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Review Article

Causes and Consequences of Nuclear Envelope Rupture and DNA Damage in Micronuclei

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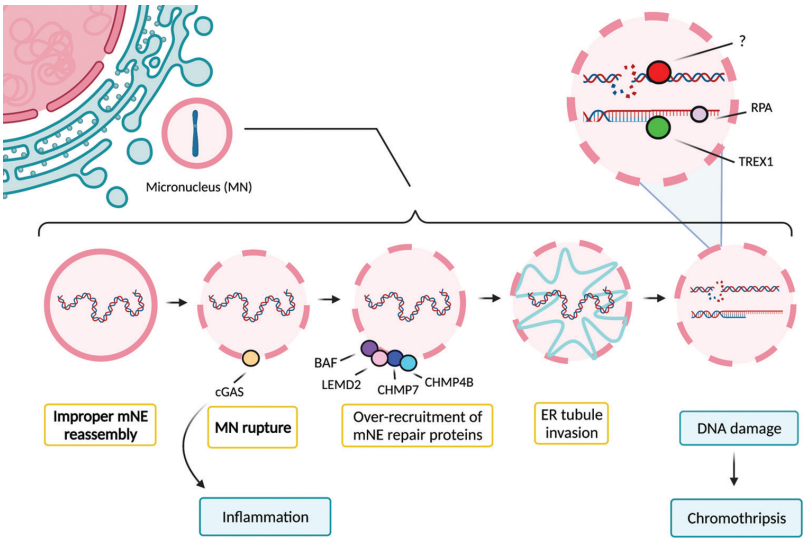
ABSTRACT

Micronuclei are small, aberrant nuclear compartments containing mis-segregated chromosomes or chromosomal fragments. During telophase, dysfunctional micronuclear envelope reassembly leaves the micronuclear envelope highly unstable and rupture-prone. Following rupture, micronuclei attempt to repair membrane gaps, but the process is typically unsuccessful and may promote the invasion of ER tubules into the interior of micronuclei. These abnormalities cause ruptured micronuclei to accumulate significant DNA damage in the form of both single-stranded DNA and double-stranded breaks. Because micronuclei are capable of promoting genome instability, it is essential to understand the sources of DNA damage and the mechanism through which it arises in these structures. In this review, I will explore the causes and consequences of micronuclear envelope rupture, beginning with the processes surrounding improper micronuclear envelope reassembly. I will then discuss micronuclear envelope rupture, attempted micronuclear envelope repair and its consequences, and the proposed causes of micronuclear DNA damage.



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INTRODUCTION

The nuclear envelope (NE) maintains a semi-permeable barrier encompassing the nucleoplasm and consists of two lipid bilayers, the inner and outer nuclear membranes (INM and ONM, respectively), which are the primary regulators of compartmentalization (1). Immediately adjacent to the INM is the lamina, a dense, filamentous network of lamin proteins that provides structural support to the nucleus and aids in chromatin organization (2, 3, 4, 5). To facilitate import and export between the cytoplasm and nucleoplasm, nuclear pore complexes (NPCs) form a channel-like structure that extends through both the INM and ONM (6). Adjoining the NE, the endoplasmic reticulum (ER) is contiguous with the cytoplasm-facing ONM, while peripheral heterochromatin is contiguous with the nucleoplasm-facing lamina (7, 8). During prometaphase, the NE breaks down and leads to dispersion of soluble components into the cytoplasm and the retraction of non-soluble components into the ER (9, 10, 11).

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The NE is later reassembled at decondensing chromosomes in telophase by recruiting both ER membranes and soluble nuclear proteins (reviewed in 9).

Micronuclei (MN), while relatively uncommon in healthy cells, are found frequently in cancerous tissues (12). These compartments often arise from lagging chromosomes (LCs), which, due to varying mitotic defects, fail to migrate properly during anaphase and consequently linger in the mitotic midzone (13). In addition to LCs, MN can originate from extrachromosomal or acentric chromosome fragments, defective NE reassembly, and many more cellular abnormalities (reviewed in 14). Because the vast majority of studies involving MN artificially induce LCs through mitotic defects, this review will focus solely on MN generated by mis-segregation errors in mitosis.

During NE reassembly following anaphase, MN are unable to completely recruit necessary NE components and frequently rupture during the subsequent interphase due to the unstable nature of their only partially-formed NEs (15). Ruptured MN pose numerous concerns to genome stability, as they accumulate large amounts of DNA damage and are unable to properly execute replication, transcription, and DNA damage repair (16). MN therefore serve not only as passive indicators of chromosomal instability, but as direct contributors.

