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REVIEW ARTICLE

Impaired nuclear functions in micronuclei results in genome instability and chromothripsis

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Abstract Micronuclei (MN) have generally been considered a consequence of DNA damage and, as such, have been used as markers of exposure to genotoxic agents. However, advances in DNA sequencing methods and the development of high-resolution microscopy with which to analyse chromosome dynamics in live cells have been fundamental in building a more refined view of the existing links between DNA damage and micronuclei. Here, we review recent progress indicating that defects of micronuclei affect basic nuclear functions, such as DNA repair and replication, generating massive damage in the chromatin of the MN. In addition, the physical isolation of chromosomes within MN offers an attractive mechanistic explanation for chromothripsis, a massive local DNA fragmentation that produces complex rearrangements restricted to only one or a few chromosomes. When micronuclear chromatin is reincorporated in the daughter cell nuclei, the under-replicated, damaged or rearranged micronuclear chromatin might contribute to genome instability. The traditional conception of micronuclei has been overturned, as they have evolved from passive indicators of DNA damage to active players in the formation of DNA lesions, thus unravelling previously unforeseen roles of micronuclei in the origins of chromosome instability.

Introduction

Genomic instability is a characteristic of cancer cells and can be defined as a condition in which cells acquire alterations in the genome at a high rate. Depending on the genetic level at which these alterations occur, these alterations can be classified as nucleotide instability (NIN), microsatellite instability (MIN or MSI) or chromosome instability (CIN) (Lengauer et al. 1998). NIN includes substitutions, deletions and/or insertions of nucleotides, while MIN/MSI involves changes in the number of repeated nucleotides in the microsatellite sequences derived from mismatch–repair defects. CIN is categorized into S-CIN (segmental chromosome instability) when alterations occur in the chromosome structure or into W-CIN (whole chromosome instability) when there is a gain or loss in the number of chromosomes.

It is accepted that micronuclei (MN) constitute a good cellular indicator of CIN because most solid tumours and pre-neoplastic lesions display CIN and high frequencies of MN (Gisselsson et al. 2001a). Moreover, high frequencies of MN were found in peripheral blood lymphocytes of healthy patients that developed cancer years later (Iarmarcovai et al. 2008; Bonassi et al. 2011). For this reason, a high frequency of MN in peripheral blood lymphocytes of healthy people may also be indicative of cancer risk. Although MN are considered passive indicators of chromosomal instability, a growing body of evidence shows that the chromatin sequestered into MN can accumulate massive damage thus making researchers suspect that MN themselves could cause

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chromosomal instability. For this reason, we collected data regarding morphology and physiology of MN in an attempt to determine how MN could participate in triggering or maintaining CIN.

## Micronuclei as indicators of chromosome instability

Micronuclei are organelles very similar to the primary nuclei (PN) but smaller in size. They were first observed in erythrocytes by haematologists William Howell (1890) and Justin Jolly (1905); for this reason, MN are also referred as Howell–Jolie bodies (Fenech et al. 2011). In recent years, studies were focused on the nature of MN and the effects of different drugs on the formation of MN. It was concluded that the treatment of cells with aneugenic or clastogenic agents results in the formation of MN that contain, respectively, acentric fragments or whole chromosomes that are excluded from the daughter nuclei at the end of mitosis. Consequently, analysing the presence of MN in peripheral blood lymphocytes became an indispensable assay to assess genotoxicity (Klein and Klein 1952; Evans et al. 1959; Heddle 1973; Schmid 1975; Fenech et al. 2011). As the frequency of MN was found to be influenced by the number of cell divisions, the incorporation of cytochalasin-B in the assay provided a robust methodology for scoring MN in binucleated cells, i.e. cells that had divided once (Fenech and Morley 1985). The combination of the cytokinesis-block micronucleus (CBMN) assay with kinetochore labelling or centromeric in situ hybridization allowed scientists to determine the MN content and thus distinguish between aneugenic and clastogenic agents (Lynch and Parry 1993; Doherty et al. 1996). CBMN has been automatized using flow cytometry analysis; international regulatory guidelines recommend use of the CBMN assay, as it has been proven to be an effective tool for studying cellular and nuclear dysfunction caused by in vitro or in vivo ageing, micronutrient deficiency or excess, genotoxin exposure and genetic defects in genome integrity maintenance (Fenech 2007; Avlasevich et al. 2011).

As outlined above, cell treatment with aneugens or clastogens may derive in the formation of MN. Specifically, treatment with clastogenic agents induces double-strand breaks (DSBs) that, when unrepaired, derive in the formation of acentric fragments and chromosome rearrangements. During mitosis, the acentric fragments are unable to integrate in any of the daughter nuclei because of their incapacity to attach to spindle fibres (Fig. 1a). When the nuclear envelope is formed, they are enclosed separately from the PN and arise as MN (Heddle and Carrano 1977). Alternatively, the misrepair of two broken ends, of two unprotected ends or of one unprotected end with a broken end results in the formation of a dicentric chromosome (Latre et al. 2003; Shay and Wright 2005). When the two centromeres of a dicentric chromosome

are pulled to opposite poles, a chromatin (anaphase) bridge is formed. Bridges derived from dicentric chromosomes formed during telomere crisis frequently evolve into long chromatin bridges connecting the daughter cells (Maciejowski et al. 2015). However, chromatin bridges may break and result, at the end of mitosis, in the formation of acentric fragments that arise as MN (Fig. 1b) or nuclear blebs (micronuclei-like bodies attached to the PN by a chromatinic filament) (Hoffelder et al. 2004). The resulting broken ends are susceptible to further reorganization that can initiate a new breakage–bridge–fusion (BFB) cycle (Gisselsson et al. 2001b). Therefore, BFB cycles are considered a source of chromosomal instability in cancer cells and the resulting MN as markers of S-CIN.

