# Nuclear Mechanics and Cancer Cell Migration

8

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**Abstract**

As a cancer cell invades adjacent tissue, penetrates a basement membrane barrier, or squeezes into a blood capillary, its nucleus can be greatly constricted. Here, we examine: (1) the passive and active deformation of the nucleus during 3D migration; (2) the nuclear structures—namely, the lamina and chromatin—that govern nuclear deformability; (3) the effect of large nuclear deformation on DNA and nuclear factors; and (4) the downstream consequences of mechanically stressing the nucleus. We focus especially on recent studies showing that constricted migration causes nuclear envelope rupture and excess DNA damage, leading to cell cycle suppression, possibly cell death, and ultimately it seems to heritable genomic variation. We first review the latest understanding of nuclear dynamics during cell migration, and then explore the functional effects of nuclear deformation, especially in relation to genome integrity and potentially cancerous mutations.

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## 8.1 Introduction

Multiple processes *in vivo* require cells to move through three-dimensional (3D) tissue. Cancer cells migrate into wounds during healing (Clark et al. 1982) and into vessel-adjacent matrix during angiogenesis (Lamalice et al. 2007). Leukocytes squeeze through capillaries as small as 2–3 μm in diameter and extravasate into sites of tissue damage or infection as part of the innate immune response (Luster et al. 2005). Embryogenesis involves progenitor and committed cells moving and positioning themselves in developing organs (Kurosaka and Kashina 2008). Cancer cells invade healthy tissue, penetrate basement membrane barriers, and enter distant capillary beds during tumor metastasis (Liotta et al. 1991). As the largest and stiffest organelle (Dahl et al. 2008), the nucleus has long been speculated to sterically limit a cell's ability to migrate through small, stiff pores including basement membranes that separate tissues (Lichtman 1970). In migration through constricting 3D fibrous matrix, the nucleus has been described as a "piston" that is pulled forward to establish a hydrostatic pressure



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gradient between the tight-fitting nucleus and the cell's leading edge (Petrie et al. 2014). Moreover, softening the nucleus by knockdown of key nuclear structure components can enhance the rate of migration through small constrictions (Shin et al. 2013; Harada et al. 2014), consistent with the idea of the nucleus as a physical impediment to migration.

While the nucleus affects migration by presenting a challenge to the moving cell, migration also affects the nucleus. Constriction-induced deformation causes chromatin reorganization and even nuclear envelope rupture (Denais et al. 2016; Raab et al. 2016; Irianto et al. 2016a, 2017), among other effects, with implications for important biological processes like DNA damage and repair. For an overall understanding of cell migration, it is therefore necessary to consider the role of the nucleus.

The chapter will examine: (1) the forces exerted on the nucleus during 3D migration; (2) the regulators of nuclear deformability that influence transit through small pores; (3) the impact of large nuclear deformation on chromatin and nuclear factors; and (4) the downstream consequences of physically perturbing the nuclear content, including effects on genome integrity and cell cycle progression. A main goal of the chapter is to introduce some of the biophysical processes relevant to nuclear dynamics during cell migration, while also highlighting the functional effects of nuclear deformation on the biology of the cell.

## 8.2 Structure of the Nucleus

Although nuclear sizes vary among and even within cell types, the nucleus is typically the largest cellular organelle, with a diameter of ~5–20 μm (Dahl et al. 2008). In cells imaged *in situ* or grown in 3D scaffolds, the nucleus tends to be round or ovoid, whereas 2D culture drives cell spreading and nuclear flattening (Khatau et al. 2009). The nucleus—along with other, smaller organelles (<1–2 μm) and cytoskeletal filaments—is embedded in the cell's gel-like cytoplasm. The cytoplasm, cytoskeleton, and plasma membrane are easily deformed and rearranged during constricted migration such that cytoplasmic protrusions can squeeze into channels of even submicron diameter (Wolf et al. 2013). By contrast, the nucleus is 2–10 times stiffer than the surrounding cell body (Guilak et al. 2000; Caille et al. 2002), making its constriction a more torturous—and rate-limiting (Davidson et al. 2015)—step in the process of 3D migration.

The nuclear envelope, which defines the boundary of the nucleus, consists of two closely apposed lipid bilayers: the inner nuclear membrane (INM) and the outer nuclear membrane (ONM). Both are continuous with the endoplasmic reticulum. Just below the INM is the nuclear lamina (Fig. 8.1), a dense meshwork of intermediate filament proteins (lamins) that confers mechanical support and stiffness to the nuclear envelope (Ungricht and Kutay 2017). Together, the envelope and lamina surround the nucleoplasm, the genome (i.e. chromatin), and various subnuclear bodies—mostly ribonucleic protein complexes like nucleoli, promyelocytic leukemia (PML) nuclear bodies, Cajal bodies, and splicing speckles (Martins et al. 2012).