These structures further impair genome stability by promoting chromothripsis, in which chromosomes or chromosomal regions fragment then repair in a highly error-prone manner, leading to large-scale chromosomal rearrangements (17). Chromothripsis can provide cancerous cells with a selective advantage as it accelerates their genomic evolution and increases their chance of obtaining an advantageous mutation (18, 19, 20). Further research of MN is therefore of great interest, as it may reveal critical insights into the etiology of oncogenesis and cancer mutagenesis. Despite promoting DNA damage and rearrangement, MN may also trigger anti-tumor immunity through cGAS-mediated inflammation and thus may have an inhibitory effect on cancer progression as well **(**21, 22).

This review seeks to explore the sources and the underlying mechanism of DNA damage in MN. I will first compare the composition of the micronuclear envelope (mNE) to that of the primary nuclear envelope (pNE) and assess proposed causes of defective mNE reassembly. I will then review the varying definitions of mNE rupture and examine possible triggers of interphase rupture. Next, I will discuss defects in mNE repair processes, possible causes of ER tubule invasion of MN, and alternative NE repair pathways. Lastly, I will explore proposed causes of both single-stranded DNA and double-stranded breaks in MN before concluding with a discussion of the broader outcomes of MN rupture.

THE MNE ASSEMBLES IMPROPERLY AND IS RUPTURE PRONE

MN and Primary Nuclei Differ in NE Composition

Due to reassembly defects, the mNE envelope has a unique protein composition, particularly in regards to the lamina, nuclear pore complexes, and LEM-domain proteins. The following section will examine the functionality of each NE component listed above and will summarize their status at the mNE envelope in comparison to that of primary nuclei (PN).

The nuclear lamina, a thin, fibrous meshwork composed of lamin proteins, tethers the NE to heterochromatin ends and provides structural support to the nucleus (3, 4, 5). It plays a role in many key nuclear functions, including genome organization, gene expression, DNA replication, and nuclear compartmentalization. The lamina is composed of three types of proteins: Lamins A, B, and C. For the purposes of this review, Lamins A and C will be grouped together as Lamin A/C, as both are transcribed from the gene LMNA and differ only by alternative splicing (23).

Numerous studies have discovered deficiencies in mNE lamina composition, particularly in the assembly of Lamin B1. Micronuclear Lamin B intensity is significantly lower than that of PN, and many MN are completely deficient for Lamin B (15, 24, 25, 26). Ruptured MN similarly lack Lamin B (16). However, results of Lamin A/C presence at MN vary between studies. Some have found that Lamin A/C intensity and frequency of presence is comparable to that of PN in MN induced by Nocodazole and Colchicine (15, 25). Contrarily, about one third of MN induced by Paclitaxel lack Lamin A/C (26), and nearly all ruptured MN in KIF18A KO cell lines were deficient for the protein (27). These differences in results may be associated with the type of treatments used to induce MN, as both studies that observe typical Lamin A/C levels at MN use microtubule depolymerizing agents.

Nuclear pore complexes (NPCs) allow import and export between the cytoplasm and nucleoplasm and are critical for proper localization of various proteins, RNAs, and more. These complexes consist of a nuclear ring and basket, a scaffold, a central channel, a cytoplasmic ring, and cytoplasmic filaments (6). The NPC traverses both the INM and ONM, with the scaffold and central channel positioned primarily in the perinuclear space, the nuclear basket extending into the nucleus, and the cytoplasmic filaments into the cytoplasm. In MN, the nucleoporins Nup153 (in the nuclear basket), Nup62 (in the central channel), and Nup358 (in the cytoplasmic section), have been studied most extensively. Like Lamin B, assembly of these nucleoporins is often defective at MN in terms of both protein intensity and presence (15, 24, 26). Ruptured MN similarly exhibit less intense Nup153 compared to PN (16).

The Lap2-Emerin-Man1-domain (LEM-domain) proteins are a family of INM proteins with the ability to bind to both the lamina and peripheral heterochromatin (28). They are recruited to the NE by Barrier-to-Autointegration Factor (BAF) and play key roles in gene expression regulation, chromatin organization, signal transduction, NE assembly, and NE repair (29, 30, 31, 32). Within the LEM-domain protein family, Emerin and Lap2a have been studied substantially at MN. Contrary to other NE components, both Emerin and Lap2a are present and often enriched at MN (15, 24). Lap2a presence is also associated with CHMP7 enrichment, which commonly occurs at ruptured MN (33).