Certain aneugenic agents induce aneuploidy by chromosome lagging. Specifically, they prevent formation of the spindle apparatus during mitosis and, when the spindle is finally assembled, increase the frequency of the simultaneous attachment of kinetochores to microtubules from both poles (merotelic attachments). Due to their incapacity to segregate in either of the daughter nuclei, the chromatids having merotelic kinetochore orientation are lagged behind the bulk of chromosomes and sequestered into MN during the nuclear envelope (NE) formation (Fig. 1) (Cimini et al. 2002). Not only aneugens but also defects during mitotic spindle assembly, misregulation of the spindle assembly checkpoint (SAC) and the presence of supernumerary centrosomes increase chromosome lagging and, consequently, the formation of MN carrying whole chromatids or chromosomes (Marshall et al. 1996; Chan et al. 1999; Ganem et al. 2009). Moreover, it has been recently observed that distorted dicentric chromosomes that resist as unbroken units can eventually result in the formation of MN as the mitotic spindle disappears (Fig. 1b) (Pampalona et al. 2009). Under each of these circumstances, MN would be indicators of W-CIN.

Micronuclei can also derive from the aggregation of double minutes (DMs), which are autonomously replicating, acentric and telomere-free extrachromosomal bodies composed of megabases of circular DNA. Each DM contains a gene copy, and thus, together with chromosomal homogenously staining regions, DMs are the cytological manifestation of gene amplification. Considering that the amplification of oncogenes or therapeutic drug-resistant genes can play a pivotal role in the malignant transformation of cancer cells, DMs have important implications for cancer cell phenotype (Shimizu 2011) and, accordingly, are only found in cancer cells. Time-lapse experiments of cells expressing GFP-tagged DMs allowed understanding the intracellular behaviour of DMs and the generation of DM-type MN. Multiple copies of a DM can be found in a single cell; they stick to chromosomes to ensure their transmission to daughter cells during mitosis (Kanda et al. 1998). When detached from chromosomes, DMs can be sequestered into MN during either mitosis or interphase (Kanda and Wahl
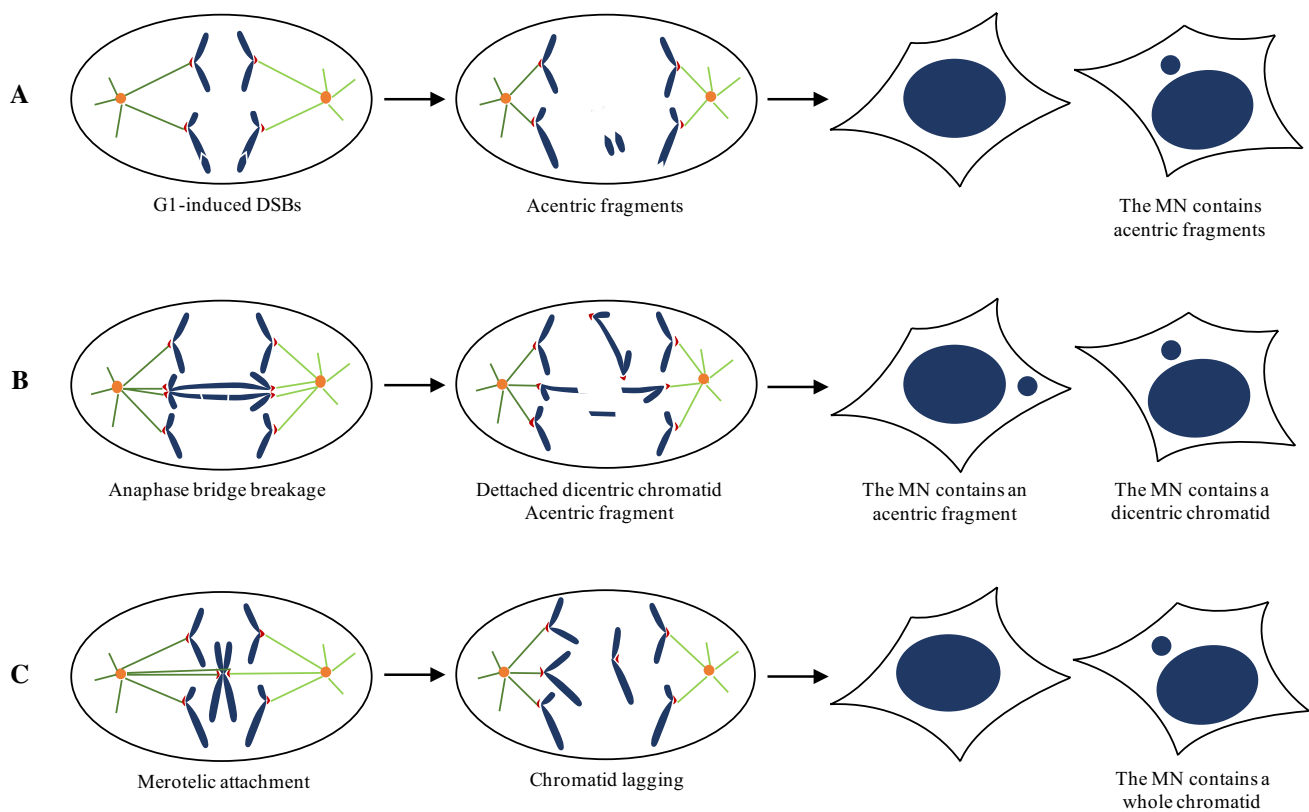
Fig. 1 Origin of micronuclei. **a** The presence of double-strand breaks in a mitotic chromosome results in the formation of acentric fragments that can be sequestered inside a micronucleus because they are incapable of adhering to spindle fibres and integrating in the daughter nuclei. **b** Torsion between the two centromeres of a dicentric chromosome derives in the formation of an anaphase bridge. One of the dicentric chromatids is detached from the microtubules and lost in a micronucleus after nuclear envelope (NE) formation. The other dicentric chromatid breaks and the resulting acentric fragment is sequestered into a micronucleus at the end of mitosis. **c** Merotelic attachment results in chromatid lagging, and after the NE formation, it is left out of the daughter nuclei and sequestered inside a micronucleus in one of the daughter cells

