cell migration, in addition to their role in transcriptional activity. Importantly, there is a strong interplay between nuclear envelope proteins and chromatin organization. Lamin A regulates dynamics of heterochromatin proteins in early embryonic stem cells [25]; lamins A/C-deficiency and mutations in the *LMNA* gene result in loss of heterochromatin [111,210]. Furthermore, lamins and lamin B receptor (LBR) play an important role in tethering specific chromatin regions to the nuclear periphery [211,212], which typically serves as a transcriptionally repressive environment [195]. LBR also interacts with heterochromatin protein 1 [213] and histones H3/H4 [213]. Lamin-associated polypeptide-2β (LAP2β) can modulate gene expression by regulating higher order chromatin structure or binding the transcriptional repressors germ cell less (GCL) [214] and histone deacetylase 3 [215], resulting in deacetylation of histone H4 [215]. Emerin can directly associate with chromatin modifiers and transcriptional repressors such as the death promoting factor Btf [216], the splicing associated factor YT521-B [217] and the transcriptional repressor GCL [218]. Given these findings, it is tempting to speculate that the altered expression of nuclear envelope proteins found in various cancers (Table 1) can directly affect chromatin organization and gene expression. Of course, the observed changes in expression of nuclear envelope proteins could also be the consequence, rather than the cause of altered chromatin organization. In this case, the changes in nuclear envelope composition could still result in further modifications of nuclear structure and organization while also directly altering nuclear mechanics.

## Conclusion and future perspectives

The field of cancer cell biology has dramatically changed since 1943, when George Papanicolaou published his book *Diagnosis of Uterine Cancer by the Vaginal Smear*, which laid the basis for the now abundant "pap smear" to detect early signs of cervical cancer. Since then, researchers and clinicians have learned not only to identify and assess cancer cells based on characteristic morphological changes, but also to peek inside the inner life of cancer cells, including their genetic changes, biochemical composition, and metabolic state. In recent years, these approaches have been complemented by a new research direction, focused on the biophysical changes in cancer cells and their microenvironment. This research has already led to striking discoveries, including the role of the extracellular matrix stiffness, composition and topology in cancer progression [219] and the characteristic difference in cell deformability of cancer cells, which may lead to new diagnostic and prognostic applications [3]. Motivated by research in other diseases (laminopathies), it is now emerging that the mechanical properties of the cell nucleus, particularly its deformability and connection to the cytoskeleton, may play a similarly important role in cancer metastasis. The idea that deformation of the large and stiff nucleus presents a rate-limiting factor during the passage of metastatic cancer cells through tight interstitial spaces or narrow capillaries has recently found increasing experimental support [19,103,165]. Given the increasing reports of altered expression and mutations in nuclear envelope proteins responsible for determining nuclear stiffness, it is intriguing to speculate that (a subset of) cancer cells may have acquired specific adaptations in their nuclear structure and mechanics to promote metastatic spreading. Nonetheless, experimental verification of this idea is still lacking. Additional experiments, using sophisticated combinations of live cell imaging and measurements of subcellular mechanics, including primary tumor (and metastatic) cells from cancer patients and complemented by *in vivo* studies in mouse models, will be required to firmly establish this hypothesis. These experiments will also have to address why some cancers frequently have increased lamin levels while others have decreased or unchanged levels, and whether such changes in nuclear envelope composition can serve as reliable prognostic markers. Given the diverse functions of lamins, it is likely that (varying) combinations of altered cellular mechanics, cell signaling, and stem cell differentiation contribute to the increasingly emerging role of lamins in cancer progression. Done correctly, such experiments have the potential to not only address these key questions but to also produce novel insights into the dynamic nature of cancer cells, which may switch between different morphological and mechanical modes depending on their current role in cancer progression. Novel technology developments to probe single cell mechanics at substantial higher throughput than traditional methods [102,5,220,221] will enable detection of rare cell subpopulations, which could play a crucial role in cancer progression. Identifying key (mechanical) parameters that govern cancer cell metastasis may reveal novel therapeutic targets for pharmacological inhibition.

These clinical translation-driven experiments should be complemented by research to address some of the more fundamental questions in cancer cell biology, including the molecular mechanisms by which cells manage to squeeze the nucleus through constrictions only one tenth the diameter of the nucleus in size, and whether induced nuclear deformations

can directly contribute to cellular mechanosensing. We are only at the beginning of a long road ahead, the destination a complete understanding of the physics of cancer progression und the underlying biology, but it will be exciting to see what is awaiting us around the next corner.