These differences in protein accumulation correspond with the pattern of core and non-core NE protein recruitment (15). Following mitosis in PN, ER membranes are recruited to and expand around decondensing chromosomes to reform the NE (reviewed in 9). This membrane expansion, however, is not uniform across the PN chromatin: membranes adjacent to the mitotic spindle/midzone region, labeled "core" regions, observe delayed assembly in comparison to "non-core" regions, which are located peripheral to the spindle/midzone (30, 34, 35). Both regions accumulate unique sets of proteins as well, with core regions recruiting BAF, Lamin A, and LEM-domain proteins and non-core regions recruiting Lamin B and NPC components. Contrarily, in MN, non-core proteins fail to assemble, resulting in a mNE composed entirely of core proteins (15).

Causes of Defective mNE Assembly

MN presence in the midzone causes NE assembly issues

It is commonly agreed that the midzone plays a role in aberrant mNE assembly, as treatments that limit exposure of LCs to the midzone also decrease the prevalence of mNE reassembly defects (15, 27). A recent study found that generating MN with KIF18A KO, as opposed to the more commonly used nocodazole treatment, increased the frequency of lamin recruitment to MN (27). KIF18A KO generates MN by causing improper metaphase alignment of chromosomes, elongated MTs, decreased tension between kinetochores, and increased MT oscillation intensity (36, 37). It may also increase the speed of chromosome movements, although results regarding speed remain controversial. Contrarily, nocodazole treatment induces MN by impeding MT polymerization.

Sepaniac et al. 2020 demonstrated that nocodazole-generated MN lag in the midzone for longer periods of time than MN generated by KIF18A KO, suggesting that prolonged time spent in the midzone may be responsible for micronuclear reassembly defects. Similarly, a study that induced MN to localize peripheral to the mitotic spindle observed restoration of mNE reassembly (15). However, it is still unclear through what mechanism the commonly observed mNE reassembly defects occur. Two main theories exist, the first proposing an Aurora B, chromosome condensation-based mechanism (38, 39), and the second proposing a physical MT barrier (15).

Proposed causes of aberrant mNE reassembly

Afonso et al. 2014 suggests that an Aurora B midzone gradient prevents chromosome decondensation, the lack of which inhibits NE reassembly. Aurora B kinase, a chromosomal passenger complex component, mediates the spindle assembly checkpoint by ensuring proper microtubule-kinetochore attachment during mitosis (40). Afonso et al. 2014 suggests that the midzone Aurora B gradient also maintains Condensin-I at chromosomes to prevent premature Lamin B and Nup107 reassembly. The study proposes that LCs, however, lag in the midzone for prolonged periods of time, unable to decondense chromosomes and recruit Lamin B and Nup107. Although this study does not account for differences in core and non-core assembly, only non-core proteins were investigated, thus it is possible that core protein assembly was functional and not affected by Aurora B.

De Castro et al. 2017 proposes that varying mechanisms exist to regulate mNE protein loading. The study suggests that kinases CDK1 and PLK1 serve as negative regulators of premature mNE reassembly, and that Lamin A loading requires loss of CDK1, while nucleoporin loading requires loss of both CDK1 and PLK1. The authors conclude that Lamin A loading is time-dependent, while NPC loading is both time-dependent and spatially regulated by a midzone pool of PLK1. Importantly, phosphorylation of PLK1 by Aurora B localizes PLK1 to the midzone (41), indicating that, consistent with Afonso et al. 2014, Aurora B loss may be necessary for complete mNE reassembly. These results suggest a mechanism in which reassembly of NPCs, but not Lamin A/C, is dependent on MN location relative to the midzone during mitosis. Because NPC components are classified as non-core and Lamin A/C as core, these differences in recruitment could extend to other non-core and core proteins so that non-core protein recruitment is spatially regulated by Aurora B and core protein recruitment is only regulated by CDK1.

It should be noted, however, that significant portions of the data from this study are derived more generally from mitotic cells and their reforming pNEs as opposed to specifically from LCs. Although the study does demonstrate that both the inhibition of PLK1 and Aurora B increases the frequency of mAB414-positive LCs, it does not explicitly compare the effect of CDK1 inhibition to PLK1 and Aurora B inhibition at these structures. While it is possible that these mechanisms of protein loading regulation extend to MN, additional research is necessary to confirm this hypothesis.