2000). During interphase (most frequently, during S phase), this phenomenon occurs by nuclear budding. The frequency of the phenomenon is dramatically increased when cells are treated with low levels of DNA replication inhibitors (e.g. hydroxyurea) or with low doses of radiation (Shimizu et al. 1998; Schoenlein et al. 2003). Alternatively, DM-type MN appear during mitosis when different DMs bind to each other forming an anaphase bridge-like structure that detaches from chromosomes and is separated from the daughter nuclei when nuclear envelope is reassembled (Tanaka and Shimizu 2000). These mechanisms could have a profound influence on determinations of cancer cell phenotype, and the presence of DM-type MN in these cells is indicative of CIN.

Most micronuclei display disrupted nuclear envelope

While hundreds of studies focused on the micronuclear content, only a few dozens have explored the micronuclear

structure. Considering that MN are frequently described as organelles similar to the cell nuclei but smaller in size, it is assumed that micronuclear chromatin is surrounded by a nuclear envelope like the PN. The NE is composed of two lipid bilayers perforated by the nuclear pore complexes (NPCs), which consist of multiple copies of different nucleoporines that serve as the primary transport gate for molecular exchange between nucleus and cytoplasm. Lining the inner nuclear membrane, the nuclear lamina is a meshwork of intermediate filaments (lamins A, B1, B2 and C) that provide structural support to the nucleus and serve as a scaffold for spatial genome organization (Dittmer and Misteli 2011).

In the late 1980s, by means of electronic microscopy, NPC distribution among MN derived from chromosome lagging was observed to be heterogeneous, and some MN displayed an incomplete NE with gaps in the double membrane and areas without lamina or condensed chromatin (Geraud et al. 1989). Lamin B1 defects were also observed in DM-type MN (Utani et al. 2007, 2011), and

immunodetection studies revealed that MN derived from anaphase bridge resolution exhibited apparently normal lamina but also displayed reduced NPC density (Hoffelder et al. 2004). Similar observations were made in our laboratory: 14 % of radiation-induced MN lacked lamin B1 and nucleoporines labelling, 54 % displayed only lamin B1 and 30 % exhibited an apparently normal NE as they displayed lamin B1 and nucleoporines labelling (Terradas et al. 2012). Therefore, defects in the structure of the NE have been described in all types of MN.

Nonetheless, the presence of an apparently normal NE in MN may not entail a functional nucleocytoplasmic transport. In this regard, the lack of nuclear proteins such as cell cycle regulator cyclin D1 or the DNA repair factor XPC in some MN drove us to speculate about possible nuclear trafficking defects. Indeed, some MN displaying normal nucleoporine staining were devoid of XPC, pointing to possible functional defects in NPC functionality (Terradas et al. 2012). Similar conclusions were reached when testing other components, i.e. 100 % of PN showed uptake of exogenous glucocorticoid receptor while only 10 % of BFB-derived MN did (Hoffelder et al. 2004). Micronuclear import capacity is also reduced in MN derived from chromosome lagging. In this type of MN, even if the NPCs assembly pathway is not defective, the NPCs are diminished and MN have a striking defect in nuclear import (Crasta et al. 2012). In fact, the misallocation of nuclear and cytoplasmic fluorescent reporters in MN derived from chromosome lagging indicates that not only micronuclear import but also micronuclear export is defective, demonstrating that MN have disrupted nuclear permeability and, consequently, a loss of compartmentalization in relation to the cytosol (Hatch et al. 2013). These authors also observed that micronuclear disruption occurs in normal and in transformed cells and is cell cycle independent, although the proportion of disrupted MN increases during interphase. Further experiments revealed that while NE ruptures in the PN are repaired within minutes, NE ruptures in MN are almost always irreversible. Therefore, MN disruption is caused by non-mitotic breakdown of the NE but does not involve cytological disintegration of the MN (Hatch et al. 2013). Non-mitotic breakdown of the micronuclear NE is characterized by chromatin compaction and a failure to exclude ER tubules from the chromatin mass. MN disruption has been linked to lamin B1 defects as the number of disrupted MN increased in lamin B1-depleted cells. Accordingly, subsequent expression of non-degradable lamin B1 decreased the number of disrupted MN to control levels (Hatch et al. 2013). To finish with, no correlation between MN disruption and chromatin amount exists, and accordingly, the presence or absence of a centromere does not affect MN disruption (Hatch et al. 2013). Therefore, MN disruption occurs independently of micronuclear content and could be caused by lamin B1 depletion.

