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REVIEW

Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells

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Micronuclei (MN) and other nuclear anomalies such as nucleoplasmic bridges (NPBs) and nuclear buds (NBUDs) are biomarkers of genotoxic events and chromosomal instability. These genome damage events can be measured simultaneously in the cytokinesis-block micronucleus cytome (CBMNcyt) assay. The molecular mechanisms leading to these events have been investigated over the past two decades using molecular probes and genetically engineered cells. In this brief review, we summarise the wealth of knowledge currently available that best explains the formation of these important nuclear anomalies that are commonly seen in cancer and are indicative of genome damage events that could increase the risk of developmental and degenerative diseases. MN can originate during anaphase from lagging acentric chromosome or chromatid fragments caused by misrepair of DNA breaks or unrepaired DNA breaks. Malsegregation of whole chromosomes at anaphase may also lead to MN formation as a result of hypomethylation of repeat sequences in centromeric and pericentromeric DNA, defects in kinetochore proteins or assembly, dysfunctional spindle and defective anaphase checkpoint genes. NPB originate from dicentric chromosomes, which may occur due to misrepair of DNA breaks, telomere end fusions, and could also be observed when defective separation of sister chromatids at anaphase occurs due to failure of decatenation. NBUD represent the process of elimination of amplified DNA, DNA repair complexes and possibly excess chromosomes from aneuploid cells.

Background

Almost 100 years ago, Theodor Boveri observed that cells with supernumerary centrosomes missegregated their chromosomes through the assembly of multipolar spindles and hypothesised that abnormal chromosome number might contribute to carcinogenesis (1). Although it is not known whether he had observed micronuclei (MN) in tumour cells, he certainly was aware that abnormal nuclear morphologies commonly occurred in cancer. MN, also known as Howell–Jolly bodies, were originally identified and described in erythrocytes by the haematologists William Howell and Justin Jolly and they were later found to be associated with deficiencies in vitamins such as folate and vitamin B12 (2). The association between MN expression and exposure to environmental agents was first reported in root tip cells exposed to ionising radiation (3) and MN induction by a chemical was reported 7 years earlier in Ehrlich ascites tumour cells treated with colchicine (4). It is evident even from these very first studies that multiple mechanisms might lead to nuclear anomalies that are quantified in the MN and CBMNcyt assays. The purpose of this brief review is to provide an update on current knowledge of the molecular mechanisms that are thought to cause the formation of MN and other nuclear anomalies such as nucleoplasmic bridge (NPB) and nuclear bud (NBUD). Historically, the cytokinesis-block micronucleus (CBMN) assay was developed to score MN specifically in those cells that completed nuclear division by blocking them at the binucleated stage prior to cytokinesis (5). However, as the mechanisms of MN formation and other associated biomarkers of DNA damage (NPB and NBUD), cell death and cytostasis became more evident, this method eventually evolved into the CBMN 'cytome' assay designed to capture all these events (6–9).

The origin of MN

It is now well-established that MN mainly originate from acentric chromosome fragments, acentric chromatid fragments or whole chromosomes that fail to be included in the daughter nuclei at the completion of telophase during mitosis because they did not attach properly with the spindle during the segregation process in anaphase (Figure 1) (5–9). These displaced chromosomes or chromosome fragments are eventually enclosed by a nuclear membrane and, except for their smaller size, are morphologically similar to nuclei after conventional nuclear staining.

MN from acentric chromosome or chromatid fragments

Acentric chromosome fragments originate via multiple mechanisms. Radiation biology studies over several decades have shown that misrepair of DNA double-strand breaks can lead to symmetrical and asymmetrical chromatid and chromosome exchanges as well as chromatid and chromosome fragments

(5–9). A small proportion of acentric chromosome fragments may simply arise from unrepaired double-stranded DNA breaks, but this is only likely when DNA damage load exceeds the repair capacity of the cell within a specified time frame. The propensity for misrepair of DNA breaks is enhanced if the error-free homologous recombinational DNA repair pathway is dysfunctional due to defects in relevant genes such as BRCA1 and BRCA2; furthermore, DNA breaks, which lead to MN formation, may be left unrepaired if repair enzymes in the non-homologous end joining pathway are defective (Figure 2) (10,11).