Contrarily, Liu et al. 2018 proposes that spindle MT bundles may impose a physical barrier to protein reassembly. The study found that stabilization of MTs with Paclitaxel, a MT depolymerization inhibitor, prevents non-core protein assembly at PN chromosome regions covered by MTs. Furthermore, loosening MT bundling through inhibition of the kinesin KIF4 (42) reversed micronuclear reassembly defects. Liu et al. 2018 suggests that, due to the small size and midzone location of MN, these structures are completely encompassed by MTs, which renders them unable to properly load non-core proteins.

Analysis of the proposed causes of aberrant mNE reassembly

There are two fundamental disparities between the hypotheses presented in the above three studies: (1) whether Aurora B (possibly via PLK1) or MTs obstruct mNE reassembly, and (2) whether or not reassembly is hindered by chromosome condensation. In addition to proposing MTs block mNE reassembly, Liu et al. 2018 presents evidence against the involvement of Aurora B and chromosome condensation in mNE defects.

The study found that inhibition of Aurora B at anaphase onset caused MT disassembly, indicating that reduced MT

density, as opposed to Aurora B absence, could have caused the restoration of mNE reassembly seen in Afonso et al. 2014 and de Castro et al. 2017. Because both of these papers inhibited Aurora B either at or prior to anaphase onset, it is possible that MTs in these studies were not functioning properly when data was collected. Indeed, α-tubulin presence and organization in Afonso et al. 2014 was clearly diminished by Aurora B inhibition. This phenomenon can be attributed to a positive feedback loop during anaphase involving Aurora B and MTs, in which MTs activate and maintain the Aurora B midzone gradient, and Aurora B phosphorylates proteins that regulate MT organization (43, 44). PLK1 inhibition similarly altered MT behavior by inducing monopolar spindles (39).

It should be noted that inhibition of Aurora B did not impact spindle formation and intensity in de Castro et al. 2017. However, the length of Aurora B inhibitor treatment differed greatly between the experiment assessing spindle functionality and the experiment investigating the effect of Aurora B inhibition on mNE reassembly (15 minutes and 4 hours, respectively), raising the possibility that Aurora B levels may have differed between the two conditions.

However, as discussed previously, Liu et al. 2018's primary source of evidence that MTs obstruct mNE reassembly is the restoration of non-core protein loading upon KIF4 KD. Critically, KIF4 KD resulted in changes in Aurora B localization patterns, suggesting that KIF4 KD may impact Aurora B functionality (15). In fact, studies have demonstrated KIF4 KD diminishes the activity of both Aurora B and PLK1 (45). Overall, the mitotic components assessed in these three studies (MTs, Aurora B, PLK1, and KIF4) are all interdependent on one another, thus it is difficult to deplete one without affecting another. Further research with careful experimental design is necessary to truly determine the cause of aberrant mNE reassembly.

Liu et al. 2018 also found that, contrary to Afonso et al. 2014, NPCs frequently assembled on LCs with high levels of Condensin. Afonso et al. 2014 did not explicitly study Condensin activity at LCs but did suggest that, because Condensin prevents premature pNE reassembly and is enriched in LCs, the complex may hinder mNE reassembly. Supporting this hypothesis, MN that fail to decondense chromatin often lack Lamins (27). Due to the evidence presented in Afonso et al. 2014 and Sepaniac et al. 2020, additional research regarding the role of Condensin in mNE reassembly is warranted.

High Curvature Membranes are Deficient for Lamin B

In addition to the two hypotheses discussed above, multiple studies have discovered a negative correlation between Lamin B and membrane curvature, raising the possibility that the highly curved mNE may be unable to retain Lamin B (25, 46, 47). This may be related to physical filament characteristics: Lamin B has been previously described as elastic but stiff (similar to a spring), while Lamin A has been characterized as more viscous (48). Regardless of the precise mechanism of membrane curvature-based inhibition of proper NE assembly, it seems likely that there are multiple factors limiting the assembly of Lamin B.

MN RUPTURE DURING INTERPHASE

It should be emphasized that aberrant NE reassembly is not synonymous with NE rupture: NE reassembly failure results in deficient NE composition and occurs at the end of mitosis (15), while rupture results in loss of compartmentalization and occurs at varying times during interphase (16). Because some intact MN lack Lamin B and NPCs (15), and overexpression of Lamin B2 decreases rupture frequency (16), MN with assembly defects are best considered "rupture-prone" as opposed to "ruptured." Experimental definitions of NE rupture, however, do vary. The following section will review the most commonly used rupture indicators, their mechanisms, and what specifically they indicate about the integrity of the NE.

Definitions and Indicators of NE Rupture

Nuclear localization and export signals (NLS and NES respectively), the most established indicators of NE integrity, are short amino acid sequences in cargo proteins that allow protein movement through NPCs. To assess NE intactness by fluorescent visualization, individual signals are fused to fluorescent proteins (15, 16, 25, 31, 49, 50, 51, 52, 53, 54, 55).