Overall, NE defects can be observed in MN originated from whole or broken chromosomes and in DM-type MN. These defects, which have been linked to lamin B1 depletion, derive from non-mitotic breakdown of the micronuclear NE and, consequently, result in disruption of the micronuclear nucleocytoplasmic transport. This affects the development of nuclear functions in MN.

Structural NE defects reduce DNA repair capacity of MN

Studying DNA damage repair (Box 1) in MN can be puzzling, as DNA damage is also a key event in the formation of some types of MN. Therefore, it is important to distinguish whether the damage is triggered before or after MN formation. MN carrying acentric fragments or broken dicentric chromosomes may contain DDR factors derived from the DNA damage that caused their formation. In this regard, cells with dicentric chromosomes that formed during telomere crisis develop chromatin bridges decorated with RPA, a DDR factor indicative of single-strand breaks (Maciejowski et al. 2015), and accordingly, RPA have been detected in radiation-induced MN (Haaf et al. 1999). It is known that the signalling cascade activated by DSBs is truncated in mitosis (Giunta and Jackson 2011). Thus, while signalling factors can be properly recruited to damaged mitotic chromatin before MN formation, mediators and effectors need to be recruited after mitosis, when the micronucleus is already formed. In this regard, only some radiation-induced MN, which mainly derive from acentric chromosome fragments, display γH2AX colocalizing with the signalling factor MRE11. During interphase, downstream factors, such as 53BP1, should be recruited. However, colocalization between γH2AX and 53BP1 is even lower than with MRE11 (Terradas et al. 2009). The fact that not all MN display full colocalization between γH2AX and MRE11 or 53BP1 suggests that the recruitment of DDR factors in MN is limited not only by cell cycle but also by nucleocytoplasmic transport defects.

In order to study the relationship between nucleocytoplasmic transport defects and DDR impairment in MN, we induced DNA damage on preexisting MN. Radiation-induced MN were exposed to UVC light, and the presence of NER factors together with components of the NE was analysed by immunodetection. First, we observed that 40 % of damaged MN displayed XPC labelling but only 20 % showed both XPC and XPA. XPC levels increased over time in the PN after UVC light exposure because XPC is capable of shuttling between compartments (Hoogstraten et al. 2008). Lack of XPC labelling in

most of the damaged MN indicates that these MN likely have a disrupted nucleocytoplasmic transport. Analysis of the NE components showed that although 86 % of radiation-induced MN displayed lamin B1 labelling, only 32 % showed nucleoporine staining. These NE structural defects clearly explain XPC transport impairment because only those MN with nucleoporines staining displayed XPC labelling (Terradas et al. 2012). Similar studies were performed in MN carrying whole chromosomes. Treatment of micronucleated cells with both aphidicolin (replication inhibitor) and ionizing radiation induced DSBs lesions properly signalled by γH2AX but devoid of downstream factors such as 53BP1 (Crasta et al. 2012) compatible with nucleocytoplasmic transport defects. The persistence of γH2AX in MN indicates slow or inexistent repair capacity (Terradas et al. 2009; Crasta et al. 2012), demonstrating that damage persists in MN. Overexpression of lamin B2 prevented γH2AX foci appearance, confirming that structural NE defects cause transport deficiency through the micronuclear envelope and, consequently, impair the DNA repair capacity (Hatch et al. 2013). Together, these results indicate that DNA repair functions are impaired in MN as a consequence of micronuclear envelope disruption, leading to the localized accumulation of DNA damage.

Defective DNA replication can cause micronuclear DNA damage

Accumulation of DNA damage in MN has been associated not only with defective DNA damage signalling and repair functions, but also with replication stress of the chromatin sequestered inside the micronucleus. It has recently been reported that micronuclear DNA replication is defective and asynchronous, with a delay in the initiation of DNA replication and many MN continuing replication into the G2 phase (Crasta et al. 2012). The net result is that chromosomes in MN are significantly under-replicated, with some regions of the chromosome replicated while other regions remain unreplicated. It is not clear what causes DNA replication defects. Recruitment of the origin replication complex (ORC) seems to be equally efficient in the MN as in the PN, but newly generated MN showed significantly reduced recruitment of replicative DNA helicases and replication initiation factors (Crasta et al. 2012). The authors suggest that the failure of MN to recruit replication components may be due to a defect in nucleocytoplasmic transport because they observed a reduction in the density of NPCs and in nuclear imports (Crasta et al. 2012). Indeed, disrupted MN, characterized by their loss of compartmentalization, completely lacked EdU labelling, while the majority of intact MN and all the PN did (Hatch et al.

