Consequences of a tight squeeze: Nuclear envelope rupture and repair

Philipp Isermann and Jan Lammerding

Nancy E. and Peter C. Meinig School of Biomedical Engineering & Weill Institute for Cell and Molecular Biology, Cornell University, Ithaca, NY, USA

ABSTRACT

Cell migration through tight spaces can induce substantial deformations of the nucleus and cause nuclear envelope (NE) rupture, resulting in uncontrolled exchange of nuclear and cytosolic proteins. These events can cause DNA damage and, in severe cases, nuclear fragmentation, challenging the integrity of the genomic material. Cells overcome NE ruptures during interphase by repairing the NE using components of the endosomal sorting complexes required for transport (ESCRT) machinery. Paralleling the molecular mechanism used during NE reformation in late mitosis, ESCRT-III subunits and the associated AAA-ATPase VPS4B are recruited to NE rupture sites and help restore NE integrity. While these findings are common to many cell types, they are particularly relevant in the context of cancer metastasis, where nuclear deformation and rupture could drive genomic instability in invading cells and further promote cancer progression. At the same time, inhibiting NE repair may offer new therapeutic approaches to specifically target invasive cancer cells.

KEYWORDS

confined migration; DNA damage; ESCRT; lamina; nuclear envelope rupture

Introduction

In eukaryotic cells, chromosomes are separated from the cytoplasm by the nuclear envelope (NE), which consists of two phospholipid bilayer membranes, termed the inner and outer nuclear membranes (INM and ONM, respectively), nuclear membrane proteins, the nuclear lamina, and nuclear pore complexes (NPCs). The INM and ONM join at the sites of NPCs, which regulate the macromolecular transport in and out of the nucleus. For proteins larger than 30–60 kDa, a nuclear localization sequence (NLS) or nuclear export signal (NES) is required for the transport through nuclear pores. Underlying the INM is the nuclear lamina, a dense intermediate filament meshwork that provides mechanical support to the nucleus. The main nuclear lamina components in mammalian cells are A-type lamins (lamin A and C) and B-type lamins (lamin B1 and B2).

The NE is thought to physically protect the cell’s genome integrity by creating a tightly controlled, intracellular compartment with distinct molecular composition that facilitates nuclear processes such as DNA replication, repair, and transcriptional regulation. Loss of NE integrity during interphase is likely to perturb cellular functions on multiple levels, including the RanGTP gradient across the NE required for directed protein transport through nuclear pores. Furthermore, cytoplasmic nucleases that normally protect cells from foreign DNA could potentially enter the nucleus and damage the endogenous genome. As such, it had long been assumed that NE rupture would be lethal for cells.

In recent years, however, multiple studies have observed transient NE ruptures in cultured cells during interphase that allow uncontrolled exchange of molecules between the cytoplasm and nucleoplasm. NE ruptures can be readily visualized by live cell microscopy of fluorescent proteins fused to a nuclear localization signal (NLS), e.g., NLS-GFP or NLS-RFP. These fluorescent reporters are normally localized to the nucleus, but rapidly spill into the cytoplasm upon NE rupture and are then gradually re-imported into the nucleus over the...
course of 10–90 minutes (Fig. 1). NE ruptures can also be detected by monitoring fluorescent proteins tagged with a nuclear export signal (NES), which only enter the nucleus during NE rupture and are exported back into the cytoplasm once NE integrity is restored. In addition to these fluorescent reporters, evidence of NE rupture comes from mitochondria and ribosomes that become mislocalized to the nucleus, and promyelocytic leukemia (PML) nuclear bodies found in the cytoplasm of cells that exhibited NE rupture.6,7 In cells cultured on rigid substrates, spontaneous NE rupture is observed in circa 5% of cells over a 24 h period.6,7,9

Depletion of lamins or expression of disease-causing lamin mutants that impair nuclear lamina stability increases NE rupture rates.6,7,9 Similarly, human cancer cell lines, which often have altered lamin levels,12 exhibit more frequent NE rupture than non-tumorigenic controls.7