Other mechanisms that could lead to MN formation from acentric fragments include simultaneous excision repair of damaged (e.g. 8-oxo-deoxyguanosine) or inappropriate bases incorporated in DNA (e.g. uracil) that are in proximity and on opposite complementary DNA strands. Such simultaneous excision repair events, particularly if the gap-filling step is not completed, leads to DNA double-strand breaks and MN formation (12–14). In fact, this process can be exploited to greatly enhance the lymphocyte MN assay response to genotoxic agents that mainly induce DNA adducts. This enhanced sensitivity is achieved by converting excision-repairable DNA lesions into DNA strand breaks and therefore MN by treatment with cytosine arabinoside (during G1 phase of the cell cycle), which inhibits the gap-filling step of excision repair (15). More recently, it has been shown that MN can also originate from fragmented chromosome material when NPB are formed, stretched and broken during telophase (16).

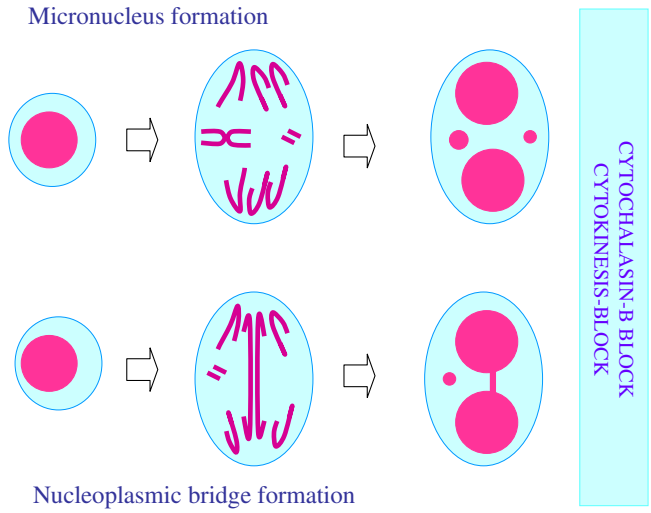

Fig. 1. MN and NPB formation in cells undergoing nuclear division. MN originate from either lagging whole chromosomes or acentric chromosome fragments. NPBs originate from dicentric chromosomes that may be caused by misrepair of double-strand DNA breaks or telomere end fusions. These events can only be observed in cells completing nuclear division, which are recognised by their binucleated appearance after cytokinesis blocking with cytochalasin-B.

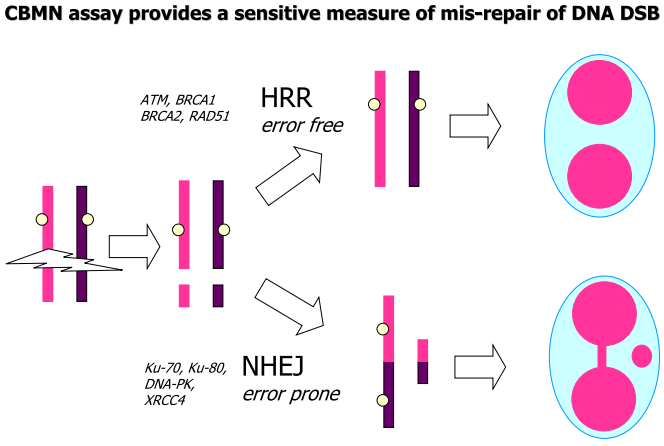

Fig. 2. Defects in genes involved in homologous recombinational repair genes (e.g. ATM, BRCA1, BRCA2 and RAD51) could be detected using the CBMNcyt assay. Under these conditions, the cell would resort to the error prone non-homologous end-joining (NHEJ) repair mechanism that is likely to lead to mis-repair of double-strand breaks in DNA, formation of dicentric chromosomes and acentric chromosome fragments, which are ultimately detectable as NPBs and MN in the CBMNcyt assay.