The nucleus mechanically couples to the cytoskeleton by way of Linker of Nucleoskeleton and Cytoskeleton (LINC) complexes (Irianto et al. 2016b). A LINC complex consists of a SUN protein that binds to the lamina and connects *via* nuclear envelope spectrin repeat proteins (nesprins) to a KASH domain on the ONM (Tapley and Starr 2013). The cytoplasmic region of the KASH domain then mediates interactions between the nucleus and the cytoplasm/cytoskeleton by tethering the ONM to cytoskeletal microtubules, actin filaments, and intermediate filaments (Tapley and Starr 2013). Numerous experiments demonstrate this physical nucleo-cytoskeletal linkage: for example, targeted laser ablation of the actin cytoskeleton causes the nucleus to move laterally and away from the culture substrate, and can even cause local nuclear deformation (Mazumder and Shivashankar 2010; Nagayama et al. 2011). And the disabling of endogenous LINC complexes results in loss of cellular mechanical stiffness comparable to the loss of stiffness observed with lam-

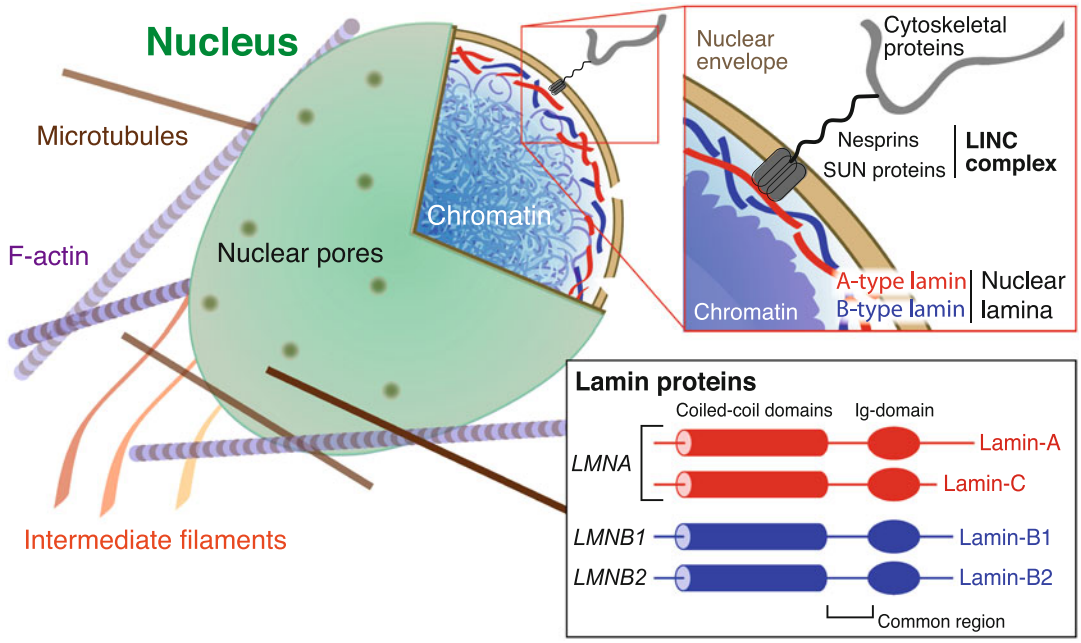


**Fig. 8.1** A-type and B-type lamins form a dense meshwork on the inside of the nuclear envelope. The nucleus mechanically couples to the cytoskeleton by way of Linker of Nucleoskeleton and Cytoskeleton (LINC) complexes, which consist of a SUN protein that binds to the lamina and connects *via* nuclear envelope spectrin repeat proteins (nesprins) to a KASH domain on the outer nuclear membrane. The cytoplasmic region of the KASH domain then mediates interactions between the nucleus and the cytoplasm/cytoskeleton by tethering the nuclear membrane to cytoskeletal microtubules, actin filaments, and intermediate filaments

ina disruption (Stewart-Hutchinson et al. 2008). Because the nucleus mechanically couples to the cytoskeleton—and ultimately, via adhesions, to extracellular matrix (ECM)—it deforms with the cell during 3D migration (Broers et al. 2004; Swift et al. 2013).

## 8.3 Deformation of the Nucleus During Constricted Migration

Different cell types employ different single-cell migration modes in 3D environments. Tissue fibroblasts exhibit relatively slow (~0.5–1 μm/minute) mesenchymal cell migration (Cukierman et al. 2001), while dendritic cells and immune cells (e.g. leukocytes) favor ~10–40-fold faster amoeboid movement (Friedl et al. 1998). Mesenchymal cell migration proceeds as follows: (1) the cell polarizes to create a leading edge that extends actin-rich protrusions; (2) the protrusions form adhesions to ECM contacts; (3) myosin II-mediated contraction of the actin cytoskeleton shortens the rear of the cell and advances the cell body; and (4) the trailing edge detaches from the substratum, allowing the cell to translate forward. Such migration can include degradation of the ECM by matrix matelloproteinases (MMPs) (Even-Ram and Yamada 2005). By contrast, amoeboid migration is typically non-proteolytic, and it involves weaker, more transient adhesive interactions with the ECM (Parri and Chiarugi 2010). In reality, migration modes exist along a continuum, and the mode adopted by a given cell in a given microenvironment seems to be determined by factors such as ECM stiffness and the intrinsic contractility of the cell (Parsons et al. 2010).

