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Causes and consequences of micronuclei

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Abstract

Micronuclei are small membrane bounded compartments with a DNA content encapsulated by a nuclear envelope and spatially separated from the primary nucleus. Micronuclei have long been linked to chromosome instability, genome rearrangements, and mutagenesis. They are frequently found in cancers, during senescence, and following genotoxic stress. Compromised integrity of the micronuclear envelope delays or disrupts DNA replication, inhibits DNA repair, and exposes micronuclear DNA directly to cytoplasm. Micronuclei play a central role in tumorigenesis, with micronuclear DNA being a source of complex genome rearrangements (including chromothripsis) and promoting a cyclic GMP–AMP synthase (cGAS)-mediated cellular immune response that may contribute to cancer metastasis. Here, we discuss recent findings on how micronuclei are generated, what the consequences are, and what cellular mechanisms can be applied to protect against micronucleation.

Introduction

Micronuclei (MN) are small nuclei-like structures formed by nuclear envelope deposition around lagging chromosomes or chromosome fragments that persist into interphase after failing to be reincorporated into a primary nucleus following completion of mitosis or meiosis. The prefix “micro” is a bit of a misnomer that would more accurately be replaced with “decinucleus” for a tenth (rather than a millionth) of the size of a typical nucleus. Accumulation of MN has been extensively used as a biomarker of genotoxic stress and genetic instability in a great variety of human and non-human models [1,2]. MN frequently possess defects in their nuclear envelope (NE), the barrier that both protects the genome from the cytoplasmic environment and mediates proper nucleocytoplasmic transport. NE rupture results in the damage of micronuclear DNA due to disruption in DNA replication and repair as well as exposure of interphase DNA/chromatin to cytoplasm [3–5]. Multiple lines of evidence have pointed to MN as a source of recently described complex genome

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rearrangements, including chromothripsis, which is a hallmark of many cancer types, especially most aggressive ones [3,5–8].

In cancer cells, MN can end up degraded by an autophagy-lysosomal pathway [9,10]. Cytoplasmic exposure of MN DNA can trigger a cellular immune response by recruiting the DNA sensor cGAS that initiates inflammatory gene expression, which has been reported to contribute to metastasis [11–13]. MN-like structures have also been described in non-dividing senescent cells under the name of “cytoplasmic chromatin fragments”, or CCFs, which are apparently formed as a result of a poorly understood process of nuclear budding. Similar to MN, CCFs lack important NE elements, such as lamin A/C or lamin B1, induce cellular immune response via cGAS, and can be cleared by autophagy [14–18].

Causes of micronuclei formation

MN arise from (1) lagging chromosomes or (2) acentric chromosome fragments that do not incorporate into daughter nuclei, but which are encapsulated into a separate (and frequently abnormal) NE [19,20]. While disruption of many aspects of spindle assembly have long been known to produce MN (especially transient exposure to drugs that affect microtubule assembly [19]), additional molecular players and mechanisms have been recently implicated in the formation of MN (see below).

1. Factors responsible for chromosome lagging include malfunctional centromeres and kinetochores, aberrant kinetochore-microtubule attachments and defects in mitotic spindle assembly. In one recent example, human somatic cells and mice lacking a functional kinesin motor KIF18A have been shown to fail at chromosome alignment, producing MN after mitotic exit [21]. Another study reported increased frequency of MN upon depletion of the deubiquitinating enzyme Cezanne/OTUD7B, implicated in the control of chromosome segregation by opposing the activity of anaphase-promoting complex/cyclosome (APC/C) [22]. Less obviously, lagging chromosomes leading to MN have been observed in the human cells bearing lysosomal defects, implicating lysosomal degradation of one or more mitotic substrates that effect chromosome segregation, including a subset of histone H3 [23] or components of the cohesin machinery [24].

2. Acentric chromosome fragments are produced by unrepaired DNA double strand breaks, some of which are related to DNA replication. As such, deregulation of numerous factors of DNA replication and repair have been demonstrated to lead to MN. Recent examples include ribonuclease RNAseH2 [12,25], MCM2–7 replicative helicase [26], DNA damage-response mediator proteins MDC1 and TOPB1 [27], DNA-directed primase/polymerase (PrimPol) [28], multiple components of the Fanconi anemia/BRCA pathway [26,29–32], Bloom syndrome RecQ-like helicase (BLM) [33], SMC5/6 complex [32], chromatin remodeling factors SSX [34] and ATRX [29], and PARP1-interacting protein KHDC3L [35]. Finally, the resolution of chromatin bridges, containing dicentric chromosomes results in the formation of acentric fragments on both sides of a cleavage furrow, producing MN in both daughter cells [36,37].