MN from malsegregated whole chromosomes

Lymphocyte MN in healthy people, not abnormally exposed to genotoxins usually originate from either acentric chromosome fragments or whole chromosome loss events at a ratio ranging between ~30:70% at one extreme to 70:30% at the other extreme depending on age and gender. In lymphocytes, MN increase with age and are generally higher in females relative to males (8). Sex chromosomes contribute the majority of chromosome loss events with increasing age (17). In females, the X chromosome can account for up to 72% of the observed MN of which 37% appear to be lacking a functional kinetochore suggesting that defects may be present in kinetochore assembly possibly due to X chromosome inactivation (18–21).

There are a range of possible molecular mechanisms that could cause chromosome malsegregation at anaphase resulting in MN formation. One of the mechanisms that may lead to MN from chromosome loss events is hypomethylation of cytosine in centromeric and pericentromeric repeat sequences such as classical satellite repeats at pericentromeric regions and higher-order repeats of satellite DNA in centromeric DNA (22,23). Classical satellite DNA is normally heavily methylated at cytosine residues, but in ICF syndrome (immunodeficiency, centromere instability and facial anomalies syndrome; Mendelian Inheritance in Man number 242860) or after treatment with 5-azacytidine (a DNA methyl transferase inhibitor), it is almost completely unmethylated (24–27). Pericentromeric heterochromatin of chromosomes 1, 9 and 16 is greatly elongated when cytosine is hypomethylated leading to either malsegregation of these chromosomes and/or their loss as MN probably due to inappropriate kinetochore assembly (23–27). Assembly of kinetochore proteins (e.g. CENPA and CENPB) at centromeres is generally affected by methylation status of cytosine as well as methylation of histones (25). In the latter case, a reduction in heterochromatin integrity might interfere both with microtubule attachment to chromosomes and with the proper sensing of tension from correct microtubule-kinetochore connections (25,28). Given the central role of kinetochore proteins in the engagement of chromosomes with the spindle, it is probable that mutations leading to defects in kinetochore and microtubule interaction dynamics could also be a cause of MN formation due to chromosome loss at anaphase (29). Other variables that are likely to increase MN from chromosome loss are defects in mitotic spindle assembly, mitosis check point defects and abnormal centrosome amplification (30,31). A recent study suggests that dicentric chromosomes resulting from telomere end fusions may often be involved in mis-segregation events; this may occur when the centromeres of the dicentric chromosome are pulled towards opposite poles of the

cell during anaphase with forces that are sufficient to detach the chromosome from the spindle (32).

Pancentromeric DNA probes are used to distinguish between MN originating from any whole chromosome loss event and MN containing acentric chromosome fragments (Figure 3). The use of chromosome-specific centromeric DNA probes allows both the determination of specific chromosome loss events resulting in MN, as well as unequal segregation of specific chromosomes among daughter nuclei even in the absence of MN formation (Figure 3).

The origin of NPB

NPB originate during anaphase when the centromeres of dicentric chromosomes are pulled to opposite poles of the cell during mitosis. In the absence of breakage of the anaphase bridge, the nuclear membrane eventually surrounds the daughter nuclei and the anaphase bridge and in this manner, an NPB is formed. NPB are usually broken during cytokinesis but they can be accumulated in cytokinesis-blocked cells using the cytokinesis inhibitor cytochalasin-B (8).

Dicentric chromosomes originate either from misrepair of chromosome breaks or telomere to telomere end fusions (5–9) (Figure 4). The latter are caused by inappropriate assembly of the telosome protein structure that encapsulates and protects the telomere either because of excessive telomere shortening or deletion and/or base damage in the telomere sequence (32–34).