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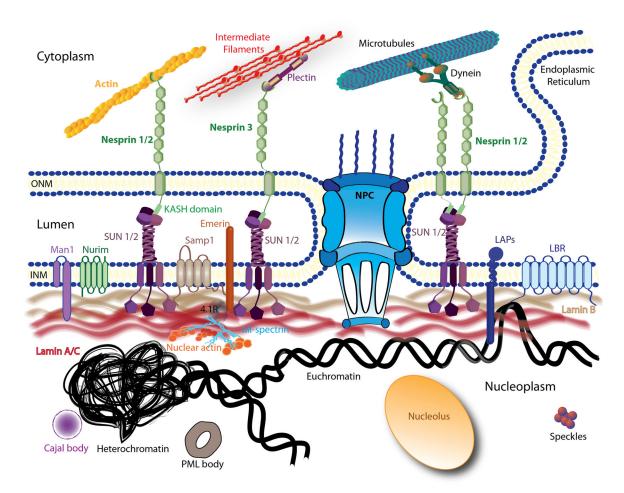
**Figure 1. Schematic overview of the nuclear structure and the LINC complex**
The nuclear envelope is composed of the inner nuclear membrane (INM) and the outer nuclear membrane (ONM) punctuated by nuclear pore complexes (NPC). The ONM is continuous with the endoplasmic reticulum (ER). Several structures of the nuclear interior are depicted here, including the nucleolus, Cajal bodies, promyelocytic leukemia bodies (PML) and speckles. Chromatin is shown in its two states, very condensed (heterochromatin) and loosely organized (euchromatin). Only a subset of nuclear membrane proteins are portrayed in this picture: lamin B receptor (LBR), emerin, MAN1, and nurim. The schematic also illustrates some of the interactions between these proteins with the lamina meshwork (lamins B and A/C). The LINC complex is represented by nesprins, Sad1p/UNC-84 (SUN) proteins and Samp1. On the outer membrane, nesprin-1 and -2 can directly bind to actin filaments or indirectly interact with microtubules through motors proteins (dyneins or kinesin). Nesprin-3 is shown interacting with intermediate filaments via plectin.
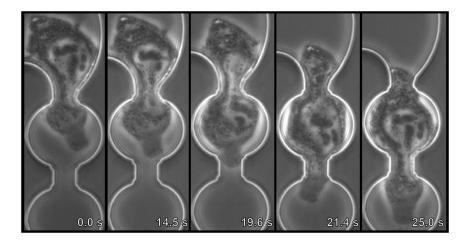
**Figure 2. Invasive cancer cell MDA-MB-231 squeezing into 8 μm width constriction**
Image sequences of a cancer cell being perfused through 8 μm-wide constriction at a pressure ( P) of 10 psi. The viscoelastic deformation as the nucleus flows through the constriction is clearly visible.
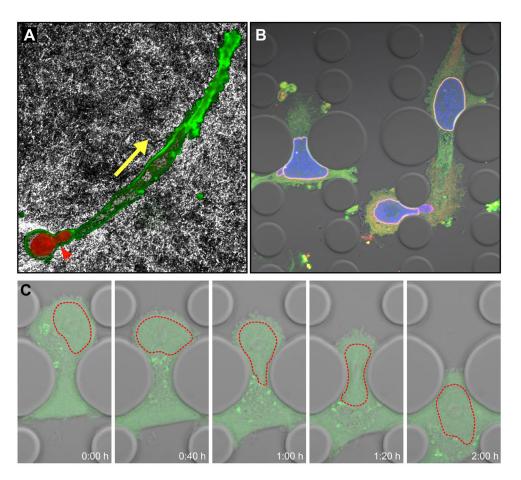
**Figure 3. Migration of cancer cell in a constrained environment**
**(A)** Fibrosarcoma cell (HT1080 cell line) migrating through a dense collagen fiber matrix. The rat tail collagen matrix was imaged by reflection microscopy; the nucleus is visible in red (DAPI), F-actin in green (phalloidin). The cell body has already advanced in the direction of migration (yellow arrow), while the nucleus is still in the process of squeezing through constrictions in the collagen matrix (red arrow head). Image courtesy of Katarina Wolf, University of Nijmegen. **(B)** Fibrosarcoma cells (HT1080) migrating through 2 μm × 5 μm and 5 μm × 5 μm constrictions in a microfluidic channel. The cytoplasm is visible in green, the nucleus in blue, and the nuclear lamina (lamin B2) in red. **(C)** Time-lapse series of MDA-MB-231 breast cancer cell expressing a green fluorescent protein migrating through a 5 μm-wide constriction in a microfluidic channel. The nucleus is outlined in red (dashed line).