Regardless of the particular motility mode, deformation of the cell during 3D migration leads to deformation of the nucleus. The first step in

the migration process, polarization, requires the cell's cytoskeleton and organelles—including the nucleus—to rearrange themselves within the cell body. In polarized mesenchymal cells, fibroblasts, neurons, and most cancer cells, the nucleus assumes a rearward position, thus establishing a leading edge-to-centrosome-to-nucleus axis along the direction of locomotion—at least on rigid substrates (Gomes et al. 2005; Gasser and Hatten 1990; Tsai and Gleeson 2005; Barnhart et al. 2010). On soft substrates, the centrosome is more random in location (Raab et al. 2012). Whereas most nuclear movements are thought to be microtubule-mediated (Luxton et al. 2010), nuclear repositioning for migration is driven by retrograde flow of actin: inhibiting myosin-II or actin with blebbistatin or cytochalasin D, respectively, is known to block actin retrograde flow, and doing so prevents rearward nuclear movement during cell polarization (Gomes et al. 2005), although cells can still migrate with myosin-II inhibition in 2D. Actin cables are coupled to the dorsal surface of the nuclear envelope by LINC complexes, as described above; these linkages transmit force from actin flow to the nucleus (Luxton et al. 2010).

After polarization, as the cell proceeds to squeeze into a tight constriction in 3D, the nucleus moves with the cell body by being either pushed or pulled. Under the pushing mechanism, the nucleus is squeezed forward by actomyosin contraction in the (detached) rear of the cell (Roth et al. 1995; Zhang et al. 2007). Such trailing-edge contraction propels nuclear translocation during constricted migration of leukocytes: myosin II-inhibited leukocytes migrating through 3D gels exhibit a peculiar elongated shape with a rounded back due to nuclear immobilization at the rear ends of the cells. Because posterior actomyosin contraction is required to retract and detach the cell membrane, myosin II inhibition renders leukocytes unable to push their large, rigid nuclei through small interstices in the gel (Lammermann et al. 2008). Similarly, in 3D migration studies of breast, brain, and other cancer cells, non-muscle myosin II localizes to the perinuclear cytoskeleton and cell posterior, and then exerts pushing forces to advance the nucleus (Harada et al. 2014; Beadle et al. 2008; Ivkovic et al. 2012). Knockdown of myosin IIB dramatically slows migration of breast cancer cells through narrow channels, whereas knockdown of myosin IIA—the non-muscle myosin II isoform that generates force during leading-edge protrusion—has little effect on migration time through the constrictions. The isoforms have almost opposite effects in 3D migration of glioma cells. Nesprin-2 provides a possible physical link between the nucleus and myosin IIB-mediated force generation (Beadle et al. 2008).

Under the pulling mechanism, actomyosin contraction physically pulls the nucleus forward during 3D migration. When Rac1 photoactivation is used to create a new leading protrusion in a crawling fibroblast (by triggering local F-actin polymerization at the front of the cell), the nucleus moves persistently toward the new leading edge without trailing-edge detachment—even when microtubules are depolymerized (Wu et al. 2014). In lobopodial fibroblasts, the pulling forces are generated by non-muscle myosin IIA-containing actomyosin bundles that form complexes with the intermediate filament protein vimentin and the LINC protein nesprin-3 (Petrie et al. 2014). Ultimately, it is likely that both pushing and pulling forces contribute—in a cell type- and migration mode-dependent manner—to the forward motion of nuclei during 3D migration.

Whether the nucleus is pushed or pulled by actomyosin, it can undergo huge deformation when constricted. Whereas the nucleus maintains its original ellipsoid shape and simply re-orients during transit through large pores in loose tissues (Friedl et al. 2011), it is severely locally compressed by small pores in dense tissues, resulting in transient shape changes (Harada et al. 2014). Reflecting the larger deformation required by smaller pores, migration speed decreases linearly with decreasing pore size (Irianto et al. 2017). Compression of the nucleus during migration is actuated by cytoskeletal forces and opposed by the geometry of ECM pores. In 2D culture on stiff glass substrates, a dome-like perinuclear actin cap largely aligns with the overall cell orientation (Khatau et al. 2009), and this cap might actively

drive nuclear shape changes during 3D migration. Moreover, intermediate filaments including vimentin surround the nucleus in a fibrous "cage" that is required for nuclear re-shaping in response to actomyosin-induced forces (Neelam et al. 2015). Both the actin cap and cage-like intermediate filaments connect to the nuclear envelope through LINC complexes; hence, LINC complex disruption impairs nucleo-cytoskeletal-mediated nuclear deformation and often causes migratory defects (Khatau et al. 2012).

Beyond single-cell migration, it should be noted that cells often maintain their cell-cell junctions and undergo collective migration, traveling in sheets, strands, tubes, or clusters (Parri and Chiarugi 2010). Such movement usually occurs along smooth ECM interfaces (Friedl et al. 2011); for example, collective migration of invasive cancer cells through tissue barriers requires MMPs to clear tracks—devoid of sterically impeding fibers—in the ECM. Multicellular invasion along these proteolytic tracks causes significantly less morphological adaptation and nuclear deformation than does single-cell migration through non-reorganized collagen (Wolf et al. 2007). Thus, the severity of nuclear deformation depends on the mode of migration—collective versus single-cell, proteolytic versus non-proteolytic.