3. Failure to properly assemble nuclei and NE upon mitotic exit contributes to MN formation. A recent study has demonstrated that DNA-binding protein Barrier-to-autointegration factor (BAF) controls nuclear formation by assembling and maintaining a group of chromosomes together into the single mass, thereby allowing formation of a single nucleus and preventing/inhibiting micronucleation [38]. Proper NE assembly has also been reported to frequently fail on lagging chromosomes. Several hypotheses underlying such incomplete assembly of a micronuclear envelope have been suggested based on signaling cascades controlled by Aurora B [19,39], but the precise mechanism(s) remain to be established. A surprising, additional "spindle inhibition model" has recently been proposed in which microtubules of the mitotic spindle directly inhibit recruitment of non-core NE proteins (such as B-type lamins, lamin B receptor) and nuclear pore complexes (NPCs), thus leading to MN with irreversible NE damage [19,40].

Besides "canonical" ways to produce MN mentioned above, an unexpected process of nuclear budding leading to MN has been observed in human cancer cells, p53-deficient human fibroblasts and epithelial cells [41]. While the mechanisms of the nuclear budding remain obscure in human cells, recent findings in Drosophila and mice point to DNA satellite binding proteins, D1 and Prod (Drosophila), and HMGA1 (murine cells) that control proper bundling of pericentromeric satellite DNA into so-called chromocenters. Aberrant D1/Prod/HMGA1 function results in nuclear budding-mediated formation of MN, unsurprisingly accompanied by marked (up to 10 fold) increase in DNA damage in both primary and micronuclei [42,43]. As nuclear budding is also the major route to form CCFs in non-dividing senescent cells [14,20], it will be intriguing to test whether similar mechanisms apply in the context of cellular senescence.

Making micronuclei experimentally

Cellular models allowing for controlled induction of MN are necessary for mechanistic studies of micronucleation and its functional outcomes (Figure 1). Antimitotic drugs impacting microtubule stability and spindle function are the most commonly used agents for inducing mis-aligned and lagging chromosomes that will become MN in the subsequent interphase. In this approach, MN are produced randomly from any mis-segregated chromosome; therefore, to analyze genomic outcomes of every single MN event, David Pellman's team invented "Look-Seq", with live imaging following MN formation and single cell DNA sequencing of both daughter cells [5]. An alternative strategy has involved the mis-segregation of a specific chromosome, the human Y, into MN after induced, selective inactivation of its centromere, and then following the consequences over several cell cycles [7,44]. Another approach employed microcell-mediated chromosome transfer (MMCT), itself an extension of a method developed in the 1970's to accomplish somatic cell genetics [45] in which single chromosome-containing MN (formed following mitotic slippage after usage of a microtubule depolymerization drug) were transferred to and followed in acceptor cells [46]. A final approach has relied on formation of dicentric chromosomes that produce MN as a consequence of fragmentation of DNA and its encapsulation into MN on both sides of a chromatin bridge [36,37].

The consequences of micronuclei

It is generally accepted that genetic material entrapped in MN is characterized by impaired and asynchronous DNA replication, as well as defects in DNA repair, which are caused by compromised integrity of NE and altered nucleocytoplasmic transport [3,5]. The latter probably arises from assembly of a suboptimal number of functional nuclear pores [3,4], possibly driven by delayed and/or inhibited nuclear pore assembly into a highly constrained envelope with a small radius of curvature that itself is prone to physical rupture. Added to this, endoplasmic reticulum (ER) invasion as a means to disrupt MN integrity has been also observed, albeit it is not clear if this directly leads to DNA damage [19]. Loss of NE integrity can also be provoked by the depletion of NE components [4,40].