Confined migration causes increased nuclear envelope rupture

While earlier studies were limited to cells in 2D culture, researchers have recently begun to investigate the role of the nucleus in 3D cell migration and uncovered that nuclear deformation can become a rate-limiting factor during confined migration.13-18 When cells encounter constrictions smaller than the nuclear cross section, the large and relatively rigid nucleus must deform substantially to pass through the available space.13,14,19 Two recent studies found that the forces exerted on the nucleus during this process frequently rupture the NE.10,11 Nuclear deformation and NE rupture were recorded in cells migrating through collagen matrices, through microfluidic devices with precisely controlled pore sizes, and through living tissues.10,11 The incidence of NE rupture increased dramatically with increasing confinement, with up to 90% of cells exhibiting NE rupture rates over 24 h when moving through pores smaller than ~6 μm² in cross section.10,11

Mechanics of nuclear envelope rupture

The formation of nuclear membrane blebs (Fig. 1) that precede NE rupture and that collapse upon nuclear membrane rupture6-11 suggest that these events are driven by an increase in intranuclear pressure due to forces, possibly generated by the cytoskeleton, that compress the nucleus. Notably, even nuclei in unconfined cells cultured on 2D substrates experience compressive forces from the contractile actomyosin filaments spanning the nucleus.8 Supporting a role for the cytoskeleton, treatment with either the myosin inhibitor blebbistatin or the actin polymerization inhibitor cytochalasin, which reduces the tension of the actin cytoskeleton, also reduces the NE rupture rates.8,10 In contrast, when cytochalasin D treated cells are subjected to external vertical confinement to match nuclear compression levels of untreated cells, their NE rupture rate rises to that of unconfined cells. This finding is consistent with a model in which NE bleb formation—and subsequently rupture—arise
from intranuclear pressure, rather than from local cytoskeletal pulling on nuclear membrane segments.8

During confined migration, the nuclear membrane bulges out and blebs at sites of defects in the nuclear lamina.7,10 It remains unclear whether these nuclear lamina defects, which precede nuclear membrane bleb formation, are caused by excessive nuclear deformation during migration or are pre-existing abnormalities in the nuclear envelope. Nuclear bleb formation predominantly occurs at the leading edge of the nucleus as it passes through a constriction, which is where the highest nuclear membrane curvature is typically found.10,11,20 The C-terminal farnesyl group of B-type lamins, along with INM proteins, tether the INM and nuclear lamina and thereby stabilize the nuclear membrane. Thus, when the pressure in the nuclear interior increases and places additional stress on the NE, local defects in the nuclear lamina, particularly the B-type lamin network, could promote nuclear membrane detachment and bleb formation in these regions. This process may thus closely resemble the mechanics of bleb formation at the plasma membrane, where local defects in the underlying actin cortex can result in pressure-induced membrane expansion and blebbing.21

Under continued confinement, nuclear membrane blebs progressively expand until NE rupture occurs, presumably once the critical area strain of lipid bilayers has been exceeded.10 It remains unclear to what extent recruitment of membrane from the endoplasmic reticulum, which is continuous with the ONM, contributes to bleb expansion and delays NE rupture. The nuclear membrane blebs are initially devoid of most NE proteins and lack nuclear pores.8,10,22 During the bleb expansion, chromatin can herniate through the nuclear lamina and protrude into the bleb.6-11 In severe cases, chromatin segments can separate from the main nucleus as cells squeeze through tight constrictions.10 Over time, lamin A frequently forms a new nuclear lamina in the bleb, whereas lamin B1 remains absent from the bleb.10 Intriguingly, after NE rupture lamin A accumulates at the rupture site, forming ‘lamin A scars’ that remain visible for hours and that may locally protect the nuclear membrane from further rupture.10,22

