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Micronuclei and Cancer

Melody Di Bona[1,2], **Samuel F. Bakhoum**[1,2]

[1]Human Oncology and Pathogenesis Program, Memorial Sloan Kettering Cancer Center, New York, New York.

[2]Department of Radiation Oncology, Memorial Sloan Kettering Cancer Center, New York, New York.

Abstract

Chromosome-containing micronuclei are a feature of human cancer. Micronuclei arise from chromosome mis-segregation and characterize tumors with elevated rates of chromosomal instability. Although their association with cancer has been long recognized, only recently have we broadened our understanding of the mechanisms that govern micronuclei formation and their role in tumor progression. In this review, we provide a brief historical account of micronuclei, depict the mechanisms underpinning their creation, and illuminate their capacity to propel tumor evolution through genetic, epigenetic, and transcriptional transformations. We also posit the prospect of leveraging micronuclei as biomarkers and therapeutic targets in chromosomally unstable cancers.

A HISTORICAL PERSPECTIVE ON MICRONUCLEI

Micronuclei are cytoplasmic structures containing entire chromosomes or chromosomal fragments, and they result from errors in segregation during mitosis. The discovery of micronuclei can be tracked down to the end of the 19th century in the pioneering work of von Hansemann, “On pathological mitoses,” where he introduced the concept that “lost chromosomes” may isolate themselves from the rest of the nucleus (1). A decade later, Theodore Boveri proposed multipolar mitosis as a potential cause of human malignancies, based on his findings in sea urchin eggs. During his experiments on interspecies crossing, he observed that some chromosomes, depending on their position in the cytoplasm, were not transmitted into daughter cells (2).

In the first decades of the 20th century, progresses in our knowledge of chromosomes and nuclear biology allowed for the first observations of interphase micronuclei. In fact, the nucleus was believed to be composed of adjacent chromosome-containing vesicles, which helped maintain chromosome individuality during interphase, enabling accurate segregation during the subsequent mitosis (3). Sometimes, one or more of these chromosomal vesicles, also known as karyomeres or idiomeres, were observed outside of the nucleus. In developmental biology, the formation of the egg of some fish species was known to rely

Co-corresponding author: Melody Di Bona, Memorial Sloan Kettering Cancer Center, 1275, York Avenue, NY 10065. mela1989@gmail.com.

upon the fusion of multiple separate karyomeres in a bigger nucleus (4). Furthermore, it was common knowledge that most of the ciliates possess two different types of nuclei, a macronucleus and a micronucleus (5, 6). In 1937, Brues and Jackson, while studying mitotic arrest, observed that colchicine induced “swelling of individual chromosomes.” This process in turn gave rise to micronuclei with a size proportional to the amount of chromosome contained (7). In 1946, Schwarz proposed in his studies on cellular gigantism that the interphase chromatin particles observed in the cytosol are the vesicular transformation of the chromosomes that were mis-segregating in the prior anaphase (8).

Akin to Brues and Jackson, many studies in the first half of the 20th century identified micronuclei as the outcome of cytotoxic agents such as x-ray irradiation (9–11) and the mitotic poisons colchicine and derivatives (7, 12–15). These early studies led scientists to consider micronuclei as potential hallmarks of pathologic conditions.

Indeed, two papers in 1930 studied the phenomenon of extrusion of nuclear material in the cytoplasm and sometimes in the extracellular matrix, naming it “chromidia extrusion.” This process, which correlated with nuclear constriction and multinucleation, was observed in several tumor cells (16, 17). A correlation between the presence of genetic material in the cytoplasm and malignancy started to be drawn, concurrently with the rising interest in mitotic abnormalities as key features of cancer. In truth, both these processes can now be seen as the two faces (interphase and mitosis) of the same process (the separation of genetic material from the main nucleus).

Koller was one of the first, in 1943, to propose the concept of “chromosome stickiness” as a cause for abnormal mitosis (18). Sticky chromosomes were fused together and unable to correctly segregate, giving rise to either a lagging chromosome, left behind in anaphase and thus separated from the rest of the nucleus (19), or to chromatin bridges, known to undergo fragmentation resulting in micronuclei (15). Less than a decade later, Ludford clearly showed the presence of micronuclei in samples derived from mammary carcinoma, sarcoma, and mouse adenocarcinoma, from which he hypothesized aberrant mitosis derivation (20).

Until the end of the twentieth century, the scientific community considered micronuclei as miniaturized nuclei structurally identical to the primary nucleus (21), an error that can be ascribed to the poor resolution of the then-available tools and a lack of functional studies. Nonetheless, emerging evidence suggested that some micronuclear chromosomes might undergo aberrant processes. Micronuclei were in fact divided in categories depending on their ability to synthesize nucleic acids (22). Several studies in the years between 1950 and 1970 attempted to understand transcription in micronuclei and its relationship to the chromosomal regions in charge of the nucleolar formation, called the *nucleolar organizer* (21, 23, 24). The studies on micronuclear transcription and replication though, as well as the ones investigating the viability of cells containing micronuclei, reported conflicting results (25–27), probably because of the high variety of cell types, study time points, and methods used. Nonetheless, thanks to these studies, the discovery of chromosome pulverization – or shattering – in micronuclei was first made in 1968 (28). We now know that this process is a key step toward the formation of complex chromosomal rearrangements known as chromothripsis (29, 30). In an elegant work (28), Kato and colleagues proposed

pulverization as a consequence of late chromosomal condensation. In this model, the uncondensed and thus vulnerable chromosomes are exposed to the cytoplasmic conditions typical of mitosis, leading to their damage and shattering.

This hypothesis, although not universally valid, has fascinating parallels with the knowledge that we have since accumulated in the following 50 years: it is indeed the exposure of the micronuclear chromosome to the cytosolic milieu, through rupture and collapse of the micronuclear envelope during interphase, that raises several pathologic responses which drive tumor progression (31). Our knowledge of mitosis and the biology of the nucleus has progressed over the past decade, allowing us to draw a clearer picture on the processes underlying the biology of micronuclei and how they influence the pathologic crosstalk between cancer cells and the tumor microenvironment. Nonetheless, the reasons underlying micronuclear membrane collapse and its cellular consequences remain as important and unresolved questions. Given the importance of micronuclei for tumor evolution, we will aim to provide a comprehensive review of micronuclear biology and its clinical relevance, merging mechanistic and cell biology with therapeutic and translational considerations. We will place particular emphasis on the central event of micronuclear rupture and its consequences and discuss how these small, yet important features of cancer cells might drive tumor progression and highlight novel approaches for therapeutic intervention. Finally, we will end by discussing the possibility of using micronuclei as potential biomarkers of the dynamic and ongoing process that defines chromosomal instability (CIN) in cancer.