NE rupture in MN is almost always irreversible [4,40] and abruptly exposes MN DNA to the cytoplasm. Sites of MN rupture accumulate components of the NE repair machinery (including endosomal sorting complexes required for transport-III (ESCRT-III)), which paradoxically further exacerbate NE rupture, instead of repairing it [47,48]. In Drosophila, an amazing process has been reported in which lagging acentric chromosomes are transferred through NPCs and subsequently re-integrated into newly formed nuclei, thus preventing MN formation [49]. This process depends on ESCRT-III along with BAF [49], with the latter also being implicated in NE repair in mammalian cells [50]. In human cells, chromosomes within MN were reported to accumulate decreased levels of important kinetochore assembly factors over several divisions, thus leading to recurrent mis-segregation of these chromosomes into MN over several cell cycles [51]. Taken together, the evidence supports what most would have thought all along: chromosomes in MN exhibit marked DNA damage and serve as a source of genome instability (Figure 2).

Micronuclei as drivers of chromothripsis

Over the last decade MN have been extensively studied in relation to chromothripsis – a massive, clustered chromosome rearrangement usually involving a whole, or a piece of a single chromosome, apparently as the result of a one-step catastrophic event [6]. Shattering of DNA linked to MN was first reported by Kato and Sandberg in 1968 [52], but went largely unnoticed for the next 44 years until the work of Pellman in 2012 [3]. Recent analysis of 2,658 cancer genomes performed by Pan-Cancer Analysis of Whole Genome (PCAWG) Consortium established a very high frequency of chromothripsis in human cancers: high confidence chromothriptic events were observed in 29% of the samples, reaching 100% and 77% in liposarcomas and osteosarcomas, respectively [8]. MN have been shown to be the major initial site of chromosome shattering of the MN content, followed by aberrant reassembly of resultant fragments [5,7,40].

Chromothriptic shattering of DNA within MN remains incompletely understood mechanistically. Potential explanations typically are based on the assumption that chromosome shattering takes place in ruptured MN and occurs via formation of numerous DNA double strand breaks [3]. According to one scenario [3], NE rupture destroys the balance in housekeeping processes of DNA replication and repair leading to the accumulation of DNA double strand breaks. Another possibility implies the disruptive action

of external factors, including putative, yet to be identified, cytoplasmic nucleases, which under normal circumstances have no access to interphase chromatin, but will enter ruptured MN and can induce DNA double strand breaks during interphase.

One nuclease, three-prime repair exonuclease 1 (TREX1), has been implicated in inducing chromothripsis in a model of chromosome bridge resolution, whose subsequently formed MN are also characterized by NE rupture [53,54]. However, a different study argued directly against such a role for TREX1, demonstrating instead that breakage of DNA within a bridge required mechanical forces from the interphase actin cytoskeleton rather than a nuclease [36]. Furthermore, overexpression of TREX1 did not increase DNA damage upon NE rupture in a model of constricted cell migration [55]. Finally, given that TREX1 is well characterized as an ***exo***nuclease [56], in contexts where TREX1 does contribute to DNA fragmentation it is likely that initiation of DNA double strand breaks requires other nuclease(s) (or other events) to act in concert with TREX1 [53].

Micronuclei and cGAS

One of the major recent breakthroughs in the field of immunology has been the discovery of the cytosolic DNA-sensing pathway that initiates and controls cellular immune response to potentially pathogenic DNA exposed to the cytoplasm [57]. The central component of this pathway, cyclic GMP–AMP synthase (cGAS), binds to cytoplasmic DNA and produces the second messenger cyclic GMP–AMP (cGAMP) that in turn activates innate immune responses, including the induction of interferons via the Stimulator of interferon genes (STING) (Figure 3). Initially observed upon radiotherapy [11] and in a model of monogenic autoinflammation [12], the recognition of DNA in ruptured MN by cGAS and subsequent activation of STING was later expanded to multiple models of micronucleation, including BLM-deficient human fibroblasts [33], BRCA2-defective human cancer cells and mouse embryonic fibroblasts [30], bacteria infected murine macrophages and human cancer cells [58], cultured primary human breast tumors and patient-derived xenografts [59]. In line with strong association between inflammation and cancer, MN-induced cGAS activation has been further linked to cancer progression, promoting cellular invasion and metastasis in a STING-dependent manner [13]. In an interesting twist, cGAS itself has been shown to exacerbate DNA damage, thus further contributing to tumorigenesis [60]. In particular, genotoxic agents (such as etoposide, hydrogen peroxide and camptothecin) trigger nuclear translocation of cGAS and its subsequent recruitment to DNA damage sites, where it counters the assembly of the PARP1-Timeless complex, thus preventing efficient homologous recombination [60].