An NLS allows movement of proteins from the cytoplasm to the nucleus through an importin and Ran-based mechanism. In the cytoplasm, nuclear import proteins Importin-α and Importin-β complex with an NLS-containing cargo protein and proceed through the NPC (56, 57). Inside the nucleus, RanGTP dissociates the complex, releasing the imported protein. In NLS-based definitions of rupture, NLS is lost from the nucleus, indicating that proteins containing an NLS can diffuse out of the nucleus (15, 16, 25, 31, 49, 50, 51, 52, 53, 54, 55).

An NES allows movement of proteins from the nucleus to the cytoplasm through an exportin and Ran-based mechanism. In the nucleus, exportin XPO1 complexes with RanGTP and a NES-containing cargo protein and the trimeric complex is exported through an NPC (58, 59). Upon arriving in the cytoplasm, RanGTP is hydrolyzed into RanGDP and the entire complex disassembles (60). In NES-based definitions of rupture, NES is gained in the nucleus, indicating that proteins containing NESs can diffuse into the nucleus (50).

Barrier-to-autointegration factor (BAF) presence can also be used to indicate rupture. BAF is a DNA-binding protein involved in chromatin organization, NE reformation, and NE repair (61, 62). The protein is present within the cytoplasm and nucleus in both phosphorylated and non-phosphorylated states (63, 64). Importantly, a pool of cytoplasmic, unphosphorylated BAF serves as a first responder to NE rupture sites, binding to exposed chromatin and initiating the NE repair pathway (31, 32). In BAF-based definitions of rupture, hyper-accumulation of BAF at the NE indicates rupture site presence (15).

cGAS, which binds to and triggers an inflammatory response upon detection of cytosolic DNA, is used as a rupture

indicator as well. The protein commonly initiates such responses for invading viral DNA, but because it is unable to distinguish between foreign and non-foreign DNA, it can also cause autoimmune responses upon contacting self-DNA in the cytosol (65). During mNE rupture, DNA may exit the nucleus or be exposed to the cytoplasm, providing a substrate for cGAS activation (66). Although cGAS is present in the nucleus (67), exposure of DNA to the cytoplasm results in significant increases in cytoplasmic cGAS localization to MN (51), thus cGAS presence at MN can indicate a rupture event.

H3K9Ac, an indicator of active transcription, can also be used to assess NE integrity (68, 69). Following NE rupture, MN lose the ability to transcribe DNA and therefore also lose the nuclear presence of H3K9Ac (16, 51). Although H3K9Ac-based indication of rupture is accurate, it is somewhat less stringent in regards to rupture timing than other methods, likely because it signals loss of transcription resulting from NE rupture as opposed to directly signaling NE rupture (70).

Triggers of MN Rupture

The direct triggers of interphase MN rupture are poorly understood. PN rupture, however, has been researched in significantly more depth, and it is possible that MN rupture is caused by similar mechanisms. The following section will examine two causes of PN rupture that may also be responsible for MN rupture: actomyosin-induced NE stress (71, 72) and nuclear migration (73).

To regulate primary nucleus shape, the cell employs a perinuclear actomyosin cap spanning the NE that controls to what extent the nucleus is compressed (71, 72). Because the NE contributes to chromatin organization, proper shape regulation is necessary for appropriate gene expression (74, 75). In cells deficient in Lamin B, however, pressure exerted by actomyosin can overstress the already weakened NE, leading to rupture (54). Accordingly, treatment of Lamin B1-deficient cells with Cytochalasin D, an inhibitor of actin association and polymerization, was found to decrease NE rupture frequency. Importantly, Cytochalasin D had no impact on the frequency of MN rupture, indicating that MN rupture occurs independently of actin. Similarly, treatment with Latrunculin A, another actin inhibitor, also had little effect on MN rupture frequency (15). It is therefore unlikely that an actomyosin-based mechanism causes MN rupture.

Nuclear migration can cause NE rupture as well, and may also be responsible for MN rupture. Nuclear mobility, mediated by microtubule motor proteins Kinesin-1 and dynein, is critical for a variety of cellular processes, including cell division, cell migration, establishment of cell polarity, and differentiation (76, 77, 78, 79, 80). A recent study found that depletion of Kinesin-1 subunit Kif5B in Lamin A KO cells reversed the presence of chromatin protrusions and cGAS activation at nuclei, suggesting that Kinesin-mediated nuclear migration may cause NE rupture in cells lacking a stable lamina (73). Research in MN mobility and interactions with motor proteins is still very limited, but it is possible that MN could migrate within the cell and trigger rupture of the mNE.