MECHANISMS OF MICRONUCLEI FORMATION

As previously discussed, although micronuclei typically arise from mitotic chromosome mis-segregation (29, 32), they can also result from interphase nuclear blebbing and budding (16, 33). The formation of cytosolic chromatin fragments through budding is a process typical of senescent cells (34), but it can occur in some cancer cells undergoing stresses such as nuclear confinement (35), replication stress (36), or proteotoxic stress, the latter in particular when nuclear integrity proteins are involved (37). Nuclear budding might represent a mechanism of elimination of defective genetic material, either too damaged to be repaired (36), or present in aberrant quantities (38).

We will focus the remainder of this discussion on micronuclei that are derived from chromosome mis-segregation due to mitotic errors, which represent the vast majority of micronuclei in cancer cells. Lagging chromosomes represent a significant proportion of segregation errors; these are chromosomes mal-attached to the mitotic spindle and thus often retained in the spindle midzone while the remaining chromosome masses move toward the spindle poles (39–41). Another form of mis-segregation is the formation of acentric chromatin fragments and chromatin bridges, which usually originate from unrepaired or mis-repaired DNA breaks, respectively. Chromatin bridges are the product of the mitotic spindle pulling single, nondisjointed pairs of chromosomes or dicentric chromosomes in opposite directions (42). These bridges undergo breakage and disintegration, and the resulting chromatin fragments can also be encapsulated into micronuclei (43, 44).

Micronuclei formed from lagging chromosomes typically possess centromeres (45), whereas those derived from DNA fragmentation often do not (referred to as acentric; ref. 46). Micronucleated whole chromosomes might be more likely to re-integrate into the primary nucleus during the subsequent mitosis, because the centromere—if it remains intact—can catalyze their attachment to the mitotic spindle, whereas the acentric fragments might perpetually mis-segregate unless they hitchhike on other chromosomes.

Although nearly random, some studies have shown a bias in the identity of micronuclei-encapsulated chromosomes. For example, Saunders and colleagues found that micronuclei formed by chromatin bridges in oral squamous cell carcinoma often contain chromosome 11 sequences, probably because of a fragile site in its long arm (43). Similarly, Falck, Catalan, and Norppa, studying lymphocytes, showed that the X chromosome lagged more frequently than the autosomes, and as such, it was more often found in micronuclei. Because the X chromosome was located further away from the remaining chromosomes in mitosis, they suggested that chromosome mis-segregation might depend on the mitotic spindle position (47, 48).

THE VARIOUS FATES OF A MICRONUCLEUS

Micronuclei can undergo different fates; although the focus of this review is on micronuclear rupture, discussed in a separate section, we will provide a brief overview of all the potential and interrelated outcomes of a micronucleus (Fig. 1), referring the readers to Hintzsche and colleagues (49) for a more complete overview.

Reincorporation into the Primary Nucleus

Micronuclei can be reincorporated into the primary nucleus after cell division (Fig. 1A; refs. 50, 51). Approximately 25%–50% of micronuclei undergo reincorporation (52), genetically fixing the changes that happened to the DNA while harbored in the micronucleus. These changes include, among others, mutations and complex rearrangements arising from defective repair and replication and epigenetic changes in chromatin structure (53, 54). As such, what happens in the micronucleus might persist long after the reincorporation of the chromosome back into the primary nucleus and be subsequently propagated to daughter cells.

Persistence and Independent Replication

A significant proportion (~50%) of micronuclei persists in cells. The micronucleated chromosome usually continues to be mis-segregated in the following mitoses because of defects in the proper assembly of kinetochores, ascribed to low levels of essential kinetochore proteins (52). The micronucleated chromosome thus is likely to undergo cycles of mis-segregation and re-encapsulation in micronuclei in one of the daughter cells (Fig. 1B; refs. 50, 51, 55, 56). A recent work from the Ly lab shows that acentric, shattered chromosomes follow a pattern of asymmetric inheritance in mitosis. In fact, the DNA damage response complex CIP2A-TOPBP1 localizes to DNA lesions in acentric fragments, clustering together and leading to their segregation in the same daughter cell. This finding explains the asymmetric chromothripsis patterns often observed in cancer cells (57).

When two copies of the micronucleated chromosome are produced by independent replication, chances are that each daughter cell will contain a micronucleus (Fig. 1C). However, given that chromosomal instability is often associated with aneuploidy, it is more likely that the micronucleated chromosome undergoes mitosis without being replicated. Studies on DNA replication in micronuclei have yielded contradictory results; although some have reported a delay in DNA synthesis and decreased replication efficacy (22, 52), others have argued that micronuclei replicate normally and in synchrony with the primary nucleus (14, 58, 59). The reason of this controversy may lay in the variety of models studied, as well as in the lack of differentiation between subtypes of micronclei. For instance, Okamoto and colleagues show that DNA replication seems to be specific to micronuclei possessing an intact lamina (60).

Elimination and Extrusion from the Cell

In rare instances, chromatin, and more specifically micronuclei, can be extruded from the cell (Fig. 1D; refs. 16, 24, 61, 62). However, it remains to be determined whether this process is distinct from a neutrophil-like extrusion of genetic material (63) or whether it is mediated by extracellular vesicles. In fact, recent evidence suggests that in ovarian cancer, micronuclei are an important source of genomic content for exosomes (64). Yet, it is unclear whether this is the result of direct bulk micronuclear extrusion or if the micronucleus first undergoes rupture and then the fragmented micronuclear chromatin is packaged and exported in exosomes. Another proposed mechanism for micronuclear elimination is through autophagic degradation. Autophagy can recycle components of the nuclear lamina (65), and recent evidence suggest that a subset of micronuclei colocalize with LC_3 and is thus cleared by autophagy-dependent degradation (66). However, little evidence exists to show that micronuclear LC_3 localization can lead to clearance of the entire micronucleus as opposed to minor components thereof. In addition, the selective pressures to retain or dispense of micronuclei might be context dependent. In normal cells, micronuclei can serve as a trigger for immune activation and clearance as well as a stress response signal, whereas in cancer cells, micronuclei have been shown to promote protumor inflammation, epigenetic alterations, and catalyze large-scale genome evolution.

MICRONUCLEAR ENVELOPE RUPTURE: A SEMINAL EVENT

Envelope rupture represents the fate of around 40%–50% of micronuclei at any given time (67). Despite its prevalence, this process is not well characterized. Several studies have attempted to elucidate the processes underlying micronuclear rupture, but the exact mechanism remains elusive. It might be useful in this regard to compare micronucleus and primary nucleus, two similar yet different subcellular organelles. Whereas micronuclei rupture and collapse, the primary nucleus rarely encounters such a fate. The underlying cause of micronuclear envelope rupture might be uncovered through a more rigorous understanding of what distinguishes a micronucleus from its primary nucleus counterpart.

The first obvious difference between the two structures, as suggested by the name, is the size. In fact, sites of high curvature lack the structural protein Lamin B1 and are thus more

fragile (68). Micronuclei, being smaller, have a higher membrane curvature than primary nuclei and thus might generally have less Lamin B1 and a weaker lamina.