Evidence for regulation of cGAS by chromatin was reported in 2019. cGAS was found in the primary nucleus, where it was tightly bound to nucleosomes, a tethering that apparently keeps cGAS in a catalytically inactive state [61,62]. Subsequent structural studies identified that, in the nucleus, cGAS interacts with a negatively charged acidic patch formed by histones H2A and H2B which blocks cGAS dimerization and keeps it inactive [63–67]. Identification of BAF, another DNA-binding protein, as a competitor and inhibitor of cGAS added a further level of complexity to nuclear regulation of cGAS function [68]. Future studies are now essential to understand how chromatin in ruptured MN activates cGAS, including experimentally testing if the ratios between cGAS, BAF, and nucleosomes might

be very different in MN as compared to primary nuclei, thus preventing cGAS from nucleosome- and BAF-mediated inhibition.

Senescence-associated cytoplasmic chromatin fragments (CCFs)

Cellular senescence is an irreversible cell cycle arrest, traditionally considered as a means to block proliferation of damaged cells and characterized by dramatic increase in secretion of pro-inflammatory cytokines and extracellular matrix-remodeling factors, defined as "senescence-associated secretory phenotype" (SASP) [69]. Senescent cells frequently possess extranuclear DNA in the form of so-called "cytoplasmic chromatin fragments", or CCFs, that are believed to originate through budding from the primary nucleus and bear marked defects in the integrity of their NE [14]. Similar to MN, CCFs have been reported to activate cGAS-STING pathway both *in vitro* and *in vivo*, including primary human cells and mouse models [15–18]. Importantly, cGAS-STING participates in the regulation of cellular senescence by triggering the production of SASP factors [15–18]. Both MN and CCFs can be recognized by autophagy-lysosomal machinery leading to their degradation [9,14]. This feature is evolutionary conserved, as autophagic clearance of MN and nuclear material has also been reported in yeast, nematodes, and frogs [70–72]. According to one study in cancer cells, MN and CCFs were eliminated by autophagy provoked by replicative stress, which resulted in cell death, therefore protecting against genome instability [10]. Another group reported that autophagy-mediated clearance of CCFs repressed senescence by preventing CCF-induced cGAS-STING activation and SASP [18]. In this regard, autophagy emerges as a protective pathway against MN and CCFs. This defense mechanism might be particularly efficient if genetic material within MN/CCFs contained the only copies of essential genes, leading to the death of cells in which MN/CCFs were consumed by autophagy (Figure 3).

In addition, developmentally programmed elimination of chromosomes or chromosome fragments via MN has been reported in plants, frogs and nematodes, indicating that MN formation and clearance can function as a naturally occurring mechanism for irreversible silencing of gene expression [71–73]. For future therapeutic strategies, it will be important to unravel the specifics of MN/CCFs recognition by autophagy, especially taking into account recent identification of nuclear-specific autophagy receptors in yeast [74,75].

Concluding remarks

Over the last decade, the traditional view of MN/CCFs as mere markers of genotoxic stress has evolved into a much more complex picture with MN/CCFs playing central and active role in cancer and senescence. Better understanding of precise molecular mechanisms controlling MN/CCFs will be critical to therapeutically target these cellular structures. The latter is even more important, as currently used therapies acting on the level of DNA are known to promote micronucleation. Rapidly developing MN models will serve as a basis to unravel pathways and players inducing complex genome rearrangements, such as chromothripsis, that are being increasingly appreciated as a common feature of aggressive cancers. In particular, MN models will provide mechanistic insights into how chromosomes are shattered and re-build in chromothripsis.

The involvement of autophagy in the clearance of pathological MN/CCFs as well as in developmentally programmed elimination of MN is of particular interest, as it suggests a path to specifically target and destroy MN and CCFs. As to how to prevent MN/CCFs or curtail their pathological consequences, new studies will likely focus on the mechanisms of NE repair and nucleocytoplasmic transport, regulation of cellular immune response and identification of cytoplasmic factors promoting DNA damage in MN.