AN ATTEMPT AT MNE REPAIR

Repair of NE ruptures, as well as lysosome and plasma membrane ruptures, is typically promoted by the ESCRT-III complex (endosomal sorting complex required for transport), which restores the contiguity of lipid membranes (52, 53, 81). ESCRT-III-mediated repair can be categorized into two processes: the ESCRT-III recruitment pathway and the sealing of membrane gaps (82). ESCRT-III is also recruited to ruptured MN, although MN rupture is frequently irreversible due to problematic repair pathway alterations that cause unrestrained ESCRT-III activity at the mNE and ultimately result in ER tubule invasion of MN (16, 33, 50). The following section will provide an overview of the typical NE repair pathway and sealing process and will then discuss repair pathway complications and their consequences in MN.

Typical NE Repair Process

Unphosphorylated, cytoplasmic BAF is recruited to rupture sites first, binding with dsDNA exposed to the cytosol and initiating the NE repair pathway (31, 32). BAF also forms a dense diffusion barrier that limits nuclear entry of objects greater than at least 49 nm in diameter to minimize improper exchange of materials between the cytoplasm and nucleoplasm (83). It should be noted that in plasma membrane repair, Ca2+ influxes are responsible for initiating the repair pathway (84, 85). However, it has recently been shown that Ca2+ is not involved in NE rupture signaling, further suggesting that BAF serves as the primary rupture site detector (86).

BAF then recruits the INM protein LEMD2 to rupture sites (31, 50, 86, 87, 88). Upon exposure of the WH domain of LEMD2 to the cytosol, CHMP7, an ER-residing protein that serves as an ESCRT-III adaptor, diffuses into the nucleus and complexes with LEMD2 at the rupture site (50, 86, 88). The LEMD2-CHMP7 complex next recruits the ESCRT-III complex, which includes proteins CHMP4A-C, CHMP1A-B, CHMP2A-B, CHMP3, CHMP5, and IST1 (86, 88, 89). When the ESCRT-III subunit CHMP4B successfully complexes with LEMD2-CHMP7, the final membrane repair protein, AAA-ATPase VPS4 is recruited to the rupture site (86, 88).

At the rupture site, ESCRT-III subunits form a spiral-like structure spanning the membrane gap (82, 90). To re-seal the ONM/INM, the ESCRT-III filaments, with the help of VPS4, gradually constrict until the membranes fuse (91). VPS4 then disassembles the LEMD2-CHMP7-CHMP4B complex (88), and CHMP7, which contains an NES, exits the nucleus by XPO1-mediated export (50, 86).

mNE Repair Process and ER Tubule Invasion

However, the micronuclear repair pathway experiences complications that lead to excess presence of CHMP7 and subsequent over-recruitment of the ESCRT-III subunit CHMP4B (33, 50). This excessive CHMP7/4B presence has been found to promote nuclear membrane deformation and is also associated with ER tubule invasion of the nucleus (50, 86). Excess presence of CHMP7/4B proteins could result from either

CHMP7 over-recruitment and/or CHMP7/4B persistence at the mNE. Vietri et al. 2020 recently demonstrated that, due to their small size, MN are easily overloaded with CHMP7 and are thus unable to restrict LEMD2-CHMP7 complexes to the rupture site. These complexes subsequently spread across the entire mNE, recruiting excessive levels of CHMP4B/ESCRT-III. This inability to restrain CHMP7-LEMD2 complexes is likely the primary driver of excess CHMP7/4B presence. However, it may also be possible that LEMD2-CHMP7-CHMP4B complexes are not properly disassembled by VPS4 and, as a result, persist in the micronuclear interior. Although VPS4 does function at least partially at MN (33, 50), comparisons to PN suggest that the micronuclear complex disassembly process may be impaired or not fully executed by VPS4 (33). Additional research, such as utilizing VPS4 over-expression, may illuminate this protein's role at MN.