Lamin B1, an important component of the nuclear lamina, and together with Lamin B2 and Lamin A/C, it is often underrepresented in micronuclei (67, 69). Whereas overexpression of Lamin B1 induces primary nuclei abnormalities, overexpressing the sister protein laminB2 reduces micronuclear envelope rupture (67), suggesting inherent Lamin B deficiency in micronuclei at baseline. In contrast to micronuclei, Lamin A/C appears to play a more central role in maintaining primary nuclei membrane integrity (70). In general, the primary nuclear envelope is thought to be more robust to perturbation of lamina components, and nuclear atypia is seen when multiple lamin proteins are codepleted (71). In addition to reduced lamin levels, a recent study from the Hatch lab revealed that gene density strongly correlates with the propensity for the micronucleus to rupture (72), suggesting that the encapsulated chromosome determines the micronucleus fate.

The paucity of Lamin B in micronuclear envelopes suggests that the envelopes of the primary and micronucleus might have fundamentally distinct protein composition. Indeed, Pellman and colleagues demonstrated that envelopes of micronuclei derived from lagging chromosomes lack key components of the nuclear pore, specifically the “noncore” protein subunits (73). The authors causally link this finding, imputed to interference from high density of microtubules, to reduced nuclear pore-mediated import, and thus overall levels, of micronuclear LaminB1 (44, 74). Defective micronuclear import might also provide an explanation for flawed replication and transcription that are often observed in micronuclei (75). According to the authors, the micronucleus fate is predicated on the position of the mis-segregated chromosome relative to the anaphase spindle. In fact, chromosomes that are near the spindle midzone, where there is a high density of microtubules, are more likely to yield micronuclei with defective nuclear pores compared to those that mis-segregate near the spindle poles. As such, peripherally derived micronuclei are surrounded by a relatively more functional nuclear envelope (73).

Furthermore, a key difference between primary nuclei and micronuclei is the duration of rupture. In primary nuclei, the loss of compartmentalization is transient, lasting for a few minutes to perhaps one hour, which stands in stark contrast to micronuclei, which often collapse irreversibly upon rupture. This implicates key differences in the envelope repair mechanisms that are clearly lacking in the micronucleus. Cells have evolved two main mechanisms of nuclear membrane repair, with partially redundant but synergic functions (Fig. 2; refs. 68, 76–78). These mechanisms have in common the protein Barrier-to-Autointegration Factor (BAF), indicated by current prominent models of nuclear rupture dynamics (79) as the first responder after nuclear envelope rupture. BAF is a small protein (25 kDa) with a DNA-binding domain and a binding site for LEM-domain proteins. Depending on the subcellular localization, BAF forms two distinct subpopulations with different functions: nuclear BAF has a structural role, whereas cytoplasmic BAF (mostly ER-bound) is involved in nuclear envelope repair. Upon nuclear rupture, unphosphorylated cytoplasmic BAF is engaged at the rupture site, mainly through its DNA-binding domain. There, it recruits the ER membrane to repair the damage. In addition to this direct repair activity, BAF also interacts, through the LEM-binding site, with LEM-domain proteins of

the inner nuclear membrane such as LEMD2 and Emerin, facilitating their localization to the site of rupture (76). This process is fundamental to the recruitment of the ESCRT-III complex, the central component of the second nuclear envelope repair mechanism. CHMP7, the scaffolding subunit of the ESCRT-III complex, localizes to the rupture site in a BAF-dependent manner (76) and is retained in the nucleus through interaction with LEMD2 (80, 81). Once the CHMP7–LEMD2 interaction is stabilized, the rest of the ESCRT-III complex assembles and seals the membrane, similarly to what happens in postmitotic membrane resealing (Fig. 2; refs. 78, 81–83).

In micronuclei, the situation is quite different. Recent studies have revealed altered functionality of the ESCRT-III complex. CHMP7 and other components of the ESCRT-III complex are often abnormally accumulating at ruptured micronuclei (84). This hyperactivation of the complex and subsequent over-zealous repair is believed to result in catastrophic membrane collapse (85). In addition, the presence of CHMP7 in ruptured micronuclei, and thus the consequent activation of the ESCRT-III complex, has been associated with increased DNA damage (84), depicting the ESCRT-III machinery at micronuclei as a double-edged sword. Similarly, BAF is enriched at sites of micronuclear fracture (86) and is probably mediating the phenomena of ER-tubule invasion observed at ruptured micronuclei (67), but why it fails in sealing the micronuclear membrane remains unclear.

CONSEQUENCES OF MICRONUCLEAR RUPTURE

Micronuclear rupture represents a fundamental step in cancer progression. This event can catalyze rapid genome evolution and epigenetic reprogramming and can activate immune signaling pathways, and as such, has been associated with metastatic progression, promoting tumor cell survival and the rapid acquisition of genomic heterogeneity. Over the past years, our understanding of the aberrant processes linked to micronuclear rupture in cancer has significantly grown. Below, we discuss the key cellular events that take place as a result of micronuclear envelope rupture.

DNA Breaks and Chromothripsis

Through the study of cancer genomes, Peter Campbell and colleagues recently discovered and characterized the phenomenon of chromothripsis (87). It involves clustered and complex rearrangements spanning individual chromosomes or chromosome arms. Burgeoning evidence now suggests that chromothripsis, being a cataclysmic event impacting several genes at once, might play a critical role in tumor evolution. Chromothripsis, which derives from the Greek *chromos* for chromosomes and *thripsis* for shattering, traces its mechanistic origins in micronuclei. In fact, the authors interpret the observed genomic rearrangements as a desperate attempt from the DNA repair machinery to stitch together a chromosome that has been, as the name suggests, shattered in tens to hundreds of pieces (Fig. 3A; ref. 87). This observation, made possible by modern genomic tools, is the consequence of a process known in the 1970s as chromosome pulverization (28). The Pellman group rekindled the interest in chromosome pulverization by linking it to micronuclear envelope rupture and chromothripsis. In fact, they showed not only that unrepaired DNA damage

in micronuclei is the cause of chromosome pulverization (29) but also that chromosomes contained in ruptured micronuclei after S-phase entry exhibit all of the features observed in chromothripsis events (88). In this seminal article, Pellman and colleagues developed an innovative method to selectively sequence cells with ruptured micronuclei and called it Look-Seq. Using fluorescence live-cell imaging (Look), it was possible to select single cells containing a micronucleus (visualized by GFP-tagged H2B) that underwent rupture (identified by a loss of RFP containing a nuclear localization sequence) and follow them undergoing mitosis. At mitotic exit, the daughter cells can be separated and their genome sequenced at single-cell resolution (Seq). Thanks to Look-Seq, the authors were able to identify genomic rearrangements specifically derived from micronuclear rupture and characterize them (88). Although the authors recognized the possibility that chromosome shattering could happen also in unruptured micronuclei as a result of late replication, a variety of studies confirm the link between micronuclear envelope breakdown and extensive DNA damage and chromosome shattering (Fig. 3B; refs. 67, 84, 85, 89, 90). Moreover, evidences suggest that ruptured micronuclei represent the source of double minute chromosomes, small circular extrachromosomal DNA molecules containing highly amplified oncogenes, also known as extra chromosomal circular DNA (ecDNA; Fig. 3C; ref. 87). The relationship between micronuclear envelope collapse and DNA damage highlights once more the importance of micronuclear envelope rupture in catalyzing massive rearrangements conferring strong selective advantage.