## 8.4 Regulators of Nuclear Deformability

Nuclei have viscoelastic properties (Guilak et al. 2000; Dahl et al. 2005), meaning that they exhibit stress relaxation: when a constant deformation is applied, the resulting mechanical stress on the nucleus decays over time. They also exhibit a creep response such that when a constant stress is applied, the nucleus continues permanently to deform. Viscoelastic materials are often modeled as a network of elastic springs and viscous dashpots. For example, in the three-component standard linear solid model, which is designed to show exponential stress relaxation and exponential creep, a spring is placed in parallel with a "Maxwell arm" consisting of a spring and dashpot in series (Meidav 1964). This model has been applied to isolated articular chondrocyte nuclei pulled by constant suction pressure into micropipettes (Guilak et al. 2000). However, spring-dashpot models are limited in the case of nuclei because nuclear stress relaxation and creep occur over many decades of time. To accurately model viscoelastic behavior on such timescales would require a very large (physically meaningless) number of spring and dashpot elements, which could increase mathematical complexity to the point of impracticality (Lange and Fabry 2013).

As opposed to a superposition of very many exponential response functions, a power-law model provides a simpler and more physically meaningful approach to describe nuclear mechanics under deformation. Indeed, micropipette aspiration and atomic force microscopy (AFM) assays indicate that isolated intestinal epithelial cell nuclei exhibit power-law rheology (Dahl et al. 2005). The creep compliance $J(t)$ of the nucleus—that is, the ratio of nuclear strain to applied stress as a function of time $t$—is given by

$$J(t) = J_0 \left( \frac{t}{\text{sec}} \right)^{\alpha} [=] \frac{1}{\text{kPa}}, \qquad (8.1)$$

where the prefactor $J_0$ corresponds to the inverse of the dynamic shear modulus $G$ measured at a frequency of 1 rad/s (Dahl et al. 2005; Lange and Fabry 2013; Hildebrandt 1969). The exponent $\alpha$ depends on the dynamics of the force-bearing elastic structures of the nucleus (Lange and Fabry 2013)—in particular, the lamina and the chromatin, as we will describe below. A purely elastic solid would have a power-law exponent of $\alpha = 0$, while a purely viscous fluid would have an exponent of $\alpha = 1$. The measured value for isolated nuclei from intestinal epithelial cells is $\alpha \approx 0.2$–$0.3$ (Dahl et al. 2005).

Power-law rheology could have a number of important consequences for nuclei undergoing constricted migration. First, in a material with a power-law exponent of $\alpha \approx 0.2$–$0.3$, mechanical stresses decay slower than exponentially, but they do become small for large enough $t$. To illustrate, if the effective stiffness of a nucleus is 1 kPa

when measured at a frequency of 1 Hz, then the same nucleus should have an effective stiffness of only ~0.3 kPa when measured at 0.01 Hz. Thus, ignoring active mechanics, as the speed of nuclear movement decreases, so do the movement-resisting forces that arise from nuclear deformation (Lange and Fabry 2013). Second, the power-law behavior of nuclei has implications for chromatin organization during migration. Other systems with power-law rheology, such as microgels, have an essentially infinite number of intermediate conformations corresponding to infinite relaxation modes or timescales. It seems likely that nuclear components at different length scales—from nucleosomes to chromosomes to chromatin fibers—also have intermediate conformations of mechanical relaxation, reflecting metastable states that could impact gene expression kinetics (Dahl et al. 2005).

The power-law viscoelasticity of nuclei is determined principally by the lamina and chromatin—or at least the chromatin volume fraction. We will discuss each structure in turn. The intermediate filaments that comprise the nuclear lamina are divided into two sub-types (Fig. 8.1): A-type lamins (lamin-A and -C), which are alternative splicing products of the LMNA gene; and B-type lamins (lamin-B), which are encoded by the LMNB1 and LMNB2 genes. Although A- and B-type lamins have similar amino acid sequences and structural features, they have different post-translational modifications (Irianto et al. 2016b): the lamin-B monomer is permanently modified by addition of a farnesyl group, which is hydrophobic and tethers lamin-B to the INM (Hennekes and Nigg 1994). As a result, lamin-B is less mobile and dynamic than mature lamin-A (Shimi et al. 2008), from which the farnesylated C-terminus is cleaved (Irianto et al. 2016b). Like other intermediate filament proteins, including keratin and vimentin, lamin monomers form coiled-coil parallel dimers that assemble into filaments of ~3.5 nm thickness, organized in complex meshworks of ~14 nm thickness (Herrmann et al. 2009; Turgay et al. 2017). Cryo-electron tomography of mouse embryonic fibroblasts suggests that both lamin sub-types are present throughout the meshwork, including in densely packed and sparsely occupied regions (Turgay et al. 2017).