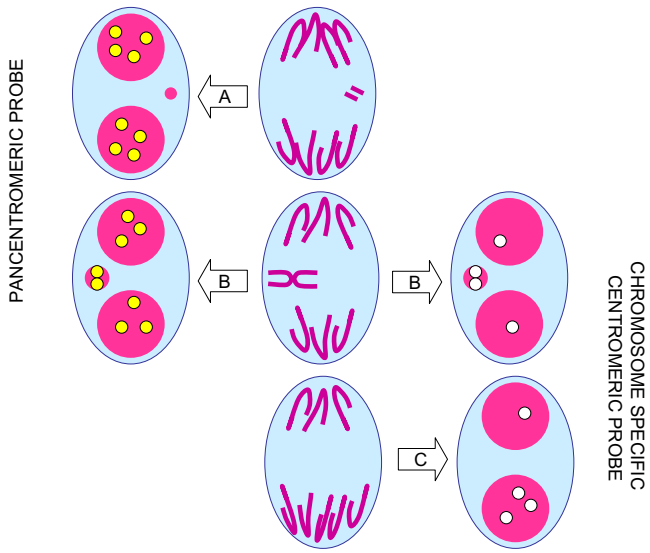

Fig. 3. The use of molecular techniques for identifying (A) an MN originating from a lagging acentric chromosome fragment, (B) an MN originating from a lagging whole chromosome and (C) non-disjunction of a specific chromosome leading to aneuploid daughter nuclei. The yellow spots in the nuclei and MN of the binucleated cells on the left of each panel show the centromeric or kinetochore pattern of staining when pancentromeric probes or kinetochore antibodies are used. The white spots in the nuclei and MN of the binucleated cells on the right of each panel show the pattern of centromeric staining when a centromeric probe specific to the chromosomes involved in MN formation or non-disjunction events is used. The example shown is for a hypothetical cell with only two pairs of chromosomes. Pancentromeric probes should be used only for distinguishing between microinuclei originating from chromosome breaks (centromere negative) and chromosome loss (centromere positive). Chromosome-specific centromere probes should be used only to measure malsegregation (due to non-disjunction or chromosome loss) involving unique chromosomes. It is important to note that pancentromeric probes cannot be used to determine non-disjunction because of difficulty in reliably counting all the centromeres within the nuclei.

Failure to cap the 3′ single-stranded G-rich overhang at the end of the telomere may result in the chromosome ends being perceived as broken DNA molecules in which case they may be repaired by the DNA end-joining mechanism and cause telomere end fusions (35,36); the latter may also be increased due to defects in recombinational repair proteins (36). The two mechanisms of nucleoplasmic bridge formation can be distinguished in binucleated cytokinesis-blocked cells using telomere probes. NPB arising from telomere end fusions are expected to be telomere positive if they retain telomere sequences and/or if the fusions are caused by telomere dysfunction due to loss of telomere-binding proteins without telomere attrition; however, if the fusion was caused due to complete erosion of telomere sequence, the NPB originating from such a mechanism can only be recognised with a specific probe that hybridises in the subtelomeric region adjacent to the telomeric repetitive sequence track (8,36–38). In contrast, an NPB caused by misrepair of DNA breaks has a low probability of occurring within the telomeric sequences and is therefore likely to be telomere negative (8,9). Furthermore, NPB arising from misrepair of DNA breaks are also likely to be associated with an MN originating from the acentric fragment generated during misrepair (9,39). However, an MN originating from an acentric fragment may not necessarily accompany a telomere end fusion event because the latter can occur in the absence of DNA strand breakage and misrepair. NPB formation has been shown to be increased by a wide range of exposures including endogenous oxidants, ionising radiation, polycyclic aromatic hydrocarbons, the cigarette smoke carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, vanadium pentoxide, as well as deficiencies in folate and selenium (9,13,39–44).