Epigenetic Reprogramming

Epigenetic abnormalities have emerged as an important driver of tumor progression (91). The development of new techniques combining fluorescence microscopy and molecular biology, such as assay for transposase-accessible chromatin with sequencing (ATAC-seq), paved the way to investigate chromatin accessibility, a feature heavily dependent on epigenetic modifications (92). Evidence suggests that micronuclei play a role in disrupting chromatin structure. Indeed, ecDNA exhibits increased chromatin accessibility and a more relaxed organization of nucleosomes compared to nuclear chromosomes, enabling high expression of the oncogenes encoded (93). Furthermore, chromosome encapsulation in micronuclei promotes rapid changes in chromatin organization. In fact, specific histone posttranslational modifications have long been used as markers of micronuclear rupture, such as loss of histone H3 acetylation on lysine 9 (H3K9Ac; ref. 67). To expand on this, Agustinus and colleagues performed a comprehensive analysis of histone posttranslational modifications in micronuclei and found that micronuclear encapsulation leads to profound changes in histones beyond individual marks (55, 56). This involves loss of acetylation at multiple lysine residues on the histone H3 tail including H3K9Ac, H3K14Ac, and H3K27Ac as well as enrichment of lysine trimethylation, most notably at H3K4, H3K9, and H3K27. Micronucleated chromosomes also exhibit loss in histone H2A and H2B ubiquitination suggesting abnormalities in multiple epigenetic-modifying enzymes. This leads to significant changes in chromatin accessibility. Interestingly, although micronuclei display overall a more compact chromatin structure associated with reduced transcription, the few genomic regions that are more accessible than in primary nuclei belonged primarily to promoter regions (55). This positional bias was attributed to enrichment of H3K4me3, a mark known to be associated with transcriptionally active genes. It is tempting to postulate

that increased accessibility of a subset of promoters in micronuclei underlies oncogenic transcription and provides the substrate for ecDNA formation.

Changes in histone posttranslational modifications persist upon the reincorporation of the hitherto micronucleated chromosome into the primary nucleus (55, 56). As such, epigenetic disruption in micronuclei forebodes heritable consequences that can lead to transcriptional silencing of entire chromosomes, suggesting that cells with identical genomic content could exhibit significantly different transcriptional output. Strikingly, long-term passage of clonal populations that underwent early micronucleation events revealed that the epigenetic abnormalities taking place in micronuclei can persist for multiple generations. Given the stochastic nature of chromosome mis-segregation and micronuclei formation, this process has the potential to generate significant epigenetic and transcriptional heterogeneity, fueling tumor evolution and plasticity (Fig. 3D).

Activation of the cGAS–STING Pathway

The DNA contained in micronuclei, beyond carrying genetic and epigenetic information, also plays an important role in activating innate immune signaling pathways. In fact, the cytosolic exposure of genetic material, because of micronuclear envelope collapse, triggers the nucleic-acid recognition and innate immune cGAS–STING pathway and downstream inflammatory signaling (Fig. 4; ref. 94). Upon DNA binding, the cyclic GMP-AMP synthase (cGAS) catalyze the formation of 2′,3′-cyclic GMP-AMP (cGAMP) from ATP and GTP (95). The second messenger, cGAMP, in turn binds to the ER-resident protein STING, inducing its oligomerization and activation; active STING recruits TBK1, IRF3, and IKK, which translocate the signal to the nucleus where it modifies gene expression through the transcription factor NF-κB (96). Under normal circumstances and in line with its antimicrobial function, activation of the cGAS–STING pathway leads to the production of type I IFN-stimulated genes (ISG), ultimately eliciting an antitumor immune reaction (65, 97). Peculiarly, the chronic activation of this pathway, as happens in chromosomally unstable cancers with a preponderance of rupture-prone micronuclei, can rewire the downstream signaling, which entails significant reduction in type I IFN response. Instead, chronic STING activation from cytosolic DNA from micronuclear origins promotes a noncanonical NF-κB and an endoplasmic reticulum stress response. This signal rewiring leads instead to epithelial-to-mesenchymal transition, immune suppression, and distant metastatic dissemination (31, 98). In addition to its cell-autonomous function, cGAS can activate STING in host and immune cells through paracrine signaling and tumor-to-host cGAMP transfer, promoting immune surveillance (99–101). In healthy cells, cGAS is tightly regulated to avoid its activation by self-DNA. In fact, chromatinized DNA exerts a strong inhibitory effect on cGAS (102, 103), as do its regulators TREX1 (86) and BAF (Fig. 4; refs. 104, 105). Nonetheless, cGAS is activated in cancer cells, suggesting a dysregulation of these inhibitory mechanisms. Moreover, the demarcation line between the interferon response and the NF- κB–mediated antitumor effect of the cGAS–STING pathway is not fully understood, as highlighted by the many contrasting results reported in literature (for more comprehensive reviews, we refer the readers to refs. 106–109). Thoroughly understanding the mechanisms underlying the activation and context dependency of the

cGAS–STING pathway would be of paramount importance to provide the possibility to pharmacologically tweak this process and evoke antitumor immunity.

Crosstalk with the Tumor Microenvironment

In the previous section, we discussed the cell-intrinsic effects of MN rupture and the importance of the cGAS–STING pathway context dependency in the interaction with the immune system. In addition, the rupture of MN and the subsequent DNA cytosolic exposure has also profound cell-extrinsic consequences. First, given the importance played by the mutational burden in the formation of neoantigens (110) and in influencing the response to PD-1 blockade (111), it would be intriguing to determine whether chromothripsis and DNA damage in ruptured micronuclei might produce mutated proteins whose derived neoantigens might elicit a T-cell response. More importantly, micronuclear rupture can catalyze the activation of pattern recognition receptors (PPR) both inside tumor cells and in the extracellular milieu. Cancer cells can export cytosolic dsDNA as a complex with proteins or associated with exosomes (reviewed in the next paragraph; ref. 112). This extracellular DNA can be taken up by cells of the immune system, eliciting an inflammatory response (Fig. 4; refs. 113, 114). Antitumor responses can arise also through export of the second messenger cGAMP in the extracellular milieu where it is imported in macrophages and monocytes (115) or where it can stimulate a STING-mediated IFN production in nontumor cells, eliciting NK cells' response (116). In addition, direct intercellular transfer of cGAMP though GAP junctions can trigger a bystander immunity (117), although the same cGAMP transfer mechanism has been shown to promote tumor progression in brain metastasis (118). Interestingly, a recent study from our lab reveals that cancer cells can bypass the antitumor immunity response triggered by cGAMP export through overexpression of the ectonucleotidase ENPP1, which hydrolyzes cGAMP into GMP and AMP. In this way, not only ENPP1 prevents cGAMP uptake from immune cells but also provides a substrate (AMP) that can be converted by the often coexpressed ectoenzyme NT5E into adenosine, a strong immune-suppressive molecule (Fig. 4; ref. 119).