Lamin-A levels vary widely across adult cell types, scaling with resident tissue stiffness (Swift et al. 2013). Meanwhile, lamin-B expression remains relatively constant such that the ratio of lamin-A to -B is highest in stiff tissues like muscle and bone, and lowest in soft tissues like brain and fat. The positive scaling of lamin-A:B ratio with tissue microelasticity suggests a possible role for lamin-A in protecting the nucleus against mechanical stresses, which are expected to be higher in stiffer tissues. Consistent with such a protective function, lamin-A confers viscous stiffness to nuclei, while lamin-B contributes to nuclear elasticity. When nuclei of diverse tissue lineage are pulled into micropipettes under controlled pressure (~kPa), each nucleus extends within seconds in a viscoelastic manner, as described above. Importantly, effective nuclear viscosity increases more rapidly than effective elasticity as a function of lamin-A:B stoichiometry. This trend suggests that whereas lamin-B functions like the elastic walls of a balloon, restoring the nucleus to its original shape in response to applied stresses, lamin-A acts like a viscous fluid that coats the walls and perhaps fills the balloon to dynamically resist deformation (Swift et al. 2013). Moreover, lamin-A knockdown is known to soften nuclei (Harada et al. 2014; Pajerowski et al. 2007), and mutations in lamin-A are associated with diseases—"laminopathies" including muscular dystrophy and premature aging (Sullivan et al. 1999). Levels of lamin-A and lamin-B are abnormal in many cancers; lamin-A is low in lung and breast tumors, for example (Irianto et al. 2016b). Lamin-A depletion had been reported to favor nuclear rupture in fibroblastic cells spread and flattened on stiff substrates but not on soft substrates where cells and nuclei are more rounded and relaxed (Tamiello et al. 2013); further study of such 2D cultures demonstrated that nuclear rupture occurs at sites of high nuclear curvature where stiff lamin-B filaments tend to detach (Xia et al. 2018).

In cell migration through small pores, the lamina regulates nuclear deformability. Lamin-A in particular is known to be rate-limiting in 3D migration of diverse human cell lines, ranging from brain and lung cancer cells to primary mesenchymal stem cells (MSCs) (Harada et al. 2014). For a given cell type, wild-type levels of lamin-A protect against stress-induced death during transit through small pores, whereas low levels cause susceptibility to stress and apoptosis, and high levels impede migration. Thus, lamin-A is a barrier to 3D migration, but it promotes nuclear integrity and survival (Harada et al. 2014).

Chromatin can also play a role in the mechanical response of the nucleus. Chromatin consists of DNA wrapped around histone octamers, and it exists in two forms: open euchromatin (low density), which contains most actively transcribed genes; and tightly packed heterochromatin (higher density), which can silence gene transcription (Dahl et al. 2008). Treatment with the deacetylase inhibitor trichostatin A (TSA) favors euchromatin organization by causing large-scale chromatin decondensation; such remodeling renders nuclei softer and more deformable. Conversely, chromatin condensation by divalent cations such as $Ca^{2+}$ and $Mg^{2+}$ results in extremely stiff nuclei with small values for the creep compliance factors $J_0$ and $\alpha$ (Eq. 8.1). The relative deformability of euchromatin structures hints that external forces—like those imposed by constricting pores during 3D migration—could easily reorganize gene-rich regions of the genome (Pajerowski et al. 2007). Moreover, although isolated chromosomes respond elastically to applied stress (Cui and Bustamante 2000; Marko 2008), chromatin within the nucleus responds by either flowing with the stress or bearing it. As observed in nuclei that lack lamins, INM chromatin tethers constrain flow and favor an elastic response to small forces. However, untethering of chromatin from the INM allows the chromatin to flow under deformation to a new, lower energy configuration (Schreiner et al. 2015). These results, which indicate that chromatin contributes both elasticity and viscosity to the nucleus, are consistent with: (1) nuclear stretching experiments in which chromatin governs elastic resistance to small nuclear deformations (Stephens et al. 2017); and (2) micropipette aspiration experiments showing that chromatin can flow, shear, and locally compact like a complex fluid (Pajerowski et al. 2007). Together, the lamina and the chromatin (especially in case of high volume fraction) determine the mechanical properties of the nucleus and the severity of nuclear deformation during constricted migration.

## 8.5 Effect of Nuclear Deformation on Chromatin and Nuclear Factors

Migration through constricting pores exerts compressive forces on the nucleoplasm, causing the internal pressure in the nucleus to rise. In regions—like the leading tip of the nucleus—where no external forces are applied, the increased internal pressure is equilibrated by an increase in the surface tension of the nuclear envelope, per the Young-Laplace equation. To relax some of this tension and lower the membrane stretching energy, a hole may form in the lamina, leading to fluid outflow from the nucleus that locally inflates the nuclear envelope. Such inflation produces a bleb, which can burst to cause leakage of nuclear factors—and even herniation of chromatin—into the cytoplasm, with corresponding leakage of cytoplasmic factors into the nucleus (Deviri et al. 2017). Indeed, in back-to-back papers from two groups (Denais et al. 2016; Raab et al. 2016), migration of various cancer cell lines, immortalized epithelial cells, and primary dendritic cells through narrow channels was shown to rupture the nuclear envelope. Rupture, which can occur even without bleb formation (Pfeifer et al. 2018), leads to exchange of nucleo-cytoplasmic contents, as indicated by cytoplasmic accumulation of GFP-NLS (nuclear localization signal) and nuclear accumulation of NES (nuclear export signal)-GFP. The resealing of nuclear envelope lesions is thought to be

mediated by endosomal sorting complex required for transport III (ESCRT III) components (Denais et al. 2016; Raab et al. 2016).