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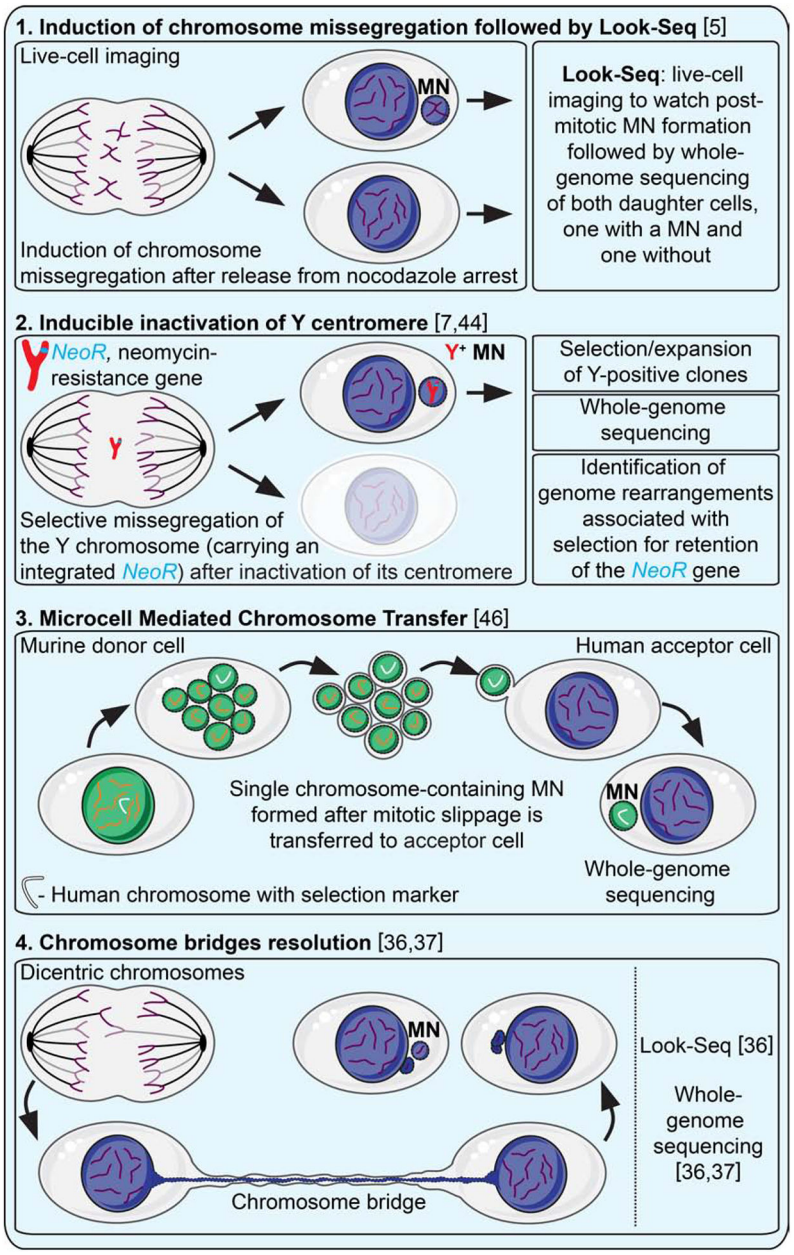

Figure 1. Research models to study cellular and genomic consequences of micronucleation.

Top to bottom: **(1)** Mitotic drug-induced chromosome missegregation followed by Look-Seq [5]; **(2)** Inducible inactivation of Y chromosome centromere resulting in Y missegregation into micronuclei (MN) [7,44]; **(3)** Generation of MN by Microcell Mediated Chromosome Transfer [46]; **(4)** Dicentric chromosomes producing MN as a result of chromosome bridge resolution [36,37]. Identification of complex genome rearrangements including chromothripsis is performed by whole-genome sequencing of single daughter cells (Look-Seq, **(1)** and **(4)**) or stable clones (**(2)**, **(3)** and **(4)**).