**Consequences of nuclear envelope rupture**

Micronuclei, composed of single chromosomes ‘lost’ from the main nucleus during improper mitotic segregation, have long been recognized as hotspots for DNA damage and chromothripsis, i.e., extensive chromatin fragmentation and rearrangements.23 Recent work suggests that micronuclei are initially protected by an intact NE, but often lose the micronuclei membrane integrity during interphase, resulting in extensive DNA defects in the micronucleus.23,24 Time-lapse microscopy and immunofluorescence staining of markers for DNA double strand breaks, such as γH2AX and 53BP1, reveal that NE rupture can similarly cause DNA damage in the main nucleus.10,11 During cell migration through small constrictions, new GFP-53BP1 foci form rapidly after NE rupture in several cell lines.10,11 In some cases, 53BP1 foci formation precedes NE rupture and may be caused by nuclear deformation.10 Furthermore, compaction of the ‘sponge-like’ nuclear interior during migration through tight spaces can temporarily displace water and soluble molecules, including components of the DNA damage repair machinery, from the confined regions of the nucleus and thereby delay or impair DNA repair.25 It is intriguing to speculate that DNA damage arising from nuclear deformation, fragmentation, and NE rupture during confined migration could contribute to the genomic instability of metastatic cancer cells.

**ESCRT-III proteins mediate nuclear membrane repair**

Stunningly, most of cells survive even repeated NE rupture and are able to restore NE integrity.6-11 Clues into the responsible NE repair mechanism have come from studying NE reformation during late anaphase, where the ESCRT machinery serves as an essential component for resealing any remaining gaps in the nuclear envelope.26-28 The ESCRT machinery was originally described as a vacuole-sorting complex,29 and subsequently recognized to mediate many additional cellular processes, such as plasma membrane repair, cytokinetic abscission, viral budding at the plasma membrane, endocytosis and secretion, and NPC insertion into the NE.30-32 In all of these processes, the ESCRT machinery mediates the membrane remodeling by promoting membrane curvature and, in most cases, driving membrane scission. The ESCRT machinery consists of multiple subunits, ranging from ESCRT-0 to ESCRT-III.ESCRT-0, -I, and -II subunits...
are often involved in cargo sorting at the vacuole and the recruitment of ESCRT-III components to this location. ESCRT-III subunits assemble into a spiral structure, in the process bending the membrane into a negative curvature and ultimately resulting in membrane scission.\textsuperscript{30,31} VPS4B, an AAA-ATPase, is recruited to the spiral structure and disassembles the ESCRT subunits after membrane scission.\textsuperscript{30,31} Intriguingly, only ESCRT-III members are required for nuclear membrane resealing during NE reformation and NE repair.\textsuperscript{10,11,26,27}

ESCRT dependent nuclear membrane repair following NE rupture (Fig. 2) appears to parallel in major parts the molecular mechanism of NE sealing during mitosis.\textsuperscript{9-11} When the NE ruptures, the newly created INM and ONM membrane ends likely fuse to each other to minimize exposure of hydrophobic fatty acid tails. This results in annular membrane channels (Fig. 2, II – III), similar in geometry to the nuclear membrane openings when the NE reforms around residual mitotic spindle microtubules in late mitosis.\textsuperscript{10,26-28} In both cases, ESCRT-III subunits and VPS4B drive the membrane scission required to separate INM and ONM and to restore NE integrity (Fig. 2, IV – VI).