2013). Therefore, the MN disruption affects not only DNA repair but also DNA replication.

Interestingly, it was observed that the replication stress that suffers the chromatin sequestered inside the micronucleus derives in an accumulation of DNA damage and extensive fragmentation of the chromosome in the micronucleus (Crasta et al. 2012). In this regard, immunodetection experiments on γH2AX revealed that a fraction of MN generated by chromosome lagging accumulated DNA damage during the S and G2 phases, while little or no damage occurred during G1 (Crasta et al. 2012). Replication-associated damage might occur from replication fork collapse and/or processing of replication intermediates by cytoplasmic nucleases. This could occur as a consequence of the non-mitotic MN disruption and loss of compartmentalization described by Hatch and colleagues (Hatch et al. 2013). Alternatively, if replication of an intact micronucleus continues into mitosis, DNA damage might occur after mitotic nuclear envelope breakdown (Crasta et al. 2012). In this case, as the MN DNA is still replicating, cytoplasmic nucleases, which cannot attack an intact chromosome, will attack the DNA in collapsed or stalled replication forks and induce additional DNA damage.

In summary, replication-associated damage in MN might occur from replication fork collapse and/or processing of replication intermediates as a consequence of non-mitotic MN disruption or mitotic breakdown of the envelope of MN containing under-replicated chromatin. In addition to replication-associated damage, the inability of MN to repair the DNA lesions evolves into an accumulation of localized damage in the micronuclear chromatin.

Accumulation of massive DNA damage in micronuclei and bridges may explain chromothripsis

The physical isolation of chromosomes within MN offers an attractive mechanistic explanation for chromothripsis (Box 2 and Box 3), a spectacular phenomenon in which massive local DNA fragmentation is generated in a single catastrophic event. In this regard, incorporation of fluorescently labelled nucleotides by terminal deoxynucleotidyl transferase reveals the massive breakage of the micronuclear content but not of the PN (Terradas et al. 2009). Similarly, MN formation by the induction of lagging chromosomes leads to mitotic cells that frequently present pulverized chromosomes (Crasta et al. 2012). Localized shattering of the chromatin confined in MN can be a consequence of defective DNA damage response and/or incomplete DNA replication (Terradas et al. 2009; Crasta et al. 2012). Localized chromatin shattering in chromothriptic events may result in complex rearrangements restricted to only one or a few chromosomes. An elegant

approach combining live-cell imaging and single-cell genome sequencing of daughter cell pairs after a single-cell division has recently shown that the formation of MN is at least one mechanism underlying chromothripsis (Zhang et al. 2015). In this study, MN were identified by live-cell imaging. The visual identification of daughter cells that arise from mothers carrying a micronucleus followed by single-cell sequencing of the daughter cell that inherited the micronucleus revealed oscillating patterns of DNA copy number levels along with complex genomic rearrangements restricted to only one or a few chromosomes (Zhang et al. 2015). The sequence identity of the chromosome mis-segregated in the micronucleus was inferred thanks to previous knowledge indicating that the chromosome in the micronucleus was under-replicated (Crasta et al. 2012). These findings suggest that the passage of chromosomes through MN can instigate chromothripsis.

Chromothripsis has also been related to nucleoplasmatic bridges. Massive localized DNA breakage observed on long chromatin bridges resulting from telomere dysfunction could also lead to highly localized breaks and rearrangements within a limited number of chromosome arms in a chromothripsis-like phenomenon (Maciejowski et al. 2015). As in the case of MN, chromatin bridges lose nuclear envelope integrity in interphase. Specifically, the intensity of lamin A/C and lamin B1 staining gradually diminished as the bridges extended and several NE components such as NPCs are not detectable in the bridge envelope (Maciejowski et al. 2015). Altered NE lead to a loss of compartmentalization in chromatin bridges as evidenced by visualization of a fusion protein formed by a fluorescent protein with a nuclear localization signal, whose intensity diminishes in the bridge and the PN and increases in the cytoplasm (Maciejowski et al. 2015). Transient lack of compartmentalization allows access of cytoplasmic exonucleases such as TREX1 to the chromatin. The action of these enzymes contributes to the generation of ssDNA and eventually facilitates resolution of the chromatin bridge by fragmentation (Maciejowski et al. 2015). Moreover, the authors documented complex chromosome rearrangements compatible with an episode of chromothripsis in half of the descendant clones sequenced in their experimental system (Maciejowski et al. 2015). Although MN were not frequently observed in this cellular model, MN may have formed after the breakage of chromatin bridges at multiple loci, contributing to chromothripsis. In fact, dicentric chromosomes bridging at anaphase may certainly be an important precipitant of chromothripsis in cancer, as this association has been observed in acute lymphoblastic leukaemia patients carrying segmental amplifications of chromosome 21 (Li et al. 2014). In these patients, repeated cycles of shattering of dicentric isochromatids that arise after the fusion of broken or uncapped sister chromatids followed by random rejoining of the fragments led to segmental

amplification. Strikingly, patients born with constitutional Robertsonian translocation between chromosomes 15 and 21 have a 2700-fold increased risk of developing acute lymphoblastic leukaemia with amplifications of chromosome 21 because their cells are more prone to form anaphase bridges. A similar pattern of segmental amplification associated with BFB cycles and chromothripsis has been observed in solid tumours (Nones et al. 2014; Ernst et al. 2016). Therefore, dicentric chromosomes bridging at anaphase may be an important precipitant of chromothripsis.