**Table 1**

Altered expression (and mutations) of nuclear envelope proteins in cancers.

| Protein | Cancer/tumor type | Reported change | Prognostic value | Reference |
|---|---|---|---|---|
| **Emerin** | Ovarian cancer | Loss of emerin | | [222] |
| **Lamin A Lamin C** | Lung cancer | Absence or very reduced expression in small cell lung carcinoma | | [177] |
| | Colonic and gastric adenocarcinomas Other cancers: Oesophagus cancer, cervical and uterine cancer, breast cancer. | Reduced levels and mislocalization (aberrant cytoplasmic immunolabelling) | | [178] |
| | Basal cell skin carcinoma | Low levels or absence of lamin A | Increased proliferation | [179] |
| | Basal cell skin carcinoma | Low levels of lamin C | Low proliferation | [179] |
| | Skin cancer | High levels of lamin A and C in the basal cell layer of the epidermis overlying basal cell carcinomas, squamous cell carcinomas and actinic keratosis (AK) | Proliferative capacity | [180] |
| | Leukemia and lymphomas | Loss of gene expression by epigenetic silencing in nodal diffuse large B-cell lymphomas and acute lymphoblastic leukemias. | Poor outcome/overall survival | [223] |
| | Colorectal cancer | Increased expression (mainly lamin A) | Promote tumor invasiveness Poor prognosis (risk indicator of tumor related mortality) | [187] |
| | Ovarian serous cancer | High levels in all stages of ovarian serous carcinomas; increased immunoreactivity in the higher stage of tumor | Correlates with advanced stage | [224] |
| | Primary gastric carcinoma | Low levels | Poor histological differentiation; poor prognosis | [186] |
| | Prostate cancer | Low expression in lower grade; increased levels in higher grade | Correlates with advanced stage | [225] |
| | Colon cancer | Low expression in stage II and III patients | Correlates with increased relapse | [183] |
| | Ovarian cancer | Heterogeneous lamin A/C protein expression pattern or absence of lamin A/C and aneuploidy | | [185] |
| | Breast Cancer | Mutated | | [190] |
| **Lamin B** | Colon cancer | Reduced expression | | [178] |
| | Colorectal carcinoma | Increased levels | | [226] |
| | Ovarian cancer | Increased levels of lamin B1 and B2 in malignant cell compared to benign | | [227] |
| | Hepatocellular carcinoma | Increased levels of Lamin B1 in cirrhotic tissue | | [228] |
| | Liver cancer | Increased levels of lamin B1 in every stage (cirrhosis, early stage, late stage); presence of soluble lamin B1 in the circulation | Potential biomarker Correlate with the tumor development | [229] |
| | Prostate cancer | Increased levels of lamin B | Correlate with the tumor development | [230] |

| Protein | Cancer/tumor type | Reported change | Prognostic value | Reference |
|---------|-------------------|-----------------|------------------|-----------|
|  | Pancreatic cancer | Increased levels of lamin B1 | Correlate with decreased levels of tumor differentiation, high metastatic potential and poor overall survival | [231] |
| **LAP2** | Malignant lymphocytes | Increased levels | LAP2β correlates with highly proliferative malignant cells | [232] |
| **Nesprins** | Ovarian cancer | Nesprin 1 polymorphism. Down-regulation of a transcript (shorter isoform) | Associated with invasive ovarian cancer risk | [191] |
|  | Colorectal cancer | Nesprin 1 is candidate cancer gene (mutated in cancer) |  | [189] |
|  | Breast cancer | Nesprin 1 (mutations) |  | [190] |
|  | Breast cancer | Nesprin 2 (mutations) |  | [189,190] |
| **NUP 88** | Ovarian cancer Different type of cancers: sarcomas, lymphomas, mesotheliomas and breast cancer | Increased levels Increased levels | Correlates with high-grade malignancies | [233] [234–237] |
|  | Colorectal cancer and hepatocellular carcinoma | Increased levels | Correlates with poor differentiation | [238,239] |
| **NUP 98** | Leukemia | Increased levels; may act as component of a chromosomal translocation |  | [240] |
| **NUP 214** | Uterine, stomach and rectal tumors, leukemias, breast cancer | Increased levels; may act as a multi –functional oncogene and as a component of a chromosomal translocation |  | [241,240, 242,189] |