In the same two papers, rupture was shown to be followed by enrichment at the envelope (Denais et al. 2016) or far from the envelope (Raab et al. 2016) of a GFP fusion of an overexpressed DNA repair factor 53BP1, but no supporting evidence of DNA damage was provided in terms of endogenous damage markers such as the standard histone γH2AX or electrophoresis of DNA fragments. The authors speculated that the observed pools of GFP-53BP1 could be due to nuclear influx of cytoplasmic nucleases, which potentially cleave the DNA and trigger a DNA damage response. However, accumulations of GFP-53BP1 could instead reflect local pooling of mobile nuclear proteins into rarefied pockets of the strongly deformed chromatin (Irianto et al. 2016a).

Other pore migration studies of two cancer lines and primary human MSCs have provided clear measures of excess DNA damage based on increased foci of γH2AX, increased foci of the upstream kinase phospho-ATM, and longer electrophoretic comets of DNA (Irianto et al. 2017; Pfeifer et al. 2018). Nuclear entry of cytoplasmic nucleases fails as an explanation for this damage, because nuclease infiltration would be expected to cause localized damage concentrated near the site of nuclear envelope rupture. However, γH2AX and pATM foci have a pan-nucleoplasmic distribution, suggesting a more global damage mechanism (Irianto et al. 2017). While it is tempting to propose that chromatin fragmentation as a nucleus enters and elongates in a small pore might account for the increased damage, this mechanism also seems unlikely given that stretched chromatin maintains its integrity. In living cells, an mCherry-tagged nuclease was targeted to a submicron locus on chromosome 1, where it causes DNA cleavage—and thus recruitment of DNA repair factors to a large region around the locus. Micropipette aspiration of these cells and their nuclei shows that the chromatin aligns and stretches parallel to the pore axis. Importantly, even though DNA within the engineered locus is cleaved by nuclease, intensity profiles of mCherry indicate continuity, meaning that integrity of the chromatin is maintained during nuclear distention. Chromatin shearing is therefore unlikely to explain the excess DNA damage that follows pore migration (Irianto et al. 2016c).

One possible mechanism is global inhibition of DNA repair. DNA breaks constantly form by various means, including replication or oxidative stress, and are repaired by dedicated factors that are often implicated in cancer (including ATM, BRCA1, etc.). Damage rate and repair rate reach a steady state dependent on the level or activity of the repair factors. A compelling hypothesis holds that constriction-induced mis-localization of repair factors causes partial depletion of repair factors throughout the nucleus, which physically inhibits repair of routine DNA breaks and leads to the observed transient increases in DNA damage (Irianto et al. 2017; Pfeifer et al. 2018). Inactivating mutations in DNA repair factors BRCA1 and BRCA2 are such well-established risk factors for cancer that they warrant surgical removal of ovary and breasts (Levy-Lahad and Friedman 2007). Mouse knockouts or heterozygous mutants for BRCA1 and BRCA2, among other repair proteins, have indeed been shown to alter chromosome copy numbers (Holstege et al. 2010). Therefore, any migration-induced physical depletion of such factors should also increase DNA damage and mutation probabilities.

Constriction mis-localizes repair factors in two ways (Fig. 8.2). First, if nuclear envelope rupture occurs, as described above, then diffusible repair factors leak into the cytoplasm for hours before ultimately relocalizing to the nucleus (Irianto et al. 2017). Second, regardless of nuclear envelope rupture, constrictions "squeeze out" all diffusible proteins from regions of high DNA compaction, such as at the entrance of a constricting pore (Irianto et al. 2016a). To elaborate, absent DNA damage, GFP-53BP1 is ordinarily diffuse in the nucleus, consistent with nucleoplasmic mobility (Bekker-Jensen et al. 2005; Pryde et al. 2005). However, during constricted migration, as the nucleus contorts to enter a pore, mobile GFP-53BP1 is significantly depleted within the