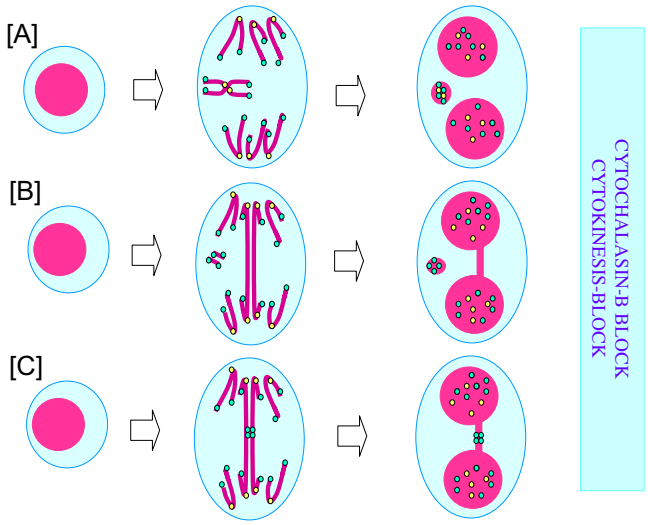

Fig. 4. Pancentromeric and telomeric probes can be used to distinguish (i) between MN originating from whole chromosome loss (A) and MN originating from acentric chromosome fragments (B) and (ii) NPBs from dicentric chromosomes resulting from misrepair of DNA strand breaks (B) and dicentric chromosomes caused by telomere end fusions (C). In the latter case, it is important to note that the telomere end fusions detectable by this system are those caused by telomere dysfunction with telomere sequence loss as a result of defects in assembly of telomere capping proteins. In the case of telomere end fusions due to complete erosion of the telomere sequence, the NPB originating from such a mechanism can only be recognised with a specific probe that hybridises in the sub-telomeric region adjacent to the telomeric repetitive sequence track. The yellow dots represent probes that hybridise to the centromeric region of chromosomes. The light blue dots represent probes that hybridise with the telomeric sequences in chromosomes.

Some new insights on NPB formation have been obtained in recent studies on cells with defects in the Bloom's syndrome (BLM) (45) and Fanconi anaemia (FA) genes (46). BLM-defective cells display a higher frequency of anaphase bridges and lagging chromatin than isogenic control cells that express the BLM protein. In normal cells undergoing mitosis, BLM protein localises to anaphase bridges. Using BLM staining as a marker, it is possible to identify a class of ultrafine DNA bridges. These so-called BLM–DNA bridges, which also stain for the PICH (Plk1-interacting checkpoint 'helicase') protein, frequently link centromeric loci and are present at an elevated frequency in cells lacking BLM. The authors of this study proposed that sister chromatid disjunction is often incomplete in human cells even after the onset of anaphase and they suggested a model for the action of BLM in ensuring complete sister chromatid decatenation during anaphase (45). The protein complexes Structural Maintenance of Chromosomes (SMCs) and their kleisin subunits also play important central roles in sister chromatid separation at anaphase (47). Two of the best-studied SMC complexes are cohesin and condensin. Cohesin is required to hold sister chromatids together; cleavage of cohesin's kleisin subunit by the separase protease triggers the movement of sister chromatids into opposite halves of the cell during anaphase while condensin is required to organise mitotic chromosomes into coherent structures that prevent them from getting tangled up during segregation (47). Inherited or acquired defects in protein complexes involved in sister chromatid separation at anaphase are likely to increase NPB formation because the sister chromatids will still be joined to each other when anaphase commences.

Similarly, FA cells have increased frequencies of MN (48) and anaphase bridges as the FA genes are involved in rescuing abnormal anaphase and telophase (ana-telophase) cells, limiting aneuploidy and reducing chromosome instability in daughter cells (46). Current models propose a cooperative role for the FA and BLM genes in preventing micronucleation, through FANC-dependent targeting of BLM to non-centromeric abnormal structures induced by replicative stress (46). Thus, after replication stress, sister chromatids are interlinked by replication stress intermediates primarily at genetic loci with intrinsic replication difficulties, such as fragile sites. Both FA and BLM are required for efficient resolution of these DNA linkages which if left unresolved would give rise to formation of NPB and MN during mitosis (46,49).

The origin of NBUD

Over the past decades, another unique nuclear anomaly known as nuclear budding has been associated with chromosomal instability events. NBUD have been observed in cultures grown under strong selective conditions, which induce gene amplification as well as under moderate folic acid deficiency (50–60). Shimizu *et al*. (54,55) used *in vitro* experiments with mammalian cells to show that amplified DNA is selectively localised to specific sites at the periphery of the nucleus and is eliminated via nuclear budding during S phase of the cell cycle. Amplified DNA may be eliminated from chromosomes through recombination between homologous regions within amplified sequences forming mini-circles of acentric and atelomeric DNA (double minutes). The NBUD are characterised by having the same morphology as an MN with the exception that they are connected to the nucleus by a narrow or wide stalk of nucleoplasmic material depending on the stage of the budding process. The duration of the nuclear budding process and the extrusion of the resulting MN from the cell have been studied in great detail by time-lapse live-cell imaging techniques (61,62). It is also reported that MN may also be formed by a budding process following exposure to γ-irradiation (56). In this process, Rad 51-recombination protein complexes are detectable throughout the entire nucleus 3 h after irradiation and then become concentrated into distinct foci before being extruded from the nucleus as NBUD. NBUD have also been shown to be formed when an NPB between two nuclei breaks and the remnants shrink back towards the nuclei (63,64,64).