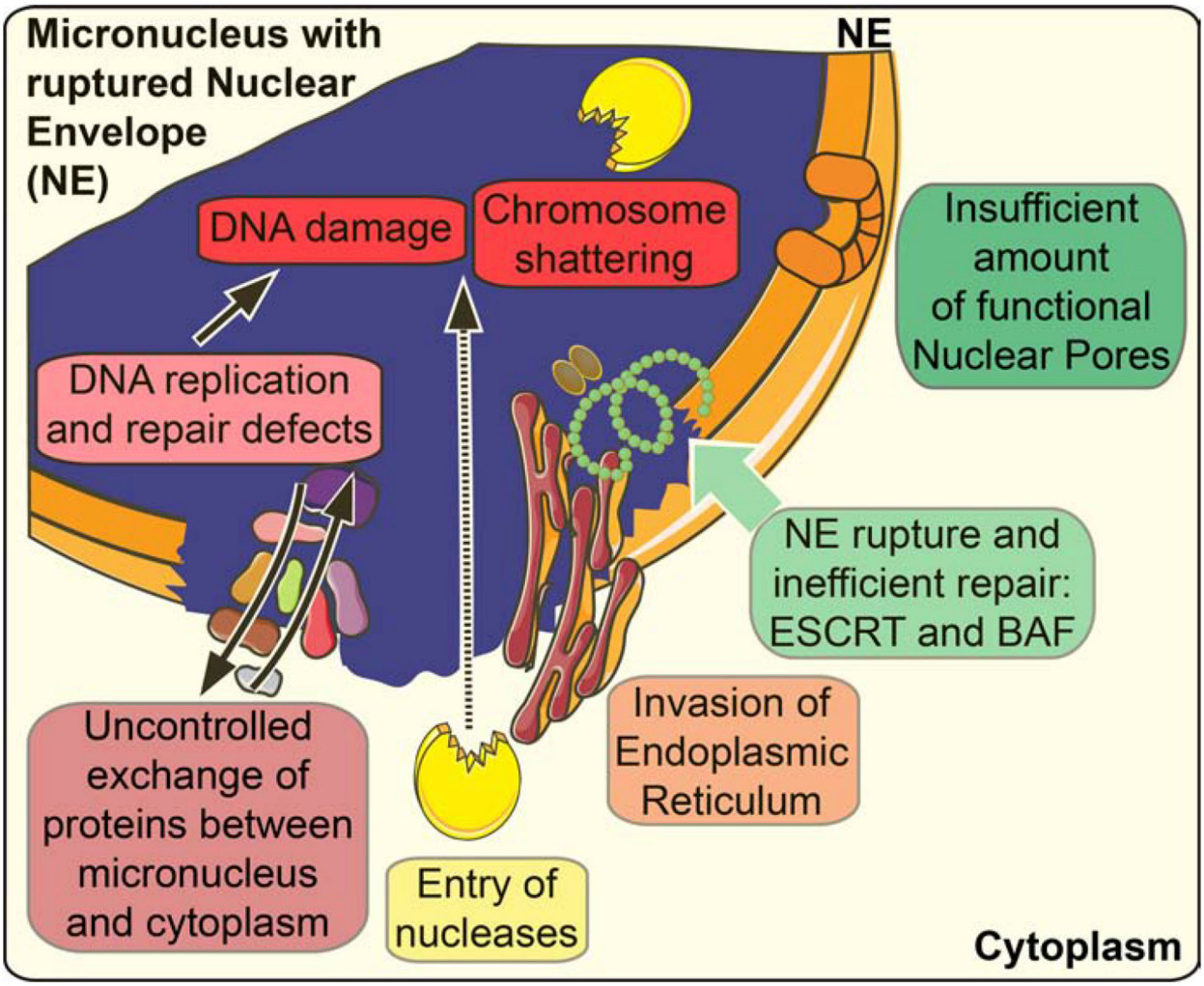

Figure 2. Nuclear Envelope (NE) rupture in micronuclei.

Ruptured MN are characterized by aberrant NE repair and Endoplasmic Reticulum invasion. NE rupture in MN results in DNA damage ultimately leading to chromosome shattering and chromothripsis. Potential contributors to DNA damage include imbalances in genome maintenance machinery and influx of cytosolic nucleases. ESCRT, Endosomal sorting complex required for transport; BAF, Barrier-to-autointegration factor.