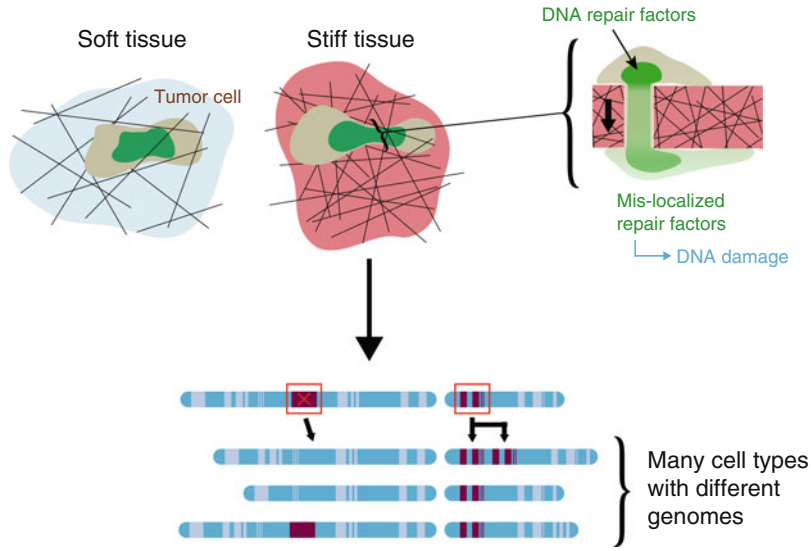


**Fig. 8.2** Since stiffer tissues tend to have higher matrix density, and thus smaller pores, cancer cells might sustain more nuclear stress during tumorigenic invasion into stiff tissues as compared to soft ones. Migration through small pores severely deforms the nuclei of invading cancer cells, which causes mis-localization of DNA repair factors *via* (1) "squeeze-out" of all diffusible proteins from regions of high DNA compaction and (2) rupture-induced leakage of nuclear proteins into the cytoplasm. Such mis-localization causes partial depletion of repair factors throughout the nucleus, which physically inhibits repair of routine DNA breaks and leads to excess DNA damage. Migration-induced DNA damage results in lasting, heritable genomic heterogeneity

constriction, in contrast to DNA or chromatin-bound proteins like mCherry-Histone H2B, which are instead enriched in the constriction. Similarly, endogenous 53BP1 (immunostained) and the additional DNA repair factors GFP-Ku70 and -Ku80—all in the mobile phase—show such striking depletion. These observations suggest that nuclear constriction excludes, and hence depletes, mobile nucleoplasmic factors from the pore. Nuclear factor segregation is also observed during micropipette aspiration: all of a dozen mobile proteins examined—including upstream DNA damage response factors (e.g. MRE11, RPA) as well as downstream factors (e.g. BRCA1)—segregate like GFP-53BP1 (Irianto et al. 2016a).

A simple model for squeeze-out of mobile nuclear factors provides insight into why segregation occurs and gives a mechanistic basis for the hypothesis that severe constriction can arrest DNA damage repair (Bennett et al. 2017). Chromatin is modeled as a solid mesh of volume fraction $f$, intermixed with a fluid of mobile nuclear proteins. For cells in static culture, chromatin has been measured to occupy $f \sim 67\%$ of the nuclear volume (Bancaud et al. 2009), so the free volume for diffusion of mobile factors is $(1 - f) \sim 33\%$. However, constriction increases the local density of chromatin by a factor of $\sim$1.25 such that inside the pore $f_{\text{constricted}} \sim 84\%$, which causes the free volume there to decrease to $(1 - f_{\text{constricted}}) \sim 16\%$. It follows that mobile factors should decline in the constriction to $(1 - f_{\text{constricted}}) / (1 - f) \sim 0.5$ of their original abundance, which agrees well with experiments (Irianto et al. 2016a; Bennett et al. 2017). This depletion of mobile proteins, including repair proteins, is compounded by rupture-induced leakage into the cytoplasm. Altogether, mis-localization of repair factors during constricted migration impedes the DNA damage response. Thus, normally occurring DNA damage—that might arise due to replication stress, reactive oxygen species, or other sources—cannot be efficiently repaired while the nucleus is inside the pore, leading to excess damage.

## 8.6 Downstream Consequences of Constriction-Induced Nuclear Deformation

Migration-induced DNA damage leads to lasting genomic heterogeneity that translates to transcriptomic and phenotypic changes. Clonal U2OS cells were subjected to three consecutive migrations through constricting pores; from among these thrice-migrated cells, the genomes of six single-cell-derived clones were quantified by SNP array analysis. Compared to the pre-migration clone, the migrated clones showed unique chromosome copy number changes and loss of heterozygosity (Irianto et al. 2017). Because the pre-migration population was 100% clonal and the migrated sub-clones exhibited unique genomic changes, it stands to reason that migration causes—as opposed to simply selecting for—genomic variation. On the other hand, selection of rare subpopulations with pre-existing genomic differences is a possible mechanism that might only be addressed by live cell monitoring of genome transitions in single cells – a method that awaits development.

The clinical implications of constricted migration causing heritable mutations are vast. Advances in whole-genome sequencing technology have allowed for complete cataloguing of the genomic changes that occur in cancers of different types (Martin et al. 2015; Schumacher and Schreiber 2015; Matsushita et al. 2016). In a meta-analysis of published cancer sequencing data (Pfeifer et al. 2017), the somatic mutation rates for 36 cancer types were culled from a number of recent papers (Schumacher and Schreiber 2015; Alexandrov et al. 2013; Lawrence et al. 2013; Martincorena et al. 2015; Martincorena and Campbell 2015; Chen et al. 2014; Shain et al. 2015), as were the stiffnesses of the healthy tissues in which those cancers arise. This meta-analysis revealed that cancers arising in stiff tissues, such as lung and skin, exhibit more than 30-fold higher somatic mutation rates than those arising in soft tissues, like marrow and brain. Although tumors often stiffen—or, less frequently, soften—their surrounding tissue over the course of tumorigenesis (Levental et al. 2010), the stiffness of a typical brain tumor microenvironment never reaches that of a typical bone tumor microenvironment, so the stiffness gradient among tissue types prevails.