A number of recent publications have provided additional insights into the molecular details of the recruitment and function of ESCRT components at the NE. One of the key nucleators for ESCRT-mediated NE remodeling is CHMP7. CHMP7 is a fusion of an ESCRT-II and an ESCRT-III-like protein,\textsuperscript{33} allowing it to bypass the upstream components of the canonical ESCRT pathway, in which ESCRT-II proteins recruit ESCRT-III subunits and VPS4B.\textsuperscript{10,27} CHMP7 is normally localized to the membrane of the endoplasmic reticulum (ER) through its curvature sensitive N-terminal membrane binding domain, but accumulates at the NE during NE reformation.\textsuperscript{28} CHMP7 initiates assembly of CHMP4B, the major ESCRT-III subunit, which ultimately drives nuclear membrane scission. Interaction of the ESCRT-III spiral with VPS4B is mediated by the ESCRT-III subunit CHMP2A, which is also incorporated into the ESCRT-III spiral.\textsuperscript{34} Interestingly, CHMP4B and VPS4B both accumulate at the rupture site within 2 min of NE rupture, and remain there for \textasciitilde 12 minutes.\textsuperscript{10} If VPS4B were only required for disassembly of the ESCRT-III complex after the membrane scission event, one would expect VPS4B to be recruited to the rupture site following CHMP4B, and not at the same time. The apparently parallel recruitment may indicate that VPS4B could be involved in remodeling the ESCRT complex even before membrane scission, and thus directly mediate the scission event, rather than only promoting ESCRT-III disassembly after membrane scission. Regardless of these molecular details, depletion of either CHMP2, CHMP4B, or CHMP7 or expression of a dominant negative VPS4B mutant delays NE reformation and NE repair, demonstrating the functional importance of these proteins in restoring NE integrity.\textsuperscript{9-11,26-28}

The mechanisms by which CHMP7 is recruited to sites of NE rupture to initiate ESCRT-III mediated NE repair are only beginning to emerge. Recent work suggests that LEM-domain proteins, normally in the INM, can recruit CHMP7 to exposed sites during NE reformation.\textsuperscript{35} In yeast, the LEM
domain proteins Heh1 and Heh2 both bind to CHM7, the yeast homolog of CHMP7, and this complex is linked to nuclear membrane sealing and the quality control of the nuclear pore complex. In interphase, CHMP7 is localized on the ER membrane, which is continuous with the ONM. Thus, CHMP7 and LEM-domain proteins, which reside in the INM, are on opposite faces of the intact NE. During NE reformation or NE rupture, however, the membrane annulus formed by the fusion of INM and ONM membrane ends (Fig. 2, III) could enable NPC-independent diffusion of membrane proteins between the INM and ONM, without control of the nuclear pore complex, and thus allow CHMP7 and the LEM-domain proteins to come into contact. The interaction between these proteins could then initiate ESCRT-III assembly and nuclear membrane remodeling. An additional signal for ESCRT-III assembly could come from the release of calcium from the ER and nuclear lumen during NE rupture, as it is implicated during in vitro NE reformation and nuclear pore formation, as well as paralleling the role of calcium influx in promoting ESCRT-III plasma membrane repair.

Conclusions and future directions

Cell migration through confined spaces, such as those found in interstitial spaces or during transendothelial migration, requires substantial nuclear deformation. The nuclear deformation can lead to NE rupture, nuclear fragmentation, and DNA damage. While cells rapidly repair the NE, enabling cell survival, the functional consequences of nuclear deformation and NE rupture remain to be assessed. In the short-term, nuclear deformation, fragmentation, and NE rupture could impact chromatin organization and transcriptional activity. In the long-term, they could promote genomic instability, particularly in proliferative cancer cells, whereas postmitotic immune cells may be less at risk. At the same time, inhibiting ESCRT-III mediated NE repair could present a novel therapeutic approach to target metastatic cancer cells. In proof-of-concept studies, treating cells with both a dominant negative VPS4B mutant to decrease NE repair efficiency and an ATM inhibitor to block DNA damage repair dramatically increased cell death after NE rupture. Since each treatment alone did not significantly reduce cell viability, it suggests that synthetic lethality was required for the effect. As both normal and cancer cells showed similar susceptibility to the treatment, future work will be needed to determine whether metastatic cancer cells have evolved unique features that enable them to withstand NE rupture and that could be exploited for specific targeting. If successful, drugs targeting such pathways could ultimately reduce metastatic cancer spreading while sparing normal cells.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

The authors are supported by awards from the National Institutes of Health (R01 HL082792 and U54 CA210184, to J.L.), the Department of Defense Breast Cancer Research Program (Breakthrough Award BC150580, to J.L.), and the National Science Foundation (CAREER Award CBET-1254846, to J.L.).

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ORCID

Jan Lammerding http://orcid.org/0000-0003-4335-8611
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