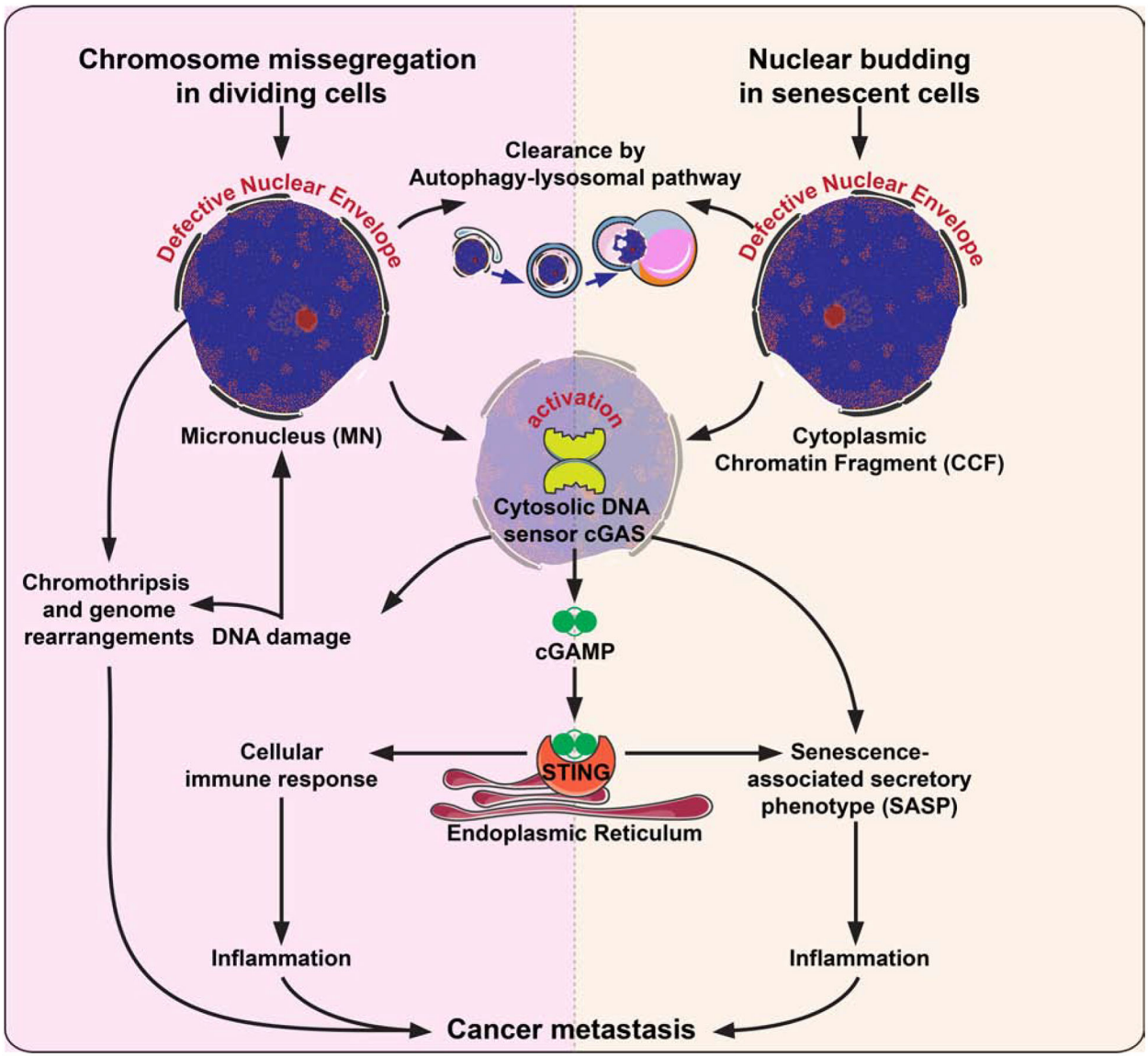

Figure 3. Unprotected chromatin in dividing and senescent cells: different causes, similar outcomes.

Ruptured micronuclei (MN) and cytoplasmic chromatin fragments (CCFs) accumulate DNA damage and trigger cellular immune response which contribute to cancer metastasis. MN and CCFs can be degraded by Autophagy-lysosomal pathway thus providing a potential therapeutic strategy. cGAS, cyclic GMP–AMP synthase; cGAMP, cyclic GMP–AMP; STING, Stimulator of interferon genes.