Micronuclei as a Putative Source of Exosomal DNA

Exosomes secreted by cancer cells contain DNA, mostly damaged and considered harmful for the cell (120), derived from all the chromosomes (121). Recent evidence showing that exosomes are loaded with the content of disrupted micronuclei (Fig. 4; ref. 64) suggests that the damaged DNA contained in cancer-derived exosomes may indeed be the result of micronuclear rupture. As exosomal secretion plays an inhibitory role on cGAS–STING activation in cancer cells (120), and enhances antitumor inflammation in dendritic cells through the same pathway (113), the pharmacologic activation of this mechanism can represent an important therapeutic strategy.

PHASE SEPARATION AND THE MICRONUCLEI

In the past few years, phase separation and membraneless organelles have emerged as important mechanisms of cellular regulation and compartmentalization (122). Furthermore, recent evidence suggests that phase separation dysregulation is involved in cancer pathogenesis and progression (123). Although there is no direct evidence connecting

micronuclear rupture to phase separation, it recently emerged that the liquid-liquid phase separation between cGAS and DNA is fundamental for cGAMP production by cGAS (124, 125) and to escape the inhibitory effect of TERX1-mediated DNA degradation (126). Moreover, phase separation has been invoked in transcriptional regulation (127) and DDR initiation (128), processes that are malfunctioning in ruptured micronuclei. Finally, heterochromatin is formed through processes of phase separation. The nucleation of phase-separated droplets of Heterochromatin Protein 1 alpha (HP1α) promotes the formation of a more condensed chromatin state (129), whereas heterochromatin maintenance is dependent on the phase separation of the p53 Binding Protein 1 (53BP1), which is also an important component of the DNA repair machinery (130). Given the high chromatin condensation observed in ruptured micronuclei (55), it is possible that abnormal phase separation plays a role in heterochromatin formation and DNA damage response deregulation after micronuclear membrane collapse.

MICRONUCLEI AS A BIOMARKER IN CANCER

Chromosomal instability is an ongoing process rather than a stable state like aneuploidy and thus can slip undetected under routinely used diagnostic methods such as sequencing. The presence of micronuclei, historical markers of DNA damage (131), is easier to detect in tissues than mitotic figures and can offer a snapshot in time of ongoing mis-segregation, thus representing a fairly reliable marker of chromosomal instability. The micronucleus assay on peripheral blood cells, in light of its minimal invasiveness and low cost, has been widely used as a marker of radiation exposure (132) and, thanks to its high sensitivity, can be used as a retrospective dosimeter of ionizing radiation (133). Given the connection of micronuclei with DNA damage, cell death, and chromosome mis-segregation, micronuclear assays are routinely used on buccal mucosal tissue to assess oral cancer risk and enabling early detection (134, 135). Moreover, the micronuclear assay on peripheral blood lymphocytes can detect high levels of micronuclei that are associated with increased pancreatic cancer risk (136).

In more recent years, micronuclei have emerged as a prognostic marker in patients with cancer (137–139) and as a potential predictor of therapeutic outcome (140, 141). Although the prospect of using micronuclei as cancer and chromosomal instability biomarkers is exciting, there might be additional layers of complexity to consider. In fact, we do not know yet if different types of CIN (eg, from spindle defects, chemical DNA damage, radiation) produce different outcomes, nor if different types of micronuclei behave in different ways. For example, micronuclei containing centromeric chromosomes might have more chances of getting reincorporated into the primary nucleus compared with micronuclei containing chromosome fragments. Another instance is represented by micronuclei derived by loss of the spindle protein Kif18a, which form stable nuclear envelopes and do not promote tumorigenesis in mice (142). In this light, micronuclei that are ruptured might have more palpable consequences during tumor progression than intact micronuclei. Given the importance played by catastrophic rupture in triggering immune responses and genomic rearrangements, incorporating a marker of micronuclear rupture in biopsies, such as a fluorescent staining of cGAS, can help not only to understand cancer stages and metastatic risks but also to predict the response to immunotherapies (119).

MICRONUCLEI AS A THERAPEUTIC TARGET

By virtue of their role as a communication hub within the tumor microenvironment and a platform for enhanced mutagenesis, micronuclei are fundamental during cancer evolution and metastatic progression. Therapies targeting micronuclear processes can thus be extremely selective for chromosomally unstable cancer cells, with limited toxicity for non-transformed cells. Moreover, several of the mechanisms dysregulated upon micronuclear rupture might represent a vulnerability for cancer cells. It is thus fundamental to include the presence of micronuclei as a determinant variable in the choice of treatment but also to develop new therapies considering the dependency of chromosomally unstable cancers on micronuclear rupture and subsequent processes. Here we will analyze how these processes can inform new therapeutic strategies and review the related ongoing therapies.

Exploiting Micronuclear Rupture for Synthetic Lethality

We discussed how cancer cells bearing ruptured micronuclei chronically activate the cGAS–STING pathway, suppressing the IFN response in favor of a noncanonical NF-κB prometastatic one. This signaling rewiring might represent an opportunity to target chromosomally unstable cancers: the ability to pharmacologically toggle the cGAS–STING response toward the IFN proinflammatory branch of the pathway in cancers that chronically activate cGAS will induce a potent antitumor response. This strategy might also override the often difficult therapeutic choice between STING agonists or STING inhibitors, the latter recently developed for the treatment of autoinflammatory diseases (143). In fact, although STING agonists displayed promising results in preclinical applications (144), their clinical efficacy remains to be proved even when combined with other immunotherapies (145).

Another important characteristic that distinguishes micronucleated cells from nontransformed ones, useful for the design of cancer-specific drugs, is the amount of DNA damage. As we previously discussed, chromosomes contained in ruptured micronuclei undergo massive DNA fragmentation and genomic rearrangements. Consequently, cells bearing collapsed micronuclei massively activate the DNA damage response (DDR) signaling, although with limited success because of the loss of compartmentalization (74). Pharmacologic inhibition of the DDR response, a common strategy in cancer treatment (146), might bring the DNA damage levels above the threshold for inducing cell death. Moreover, a recent study shows that DNA damage in micronuclei is the result of the activation of specific pathways that are inactive in primary nuclei (90). Micronuclei in fact seem to have a high rate of RNA-DNA hybrids (named R-loops), whose presence is also connected to genomic instability (147). In micronuclei, R-loops act as scaffold for the deaminase ADAR, which edits the DNA producing deoxyinosine (dI). dI is then recognized by the DNA-glycosylase MPG forming abasic sites, which in turn are cut by the endonuclease APE1. This mechanism is one of the major contributors of DNA fragmentation in micronuclei and is nearly absent in primary nuclei (90). Specifically targeting one or more of the involved players (148) is another stimulating possibility with promising low effects on nontransformed cells. While waiting for new therapies development, we posit that, given the importance of micronuclear rupture in eliciting

pathways that are specific to the micronucleated cell, considering micronuclei presence and rupture state in each patient will help inform the therapeutic choice (106).