The scaling of genomic variation with tissue stiffness suggests a possible mechanical source of cancerous mutations. One promising hypothesis implicates constricted migration of cells through stiff tissues (Fig. 8.2). Tissue stiffness increases with abundance of fibrous protein (e.g. collagen) (Swift et al. 2013), and denser collagen matrix has smaller interstitial pores (Yang et al. 2009). Therefore, when cancer cells invade normal tissue during tumor growth (Liotta et al. 1991), they generally encounter a higher collagen concentration and smaller pores in stiffer tissues than in softer ones. As discussed, squeezing through small pores—but not larger ones—severely deforms the nuclei of invading cancer cells, which stresses the nuclear lamina and causes DNA damage, heritable genome changes, and even cell death (Harada et al. 2014; Irianto et al. 2017). Thus, constricted migration through increasingly small holes in increasingly collagen-rich matrix stands as a possible explanation for the relation between mutation rate and tissue stiffness.

In a recent study, single-cell genome sequencing was used to measure copy number changes in single breast tumor cells while preserving their spatial context in the breast tissue. The authors of this study found a direct genomic lineage between the primary tumor (the ductal carcinoma *in situ*) and invasive tumor subpopulations; they concluded that the subpopulations must carry mutations from the primary carcinoma, rather than incurring new mutations during the invasive migration process (Casasent et al. 2018). However, breast is of low-to-intermediate stiffness (Lopez et al. 2011), so it makes sense, per the above hypothesis, that migration of tumor cells through breast tissue does not cause a large increase in mutational load.

Since cells need to repair DNA damage sufficiently in order to progress through cell cycle (Dasika et al. 1999), it seems plausible that DNA breaks incurred during migration—perhaps in combination with mis-localization of crucial cell

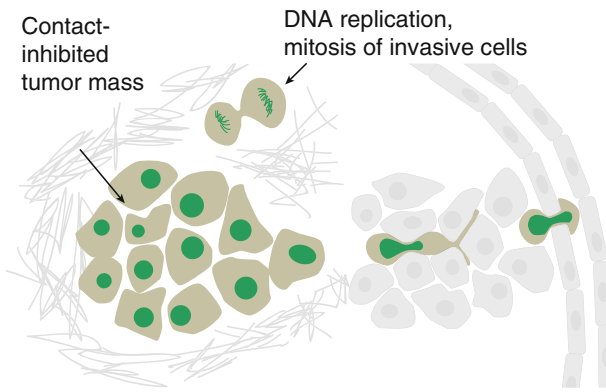


**Fig. 8.3** Invasion and proliferation are hallmarks of cancer. Invading cancer cells squeeze into regions of low cancer cell density, including nearby tissues or blood capillaries. The resulting loss of contact inhibition could in principle encourage proliferation. However, migration through 3 μm pores has been shown to cause a transient delay in cell cycle for diverse cancer cell lines, illustrating a "go, damage, and then grow" process

cycle proteins—could suppress cell proliferation. Combined EdU cell proliferation and pore migration assays show that migration through 3 μm pores indeed causes a transient delay in cell cycle for three diverse cancer cell lines (Pfeifer et al. 2018). These findings are relevant to the so-called "go or grow" hypothesis, long-debated in cancer research, which holds that proliferation and migration are mutually exclusive events (Garay et al. 2013; Giese et al. 1996). It appears that additional mutation-relevant processes are involved in a "go, damage, *repair*, and then grow" behavior, with cancer cells showing excess DNA damage and repressed cell cycle after migration. The surprising delay in growth has implications for the invasive migration of cancer cells away from a physically crowded tumor mass and into nearby stiff tissues or blood capillaries (Fig. 8.3). Moreover, the combined proliferation/migration assays also show that G1- and G2-phase cells incur a similar excess of DNA damage, suggesting that constriction-induced DNA damage occurs independent of cell cycle phase and hence independent of DNA replication (Pfeifer et al. 2018).

## 8.7 Conclusion

In this chapter, we began by describing the deformation of the nucleus during 3D migration, both due to forces imposed by the geometry of rigid pores and due to intracellular mechanisms that actively drive nuclear shape change. Then, we reviewed the latest research on the mechanical properties of the nucleus and, in particular, examined how the lamina and chromatin regulate nuclear deformability during migration through tight constrictions. Next, we considered the impact of such large deformation on chromatin and nuclear factors. Constricted migration causes frequent lamina rupture, which—along with 'squeeze-out' of mobile nuclear proteins—leads to mis-localization of crucial DNA repair factors, followed by an increase in DNA damage. Finally, we discussed some downstream consequences of constriction-induced DNA damage, namely, effects on genome integrity including possible mutations, as well as cell death and delays in cell cycle progression. Differentiation is also seen to be affected based on studies to be published soon. This chapter has introduced biophysics concepts relevant to nuclear mechanics during cell migration, while outlining some of the biological consequences of severe nuclear deformation.

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