In summary, these studies have revealed previously unforeseen parallelisms between micronuclei and chromatin bridges, both of which present difficulties in maintaining the integrity of their NE, thus facilitating nuclease intervention and extensive DNA fragmentation. Therefore, although MN and nucleoplasmatic bridges are conspicuous structures that have long been observed in genomically unstable tumour cells, the consequences of their presence have just only now started to be disclosed.

Conclusions

Although MN have been historically considered passive indicators of CIN, in light of the most recent studies, MN themselves might be a cause of DNA damage and have an important impact on the cell as eventual triggers of CIN. Recent evidences indicate that, due to disruption of the NE, MN possess defective DNA replication and repair resulting in the accumulation of massive damage, such as shattering of chromosomes that characterize chromothripsis. It is known that DNA repair defects have deleterious consequences for the cell, but micronuclear DNA lesions would not have consequences in terms of CIN if MN were eliminated from cells. However, MN seem to persist as it was described that cells carrying radiation-induced MN are predisposed to produce micronucleated daughter cells (Huang et al. 2011). Most importantly, by means of photoactivation of the micronuclear chromatin, it was revealed that a small percentage of micronucleated cells reincorporate the MN into one of the daughter nuclei after mitosis. This phenomenon occurs with significant frequency (Crasta et al. 2012). Consequently, damaged chromatin of micronuclear origin will be present in the PN. Although the damaged micronuclear chromatin would then benefit from the functional DDR of the PN, the most probable outcome will be illegitimate repair, as micronuclear chromatin has been shown to be highly fragmented, thus increasing the chances of incorrect end joining. The final outcome would likely be chromosome reorganizations susceptible of starting BFB cycles and ignition of CIN (Fig. 2). Chromosome reorganizations may also derive from the massive shattering of nucleoplasmatic bridges through two mechanisms. Firstly, through

breakage and retraction of the shattered bridging chromatin to the daughter nuclei, and, secondly, by sequestration of the shattered chromatin in MN and its reincorporation in one of the daughter nuclei (Fig. 2). In fact, sequencing of cancer genomes has shown that the physical isolation of chromosomes or chromosome arms in MN or in chromatin bridges drives chromothripsis.

The past few years have seen rapid progress in defining the mechanisms for chromothripsis. These generally highlight the deep connection between NE architecture and the maintenance of genomic integrity. Here we have reviewed how the massive and localized chromatin fragmentation observed in chromothripsis can be derived from the physical isolation of a chromosome under the particular environmental conditions of disrupted MN and nucleoplasmatic bridges. Chromothripsis is deeply connected to CIN, as it is thought to promote cancer development by leading to the loss of tumour suppressor genes, to the formation of oncogenic fusions and to oncogene amplification (Stephens et al. 2011; Rausch et al. 2012). Therefore, the DNA contained in MN is not "lost" for the cell, but the basic nuclear functions of the DNA entrapped in MN are definitely deteriorated. MN display defects in DNA replication and DNA damage signalling and response that have been associated with defects in assembly of the NE and loss of compartmentalization. If the damaged micronuclear chromatin of disrupted MN is reintegrated in the PN of daughter cells in the following interphase, new substrates for chromosome rearrangements could be available and, consequently, CIN could be ignited.

Box 1

Response to double-strand breaks and photolesions

When DNA damage is inflicted, a hierarchical cascade of proteins, known as the DNA damage response (DDR), is activated to detect, signal, and promote repair of the damage. In the front line of the DDR, multiple sensors are in charge of recognizing different DNA lesions and activating the transducers, which are kinases responsible of the downstream factors' activation that will induce a temporary delay of the cell cycle progression and promote DNA repair.

Two different cell cycle-dependent pathways respond to double-strand break (DSB) repair: the non-homologous end joining (NHEJ), which is active throughout interphase, and the homologous recombination (HR) that operates during S and G2 phases. In the NHEJ, the heterocomplex formed by Ku70 and Ku80 detects the DSB and maintains the broken ends together (Downs and Jackson 2004). Afterwards, two subunits of the DNA-PKcs transducer kinase bind to the Ku proteins and activate the downstream factors that control ends processing, DNA synthesis, resolution and ligation (Yoo and Dynan 1999; Mahaney et al. 2009). In the HR, the Mre11–Rad50–Nbs1 (MRN) complex senses the DSBs and promotes activation of the ATM kinase that, in turn, activates effectors that will promote cell cycle arrest and DNA repair by searching and copying the same sequence from the sister chromatid (Uziel et al. 2003; Kurz and Lees-Miller 2004).

One of the first substrates of both DNA-PKcs and ATM is H2AX histone, which is phosphorylated at its Ser139 (γH2AX) (Rogakou et al. 1998). Spreading of H2AX phosphorylation along the chromatin surrounding the DNA lesion provides a docking platform for recruitment of other sensors such as MDC1 and mediators such as 53BP1 and BRCA1, which in turn regulate loading of effectors (Bekker-Jensen and Mailand 2010). While 53BP1 favours recruitment of effectors from the NHEJ, BRCA1 is related to recruitment of HR's factors such as Rad51 (Bouwman et al. 2010; Chapman et al. 2013; Callen et al. 2013). It is worth noting that during mitosis, DSBs are detected by sensors of the HR, but the repair process is suppressed and thus DSBs that persisted unrepaired until mitosis or that originated in mitosis are not repaired until the subsequent G1 phase. Specifically, the signalling cascade is truncated at the MDC1 level. Thus, mediators and effectors are not recruited to the damaged site until the next interphase (Giunta and Jackson 2011).