Using centromere and telomere probes, Lindbergh *et al*. (65) investigated the mechanisms of MN and nuclear bud formation in folic acid-deficient cells. Their results suggest that NBUD and MN have partly different mechanistic origin. Interstitial DNA without centromere or telomere labels was clearly more prevalent in NBUD (43%) than in MN (13%). Telomeric DNA only or both centromeric and telomeric DNA was more frequent in MN (62 and 22%, respectively) than in NBUD (44 and 10%, respectively). Folate deprivation increased the frequency of NBUD and MN harbouring telomeric DNA, NBUD harbouring interstitial DNA and also NBUD and MN with both centromeric and telomeric DNA. According to the model proposed by Lindbergh *et al*. (65), MN in binucleate lymphocytes primarily derive from lagging chromosomes and terminal acentric fragments during mitosis; however, most NBUD originate from interstitial or terminal acentric fragments. Such NBUD may possibly represent nuclear membrane entrapment of DNA that has been left in cytoplasm after nuclear division or from excess DNA that is being extruded from the nucleus. Whether NBUD are also a mechanism to eliminate excess chromosomes in a hypothesised process known as aneuploidy rescue remains unclear as there is only limited evidence for this possibility (62,66,67). Finally, it is also plausible that NBUD might occur transiently after breakage of NPB.

Breakage-fusion-bridge cycles

MN, NPB and NBUD are nuclear anomalies commonly seen in cancer and they represent a common phenotype of chromosomally unstable cells (68–70). Chromosomal instability leads to altered gene dosage and the potential for a cell to rapidly evolve and mutate, due to its genetic plasticity, into diverse abnormal genotypes that can escape the homeostatic control mechanisms and thus become immortalised and evade the immune system (70,71). The formation of anaphase bridges or NPB is the initiating event that leads to breakage-fusion-bridge (BFB) cycles, which is a key driver of chromosomal instability in cells. When anaphase bridges break unevenly, which they almost always do, one of the daughter cells receives a chromosome with additional copies of genes and the other daughter cell loses some genes. Because such broken chromosomes lack telomere sequences at the broken end, they are likely to fuse with their replica after DNA synthesis. This perpetuates the BFB cycle into the next rounds of cell division resulting in further amplification of the genes that are close to the break point or fusion point (Figure 5). The amplified genes are eventually looped out of this aberrant chromosome by recombinational mechanisms to form minute chromosomes. The minute chromosomes can be replicated and/or eliminated by nuclear budding, transiently becoming MN before being excluded from the cell to form microcells (54,55). Live-cell

imaging studies suggest that such microcells may be recaptured by the cell (61).

In a series of studies on folic acid deficiency in long-term primary human lymphocyte cultures, we carefully quantified the interrelationship between MN, NPB and NBUD in an attempt to validate the use of these biomarkers and to determine more comprehensively the impact of folic acid deficiency on various aspects of genomic stability (58–60). Folic acid concentration within the physiological range (20–120 nM) correlated significantly ($P < 0.0001$) and negatively ($r = -0.63$ to -0.74) with all these markers of chromosome damage. The strong cross-correlation between MN, NPB and NBUD frequency ($r = 0.65$–0.77, $P < 0.001$) suggests a common mechanism initiated by folic acid deficiency-induced DNA breaks, the most plausible being chromosomal instability generated by BFB cycles (13,30).

A detailed nutrient–gene interaction model involving diverse molecular mechanisms, which could lead to MN, NPB and NBUD formation depending on folate, riboflavin and *MTHFR* (methylenetetrahydrofolatereductase) C677T genotype is described in Figure 6 (60). Analogous models via other molecular pathways (e.g. DNA repair) can be developed to explain the generation of MN, NPB and NBUD induction by exposure to specific genotoxic agents such as ionising radiation or other agents that cause double-stranded DNA breaks (7,39).