As mentioned previously, excess nuclear CHMP7/4B presence is associated with membrane deformation and ER tubule invasion of MN. A recent study found that cells expressing CHMP7 lacking NES or depleted of both VPS4 and POM152 exhibited ER fenestrations and sheet-like herniations, suggesting ER membranes may invade upon nuclear CHMP7 over-recruitment (86). Consistent with these findings, ER tubules are frequently enriched in CHMP7+ MN (33). Nuclear ER tubules also raise questions regarding the BAF diffusion barrier: tubular membranes are typically 60 - 100 nm in diameter (92), while BAF was found to limit nuclear diffusion of objects greater than 49 nm in diameter in primary nuclei (83). It is therefore unclear how ER tubules successfully enter the micronuclear interior. Perhaps only thinner ER tubules invade - tubules with diameters as small as 25 nm have been reported in human cells (93), and the BAF barrier may permit diffusion of objects this size. It is also possible that the BAF barrier is in some way disassembled or nonfunctional at MN. Regardless, very little is known about the ER tubule invasion process, and more research in this area is particularly important.

Alternative NE Repair Processes

Although ESCRT-III mediated repair is the most commonly studied NE repair process, it can only close gaps up to 60 nm (94). Penfield et al. 2020 found that larger NE gaps are instead repaired by de novo glycerolipid synthesis mediated by phosphatases lipin and CNEP-1 (95). Lipin catalyzes the dephosphorylation of phosphatic acid to form diaglycerol, shifting lipid production towards glycerophospholipids and away from phosphatidylinositol (PI) (96, 97). The study found that elevated PI levels caused by CNEP-1 or lipin depletion result in membrane invasion of nuclei. Although the rupture size at MN remains unclear, it is possible that a lipid synthesis-based mechanism is involved in mNE repair, and that the ER tubule invasion commonly described at MN could be a result of abnormalities in the CNEP-1-lipin pathway.

MN ACCUMULATE DNA DAMAGE

Following rupture events, MN accumulate DNA damage in the forms of single-stranded DNA (ssDNA), marked by RPA, and double-stranded breaks (DSBs), marked frequently by γH2AX. Although studies show this damage is linked to rupture, the precise causes are unclear. The following section will explore proposed origins of both ssDNA and DSBs at MN.

Causes of ssDNA in MN

Multiple studies have observed an association between RPA accumulation and mNE repair pathway proteins: knockdown of each LEMD2, CHMP7, and CHMP4B diminishes micronuclear RPA presence (33, 50), suggesting that ssDNA arises as a result of ESCRT-III activity or ER tubule invasion. The direct cause of ssDNA is likely the 3' cytoplasmic exonuclease TREX1, which is frequently enriched at MN and has been previously found to degrade DNA in chromatin bridges (17, 50, 51). TREX1 typically serves as a barrier against cGAS-mediated autoimmunity in the event that self-DNA is released into the cytosol, as cGAS is unable to distinguish between foreign and self-DNA (65, 98). To quickly degrade self-DNA prior to cGAS activation, TREX1 is situated just outside the nucleus in the ER, to which it is bound to by its C-terminus (98, 99). TREX1's nuclease domain-containing N-terminus faces outwards towards the cytosol (100). Due to TREX1's ER localization, it is possible that it enters MN during ER tubule invasion. Indeed, detachment of TREX1 from the ER by C-terminus deletion prevents micronuclear RPA accumulation (51). Because TREX1 is an exonuclease, it requires nicked DNA to initiate degradation (99). Although the origins of these nicks in MN DNA remain unclear, the base excision repair endonuclease APE1 may be involved (51). In summary, ssDNA likely arises as a result of TREX1-mediated degradation following ER tubule invasion and DNA priming.

Causes of DSBs in MN

While causes of DSBs in MN are far less clear, current research suggests that MN rupture, replication issues, and abnormal enzymatic activity may be involved in DSB formation. Multiple studies have discovered an association between γH2AX accumulation (an indicator of DSB presence) and mNE rupture, with γH2AX foci significantly more prevalent in MN lacking Lamin B1, lacking NLS, or containing cytoplasmic localization signal (CLS) (15, 16, 25, 46). Accordingly, Lamin B2 overexpression, which prevents rupture, decreases the frequency of γH2AX-positive MN (16). This suggests that consequences of MN rupture, such as cyto- and nucleoplasmic mixing, ESCRT-III mediated repair, and ER tubule invasion, may be involved in DSB formation. Furthermore, DSBs commonly arise during S phase in ruptured, and thus non-replicating, MN (16, 18, 101). A micronuclear attempt at replication might induce formation of stalled replication forks and other replication intermediates that ultimately lead to DSBs through processes such as microhomology-mediated break induced replication (MMBIR) (55). Still, it remains unclear whether or not MN even attempt to initiate replication to generate stalled replication forks.