Targeting Micronuclear Rupture and Subsequent Prometastatic Processes

Micronuclear rupture has important consequences for cancer progression and heterogeneity, features that are deeply involved in resistance to therapy. We postulate that therapies aiming to prevent micronuclear rupture, in combination with classical treatments, will help reduce the acquisition of resistance together with the metastasis onset. For example, recent studies suggest that both BAF and the ESCRT-III complex, ineffective in repairing the micronuclear envelope, may be somehow involved in micronuclear collapse (76, 84, 85). This eventuality has clear repercussions on the possibility of targeting these systems to prevent micronuclear envelope catastrophe and subsequent DNA damage. Moreover, BAF inhibition has already been shown to promote cancer cell death in vitro (149). Similarly, given the ESCRT-III role in repairing the plasma and nuclear membranes, and thus reducing pharmacologic cell death, targeting of ESCRT-III subunits is starting to be considered a promising cancer treatment (150).

More speculatively, we discussed cGAS activation and its regulation by phase separation (124, 126). Recently, an increasing number of drug development teams has been focusing on specific condensates modulators (151). Therapeutically disrupting phase-separated droplets might represent a novel method for cGAS inhibition. Moreover, some anticancer drugs such as cisplatin and mitoxantrone selectively partition in condensates (152); given the continuous activation of cGAS at micronuclei, the presence of highly concentrated phase-separated droplets in micronucleated cells might act as a scaffold for cancer cell–selective drug delivery.

CONCLUSIONS AND PERSPECTIVES

In this review, we discussed the biology of micronuclei with a specific focus on their frequent rupture. We discussed the proposed models of rupture together with the repair mechanisms active in the primary nucleus but flawed at micronuclei and exposed the remaining open questions in this field. We then examined the processes downstream of micronuclear collapse and the devastating consequences for patients bearing micronuclei-heavy cancers, speculating on how to exploit the existing knowledge of micronuclei for chromosomally unstable cancer treatment and detection. The study of micronuclear biology and the mechanisms underlying their collapse is fundamental to inform new strategies for the treatments of chromosomally unstable cancers. As more and more knowledge of micronuclear aberrant processes is being generated, we hope that it will be efficiently consolidated into the clinical practice, allowing advancements in the treatment of this very aggressive subset of human cancers.

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and serves on the scientific advisory board and Board of Directors (BOD) of Volastra Therapeutics, Inc., and he serves on the scientific advisory board of Meliora Therapeutics.

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Significance:

Micronuclei in chromosomally unstable cancer cells serve as pivotal catalysts for cancer progression, instigating transformative genomic, epigenetic, and transcriptional alterations. This comprehensive review not only synthesizes our present comprehension but also outlines a framework for translating this knowledge into pioneering biomarkers and therapeutics, thereby illuminating novel paths for personalized cancer management.

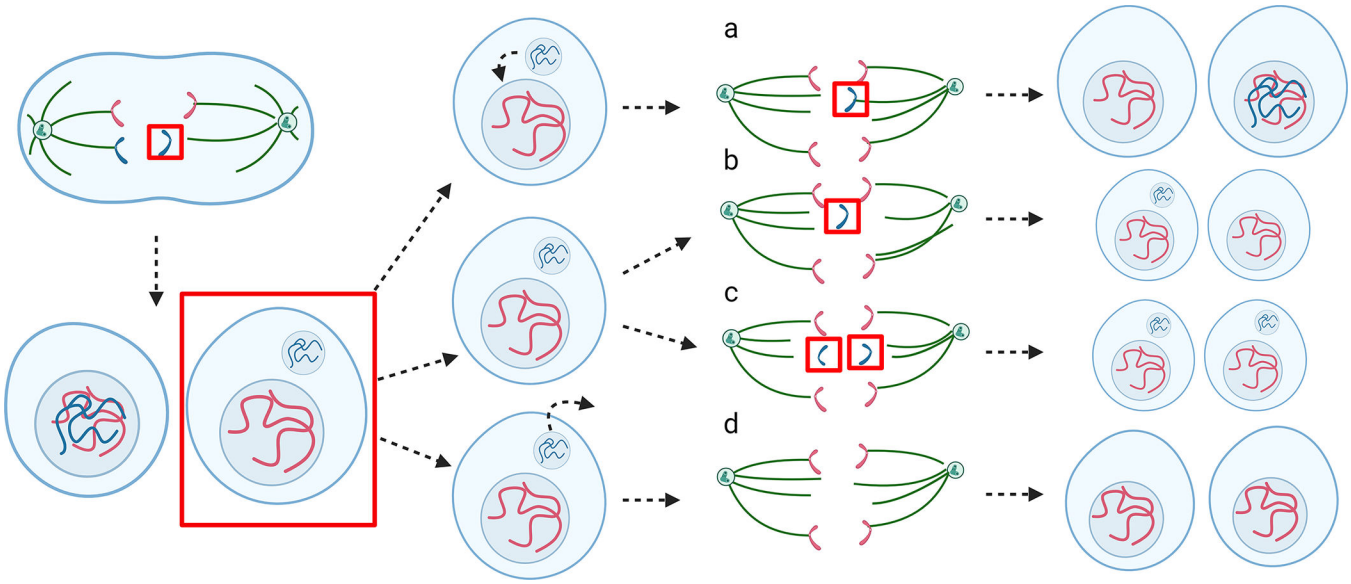

Figure 1.

The various fates of a micronucleus. Micronuclei arise from mis-segregating events where an entire or a part of a chromosome (in blue, highlighted with a red square) is left outside the primary nucleus. The micronucleus then ends randomly in one of the daughter cells; for simplicity of understanding, we consider here the option in which the micronucleus ends up in the daughter cell from which the chromosome has been mis-segregated. The micronucleus can as follows: (**A**) be reincorporated into the primary nucleus through microtubules correct attachment in mitosis, giving rise to two daughter cells (DC), one aneuploid and one diploid; (**B**) persist in a micronuclear state by mis-segregating again in the subsequent mitosis, forming a diploid DC but micronucleated and an aneuploid DC; (**C**) undergoing replication and persist in a micronuclear state through mis-segregation, forming two micronucleated, diploid DCs (for simplicity, we do not consider the possibility that one copy of the chromosome will be correctly segregating); (**D**) be extruded from the cell, forming after mitotic division two aneuploid DCs that are both missing the micronucleated chromosome. (Created with BioRender.com)