The nucleotide excision repair (NER) pathway is in charge of the repair of a wide variety of lesions like DNA adducts and helix-distorting photolesions, which are usually induced by UV light (Gillet and Scharer 2006). Depending on the transcription state of the damaged genes, different detectors are responsible for damage recognition. When genes are being transcribed lesions are detected by the RNA polymerase II itself. Otherwise, the damage is recognized by the collective action of UV-DDB and a complex containing XPC (Naegeli and Sugasawa 2011). In both cases, the transducer kinase ATR phosphorylates Chk1, initiating a signal transduction cascade that culminates in cell cycle arrest. Afterwards, different factors such as members of the xeroderma pigmentosum (XP) family unwind the dsDNA (XPB and XPD), stabilize the ssDNA (RPA), confirm the presence of the lesion (XPA), remove the affected DNA (XPG and XPF) and finally fill and seal the gap (Jones and Wood 1993; Mu et al. 1996; Coin et al. 2007; Moser et al. 2007).

Box 2

Chromothripsis and chromoanagenesis: their relevance in human cancer and constitutive disorders

Chromosome shattering occurring in a single catastrophic event (chromothripsis, from the Greek "chromo" for
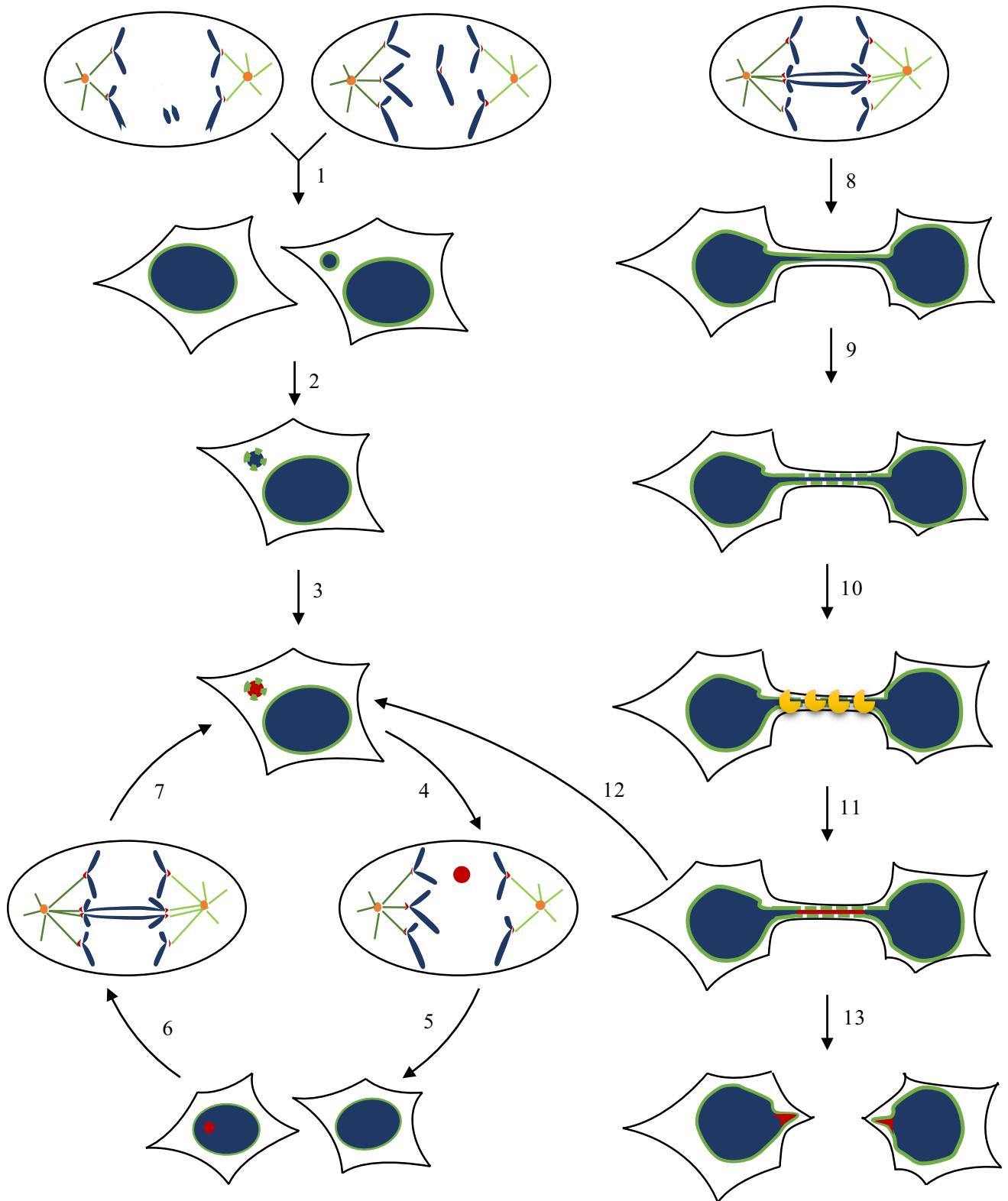
◄**Fig. 2** Micronuclei and nucleocytoplasmic bridges are related to chromothripsis and genomic instability. *1* The presence of acentric fragments or lagging chromatids derives in the formation of a micronucleus after the nuclear envelope (NE) formation and thus in a micronucleated daughter cell. *2* The NE envelope of the micronucleus is disrupted due to non-mitotic breakage (micronuclei disruption). *3* The resulting cytonucleoplasmic transport defects impair nuclear functions such as DNA repair and replication. Therefore, massive damage is accumulated due to unrepaired DNA lesions and/or stalled replications forks. *4* After the mitotic-related NE disorganization, *5* the micronuclear content can be reincorporated in one of the daughter nuclei. In the PN, where the DNA repair is properly working, the damaged micronuclear chromatin can result in chromosome reorganizations due to illegitimate repair. *6* The resulting chromosome reorganization can ignite bridge-fusion-break cycles that trigger CIN and *7* that can derive the formation of new micronuclei (MN). *8* In certain circumstances, bridging dicentric chromatids remain unbroken after cytokinesis and derive in long nucleocytoplasmic bridges between the daughter cells. *9* The NE of the chromatin bridge can also be disrupted, and consequently, *10* the bridging chromatin will be accessible for the cytoplasmic exonucleases that will induce localized DNA breaks. *11* The chromatin bridge can be broken into multiple fragments *12* and could even derive in the formation of MN. *13* Alternatively, after the breakage, the damaged bridging chromatin retracts to the daughter nuclei and, after illegitimate repair, results in chromosome reorganizations