Additionally, lower temperatures significantly decrease the percent of TUNEL-positive MN, suggesting that enzymes are necessary for DSB formation (25). APE1, TOP2B,

and TREX1 have each been indicated as potentially involved (50, 51, 102). APE1 is an endonuclease in the base excision repair pathway tasked with nicking DNA at abasic sites to allow for polymerase-mediated repair (103). Overexpression of APE1 has been reported to increase the percentage of γH2AX-positive MN (102), although a similar experiment conducted in Mohr et al. 2020 demonstrated that APE1 had only a modest effect on γH2AX accumulation. Both a pair of APE1-mediated nicks situated nearby one another on opposite strands and a single nick at a replication fork could cause a DSB (102). In order for APE1 to cause DSBs, however, abasic sites must be present in the DNA, thus differences in abasic site frequency could explain these differences in results. Abasic sites have not yet been studied at MN and warrant additional investigation.

Furthermore, topoisomerase TOP2B, which relieves torsional stress during replication and transcription by transiently generating DSBs, has been observed to colocalize with γH2AX foci in PN containing nuclear CHMP7 (50). A direct association between TOP2B and γH2AX has not yet been established, but it is possible that TOP2B could be involved in DSB formation. Additionally, although TREX1 can only generate ssDNA, it could serve as an upstream requirement for DSBs in certain cell lines. TREX1 knockdown has limited impact on γH2AX presence in RPE1, HEK293T, and U2OS cell lines, but knockdown does modestly decrease the percentage of γH2AX-positive MN in MCF10A cell lines (51). This raises the possibility that the origins of micronuclear DSBs are cell-line specific. Still, it seems that the majority do not require TREX1 for DSB generation.

OUTCOMES OF MNE RUPTURE AND DNA DAMAGE

Beyond DNA damage, consequences of MN rupture include chromothripsis and cGAS-mediated inflammation (18, 19, 20, 21, 22). During chromothripsis, significant DNA damage leads to the formation of numerous small DNA fragments detached from the larger chromosomal mass (17). Although these fragments undergo repair by non-homologous end joining, this “repair” process ligates fragments together in a random fashion with little to no respect for the original DNA sequence, resulting in a highly rearranged chromosome/chromosomal region. Such alterations can cause aberrant gene expression, improper mRNA transcript formation, and countless other abnormalities (104). Because MN tend to accumulate DNA damage, they are especially prone to chromothripsis and thus present a serious threat to genome stability (18, 19, 20).

Conversely, MN may have an anti-tumor effect by promoting an immune response through the initiation of the cGAS-STING pathway (21, 22). cGAS, briefly discussed as an indicator of mNE rupture in a previous section, acts as a sensor of cytosolic DNA and often detects micronuclear DNA following a rupture event (66). Upon activation, cGAS initiates a signalling cascade involving cGAMP, STING, and TBK1 that ultimately leads to activation of IRF3 and NF-kB, transcription factors that elicit a type I interferon response (105, 106, 107). Such a response may promote anti-tumor immunity (22, 105).

CONCLUSION

In this review, I have discussed the origins of micronuclear DNA damage through five key processes: improper mNE reassembly, MN rupture, attempted mNE repair, ER tubule invasion, and accumulation of ssDNA and DSBs. To conclude, I will suggest further directions of research that may help illuminate the mechanisms preceding and directly causing micronuclear DNA damage.

While significant advancements in the understanding of MN rupture have been made in the past ten years, many questions surrounding these structures remain. For instance, although substantial research has been conducted on processes that render MN rupture-prone, very little is known about the actual trigger of interphase MN rupture. Studies of PN suggest that nuclear migration may serve as a catalyst (73), thus future research might explore whether MN migrate within the cell, and if so, whether this migration can contribute to rupture.

Further work is also needed to fully understand the mechanism of micronuclear ER tubule invasion. Over-recruitment of CHMP7 at the NE seems to be associated with ER membrane protrusions of the nucleus (50, 86), but the cellular processes surrounding tubule recruitment and entry through the BAF barrier are still largely unknown. Because a clear comprehension of the mNE repair process is crucial to understanding ER tubule invasion, investigation into whether lipid synthesis-based repair is used at MN may also prove insightful.

Perhaps the most pressing matter in need of additional study is the precise cause of micronuclear DSBs. While ssDNA accumulation can be attributed to TREX1, causes of DSBs remain unclear. It seems likely that there is an enzymatic basis to this damage (25), thus characterizing DSB-causing proteins over-expressed at MN may hold promising results.

Ultimately, while the study of MN is only just beginning, further research may hold promising insights into specific consequences of DNA damage, as well as broader mechanisms of genome instability and mutagenesis in cancerous cells.

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None.

ETHICAL ISSUES

None.

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