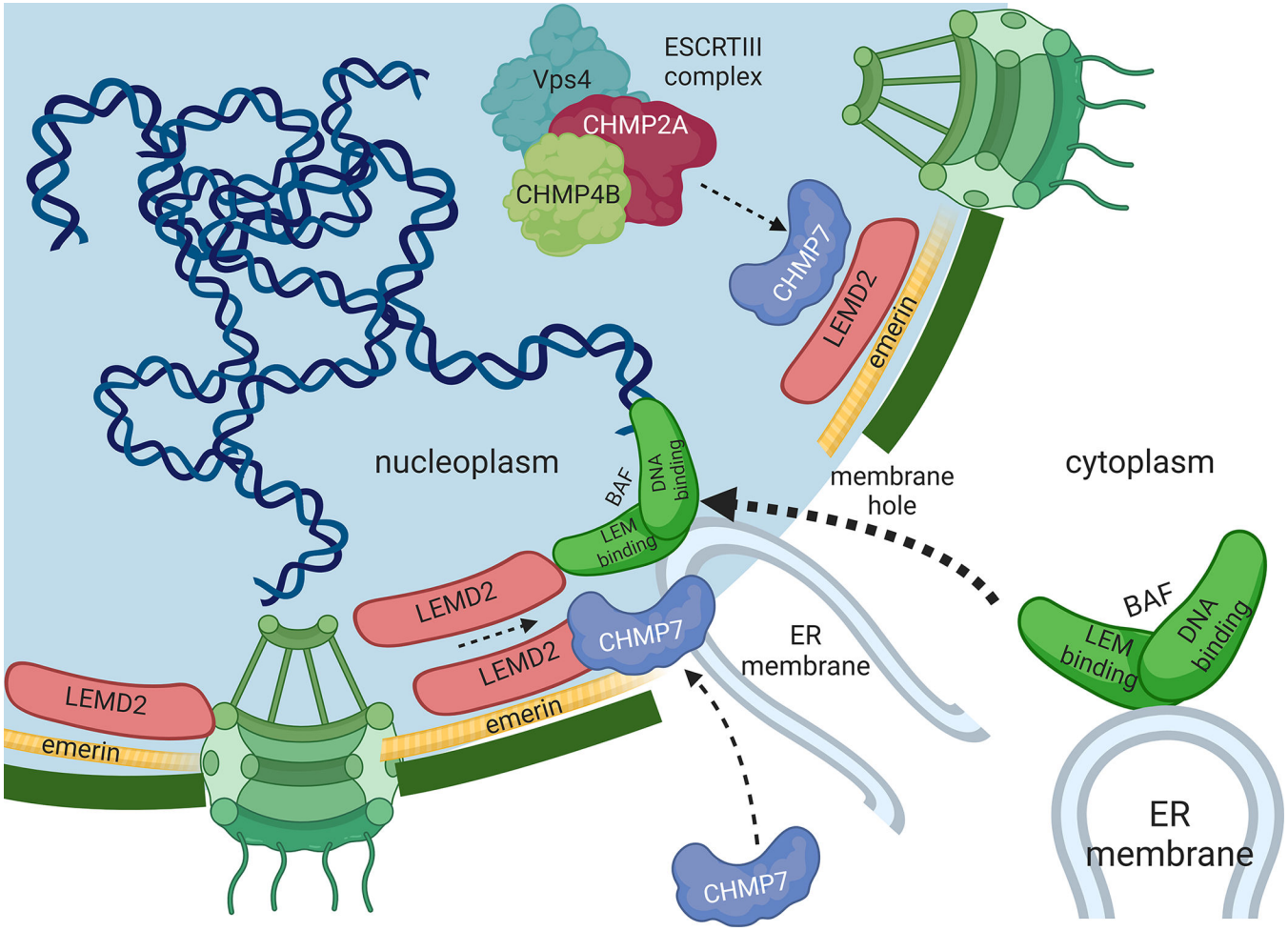

Figure 2.

Nuclear membrane repair at primary nuclei. At primary nuclei, two efficient mechanisms are in charge of repairing transient envelope rupture. The first responder is barrier-to-autointegration factor (BAF), an ER-resident membrane that localizes at the site of rupture through its DNA-binding domain, bringing at the same time ER membrane to plug the hole. With its LEM-binding domain, BAF recruits LEMD2 and other proteins of the inner nuclear membrane to the rupture site, pulling together the two sides of the membrane and providing a platform for the recruitment of CHMP7. CHMP7, upon its binding with LEMD2, catalyzes the formation of the ESCRTIII complex, which, through ATP consumption, seals the membrane in a process similar to what happens at mitotic exit. (Created with BioRender.com)