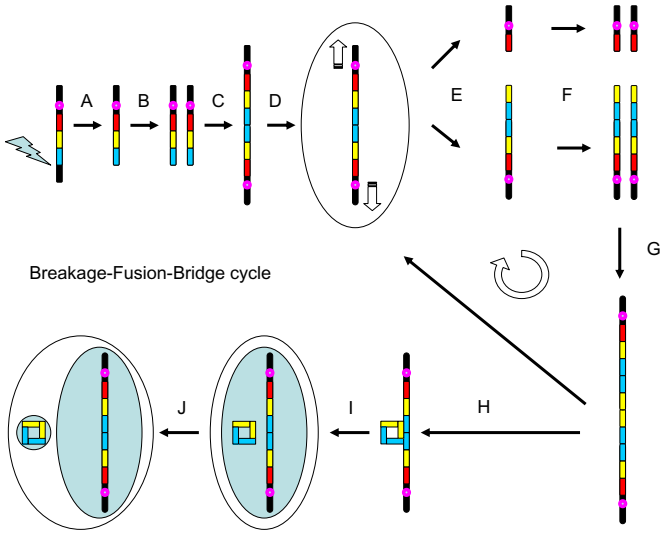

Fig. 5. Gene amplification via BFB cycles and elimination of amplified genes by nuclear budding. (A) Chromosome loses telomeric sequence on long arm due to an induced chromosome break occurring sub-telomerically. (B) The chromosome lacking telomere sequence is replicated. (C) Fusion of the chromosome ends of the sister chromosomes lacking telomeres produces a dicentric chromosome. (D) The centromeres of the resulting dicentric chromosome are pulled to opposite poles at anaphase. (E) The dicentric chromosome breaks unevenly resulting in one daughter chromosome losing the yellow and blue gene blocks and the other chromosome gaining an extra copy of these gene blocks. (F) The abnormal chromosomes are replicated in the daughter cells. (G) Because they lack telomeres, the sister chromosomes fuse again repeating the gene amplification cycle (i.e. repeating steps D–F). Alternatively, recombination may occur between homologous sequences (H) and result in the 'looping-out' of a circular acentric DNA fragment or double minute (I), which may be subsequently extruded from the nucleus via nuclear budding (J) to form an MN.

Knowledge gaps

Despite the substantial progress reported above, it is reasonable to assume that our understanding of the mechanisms that lead to MN, NPB and NBUD formation is not yet complete. For example, the effect of folate, riboflavin and selenomethionine deficiency on these biomarkers of chromosomal instability (40,60) suggests that they are highly sensitive to deficiencies of nutrients that are required for genome maintenance but the detailed molecular mechanisms of how such deficiencies cause MN, NPB and NBUD formation have yet to be unravelled. In addition, the impact of gender and common polymorphisms in genome maintenance genes in relation to susceptibility to elevated frequencies of MN, NPB and NBUD is still rudimentary (72,73). Furthermore, there remain several important unanswered questions including the following:

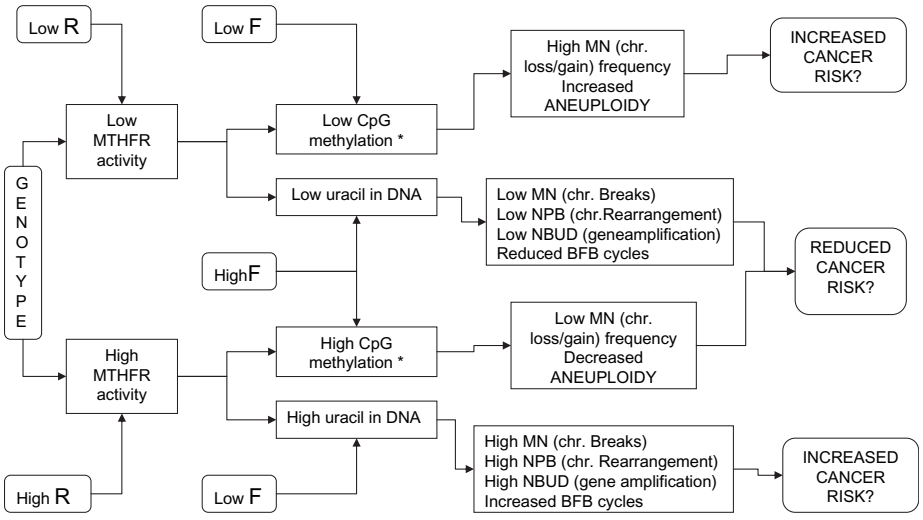

Fig. 6. Mechanistic framework explaining the molecular interrelationship between the methylenetetrahydrofolatereductase (MTHFR) genotype, riboflavin (R) and folic acid (F) with respect to CBMNcyt assay biomarkers of DNA damage and altered cancer risks. Folate and riboflavin concentration levels combined with the MTHFR genotype can affect: (i) CpG methylation and uracil in DNA, (ii) aneuploidy and MN originating from chromosome loss events, (iii) MN (originating from acentric chromosome fragments), NBUDs, NPBs and BFB cycles, (iv) initiation of cancer caused by CpG hypomethylation and aneuploidy and (v) initiation of cancer caused by increased BFB cycles, MN (originating from acentric chromosome fragments), NBUD and NPB. Asterisk represents: for brevity, other carcinogenic mechanisms induced by altered genome methylation, such as silencing of tumour suppressor genes and/or activation of oncogenes, are not included in the diagram. For more detailed explanation, refer to (60).

Table I. Molecular events associated with expression of MN, NPB and NBUD

CBMNcyt assay biomarker	Molecular events associated with biomarker
MN	Lagging acentric chromosome or chromatid fragment at anaphase. Misrepair of DNA breaks Unrepaired DNA breaks Lagging whole chromosomes at anaphase. Hypomethylation of repeat sequences in centromeric and pericentromeric DNA Defects in kinetochore proteins or assembly Dysfunctional spindle Defective anaphase checkpoint genes Unresolved replication stress intermediates
NPB	Dicentric or multicentric chromosomes with centromeres pulled to opposite poles of the cell at anaphase. Misrepair of DNA breaks Telomere end fusions due to excessively short telomeres, dysfunctional telomeres or lack of telomeres Defective separation of sister chromatids at anaphase due to failure of decatenation Unresolved replication stress intermediates
NBUD	Active process of elimination of nuclear material from nucleus. Elimination of amplified DNA possibly generated via BFB cycles Elimination of DNA repair DNA-protein complexes Elimination of excess chromosomes—may occur in polyploid cells to facilitate aneuploidy rescue. Shrinkage of the remnants of a broken NPB between two nuclei can result in a temporary NBUD on one or both nuclei.

- Which of the MN, NPB and NBUD biomarkers is most predictive of cancer and other degenerative diseases and what is the underlying molecular basis?
- Which genetic defects and their interactions are likely to increase the incidence of these DNA damage biomarkers and under which environmental conditions are these genetic effects most evident?
- Which exposure, dietary and lifestyle factors and their combinations substantially affect these biomarkers and what are the molecular mechanisms?
- Is it technically feasible to study the multiple mechanisms leading to MN, NPB and NBUD formation using multiple molecular probes simultaneously?
- Are there specific gene expression patterns that are highly correlated with the formation of MN, NPB and NBUD in cells?

Conclusion

It is evident that multiple molecular mechanisms can lead to the formation of MN, NPB and NBUD. These biomarkers are best observed in cytokinesis-blocked cells, which allow these events to accumulate in cells that have completed DNA synthesis and mitosis, which are essential for their expression. A key advantage and highlight of the CBMNcyt assay is that it allows simultaneous detection of multiple molecular events that lead to chromosome damage and chromosomal instability (summarised in Table I). This versatility of the CBMNcyt assay makes it one of the preferred methods to detect and measure chromosomal DNA damage and chromosomal instability phenotype in mammalian and human cells.

Acknowledgements

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Conflict of interest statement: None declared.

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