chromosomes and "thripsis" for shattering) is considered a key mechanism in the formation of highly rearranged chromosomes affecting one or a few chromosomes (chromoanagenesis). This phenomenon was first discovered in a patient with chronic lymphocytic leukaemia. Spectral karyotyping and fluorescence in situ hybridization experiments revealed that the patient's tumour contained 42 chromosomal rearrangements involving the q arm of chromosome 4 and demonstrated that the rearrangements derived from one parental chromosome (Stephens et al. 2011). Since these initial observations, chromothripsis- and chromoanagenesis-like patterns have been observed across many tumour types, including 25 % of bone cancers and 18 % of late stage neuroblastomas (Stephens et al. 2011; Molenaar et al. 2012; Kloosterman and Cuppen 2013; Cai et al. 2014; Waddell et al. 2015; Patch et al. 2015). Breakpoint profiling of cancer genomes reveals a lack of areas of homology or microhomology in most breakpoints involved in human cancer, pointing towards the random joining of segments as the predominant mechanism that is weaving together the segments of the shattered chromosomes (Stephens et al. 2011; Malhotra et al. 2013; Kloosterman and Cuppen 2013). In addition to cancer cells, rearrangements originated by massive and localized DNA fragmentation have been detected in patients with congenital disorders, suggesting that they may also occur in germ cells or in early embryonic stages (Kloosterman and Cuppen 2013). Key advances in the understanding of chromothripsis have been possible thanks to the development of high-resolution microscopy methods to analyse chromosome dynamics in live cells and powerful genetic analyses. Such technological advances have been essential in building a comprehensive view of the chromosome biology and the mechanisms underlying this phenomenon.

Box 3

What cellular environment favours shattering of the chromatin entrapped in the micronuclei and the bridges?

Chromothripsis is thought to promote cancer development since it can lead to the loss of tumour suppressor genes, to the formation of oncogenic fusions and to oncogene amplification (Stephens et al. 2011; Rausch et al. 2012). Because of this association, it is not surprising that genetic contexts that allow the development of chromothripsis are those typical of cancer. Notably, genome sequencing in subsets of patients with medulloblastoma links chromothripsis and chromoanagenesis with TP53 mutations (Rausch et al. 2012). Because chromosome lagging, which is capable of originating micronucleus-dependent chromothripsis, and shattering of chromosome bridges can all produce a p53-dependent cell cycle arrest (Li et al. 2010; Thompson and Compton 2010), TP53 mutations might be considered as enabling changes for chromothripsis events in cancer development.

Polyploidy, which itself can promote tumorigenesis (Fujiwara et al. 2005), may also favour chromothripsis. The connection between these two cancer-associated events can be established at two different levels. First, because whole-genome doubling events are usually accompanied by extra centrosomes, polyploidy frequently gives rise to merotelic attachments and lagging chromosomes, which are prone to form chromatin bridges and MN that favour chromothripsis. And second, the deleterious impact of massive fragmentation of chromosomes and segment loss characteristic of chromothripsis is expected to be buffered in cells that have more chromosomal sets. Indeed, a methodology enabling the reproducible generation of chromothripsis in a genetically stable cell line has evidenced that chromothripsis is more frequent after experimental transformation in cells with abnormal ploidy numbers, compared with isogenic diploid cells (Mardin et al. 2015). In summary, chromothripsis is prone to arise in cellular contexts which facilitate genomic instability, such as in the contexts of hyperploidy and p53 deficiency.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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