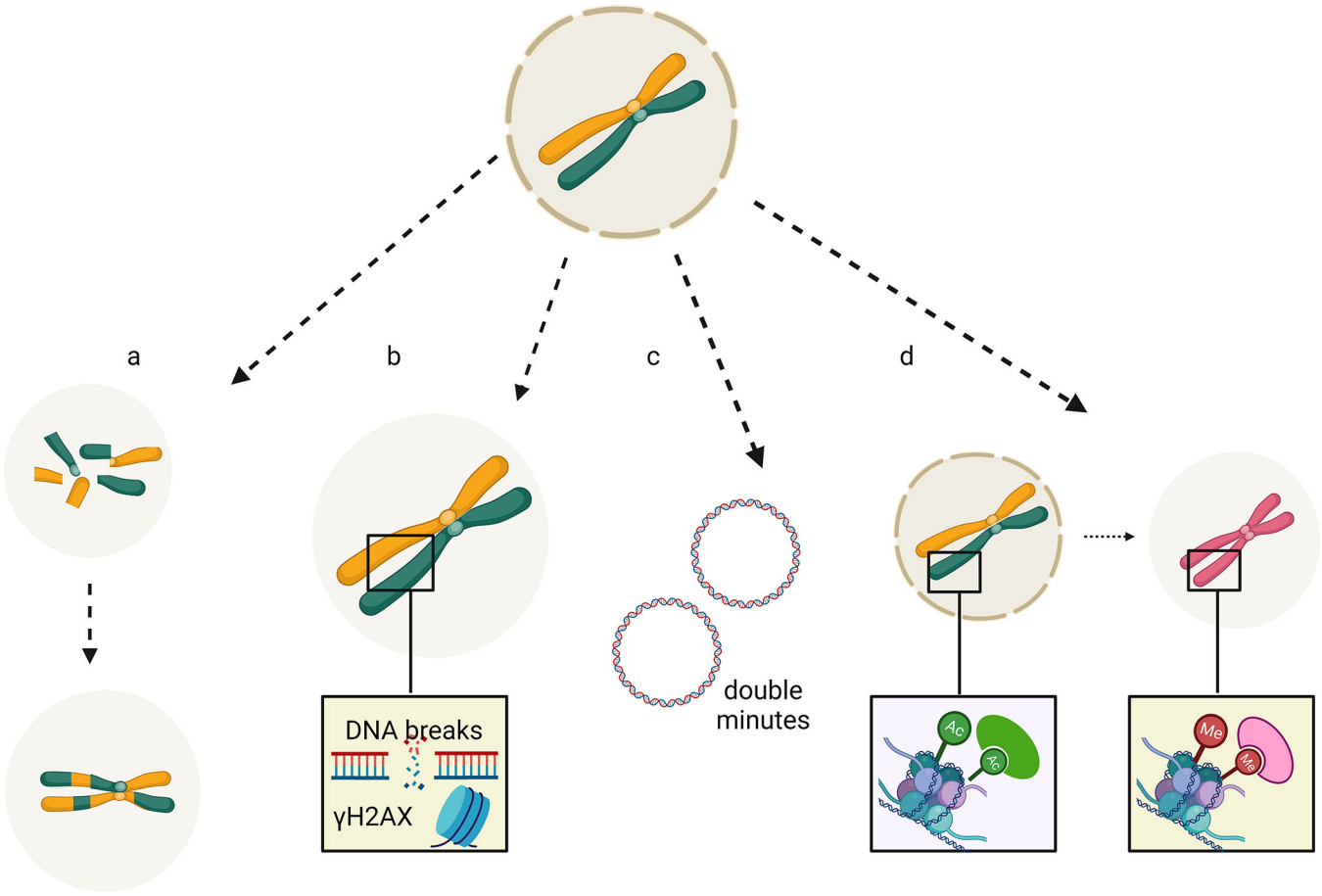

Figure 3.

Genomic consequences of micronuclear rupture. Upon nuclear envelope collapse, the genetic material contained in the micronucleus undergo profound changes and rearrangements: (**A**) the chromosome undergoes shattering and is erroneously repaired in a process called chromothripsis; (**B**) chromosomes undergo massive DNA damage that can remain unrepaired; (**C**) genomic rearrangements and amplifications of oncogenes give rise to circular oncogene-rich DNA molecules historically known as double minutes; and (**D**) epigenetic marks are modified in a heritable way. (Created with BioRender.com)

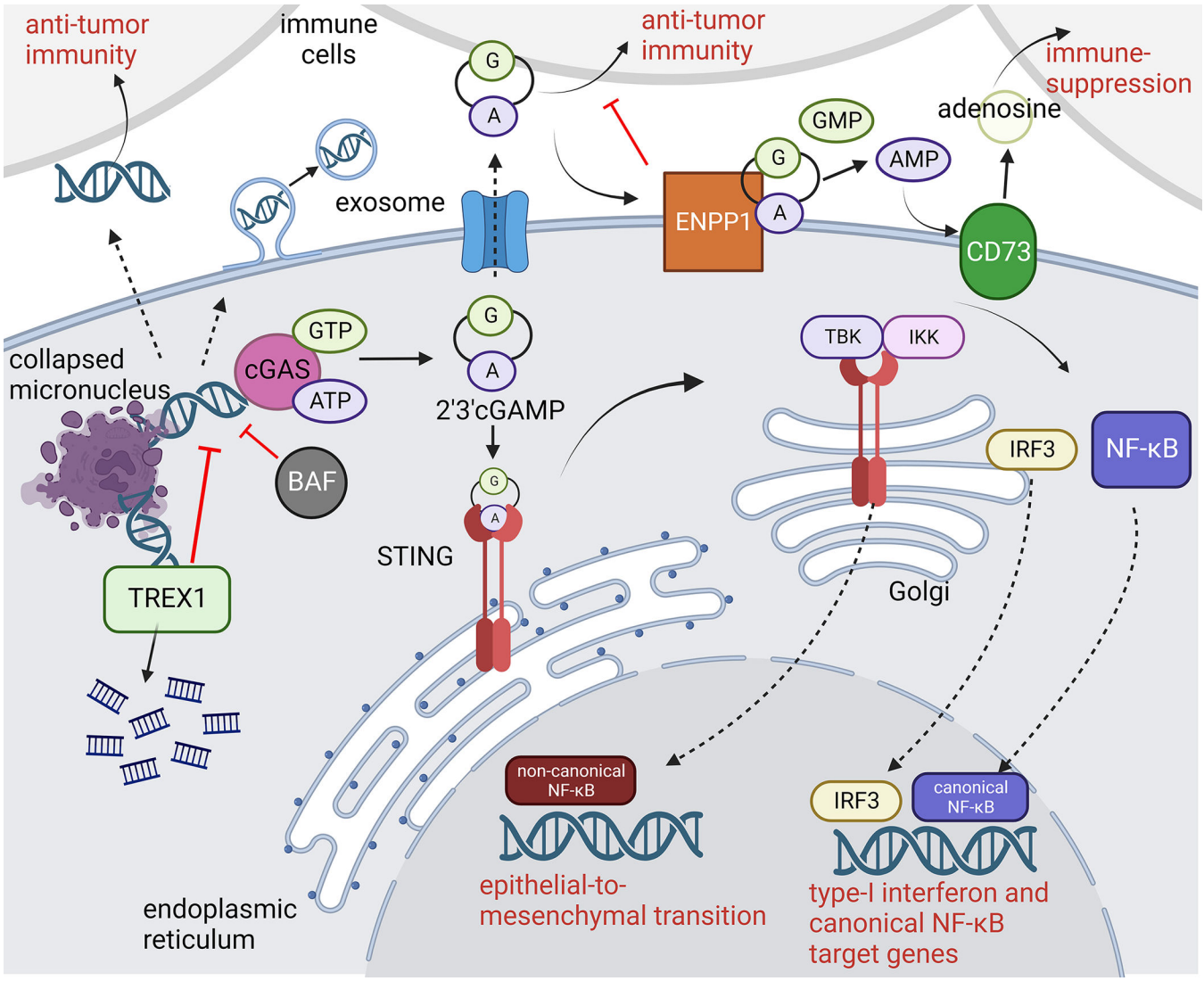

Figure 4.

Inflammatory consequences of micronuclear rupture. Ruptured micronuclei spilled their genetic content into the cytosol. The DNA can be exported, perhaps also included in exosomes, into the extracellular matrix, where it can elicit an antitumor immune response. In the cytosol, the DNA can be bound by BAF in an attempt to repair the membrane, or it can be digested by the endonuclease TREX1. Both of these mechanisms exert an inhibitory function on the other cytosolic DNA-binding protein, the cyclic GMP-AMP synthase cGAS. Upon binding with DNA, cGAS activates and produces, from ATP and GTP, their cyclic product 2′3′ cGAMP. cGAMP can be exported from the cell and taken up by the immune system, eliciting an anti-tumor immune response. If cancer cells express the hydrolase ENPP1, the exported cGAMP is converted back to GTP and ATP. ATP in turn is transformed in adenosine, a molecule with immune-suppressive function. In the cell, cGAMP activates STING and induces, its translocation from the ER to the Golgi, where it activates TBK1, IKK, and IRF3. These transcription factors activate in turn NF-κB, which, enters the nucleus and induces gene expression. Which genes are activated is highly

context dependent: in chronic settings, NF-κB shifts from inducing Interferon Stimulated Genes expression and thus antitumor immunity to a non-canonical response that activates an epithelial-to-mesenchymal transcription program and promotes metastasis. (Created with BioRender.com)