Cell-based model systems for genome instability: Dissecting the mechanistic basis of chromothripsis in cancer

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Abstract
Chromothripsis is a form of genomic instability that was shown to play a major role in cancer. Beyond cancer, this type of catastrophic event is also involved in germline structural variation, genome mosaicism in somatic tissues, infertility, mental retardation, congenital malformations and reproductive development in plants. Several assays have been developed to model chromothripsis in vitro and to dissect the mechanistic basis of this phenomenon. Cell-based model systems are designed with different strategies, such as the formation of nuclear structures called micronuclei, telomere fusions or the induction of exogenous DNA double-strand breaks. Here, we review a range of model systems for chromothripsis and the mechanistic insights gained from these assays, with a particular focus on chromothripsis in cancer.

Keywords
Cancer, chromothripsis, genome instability

1 | INTRODUCTION
Chromothripsis, originally discovered in cancer genomes,1 constitutes a new class of massive genomic rearrangements, which is characterized by the shattering of one or a few chromosomes followed by error-prone reassembly of the DNA fragments (Figure 1A). The resulting chromosomal rearrangements are restricted to a limited number of genomic regions and are most likely acquired during one or very few cell cycles.1–3 In addition to the clustering of the breakpoints, chromothriptic chromosomes are characterized by typical copy-number oscillations (Figure 1B), with 10 or more copy-number oscillations between two or three copy-number states. Furthermore, chromothriptic breakpoints show a randomness in the fragment join types as well as loss and retention of heterozygosity as commonly used minimal criteria for chromothripsis scoring.3

Cells that survive chromothripsis occasionally gain a strong selective advantage due to the simultaneous acquisition of multiple genomic aberrations, leading to the disruption of tumor suppressor genes and the activation of oncogenes. Initially identified in leukemia, chromothripsis was subsequently associated with an increasing number of tumor types4,5 as well as congenital diseases.6 Even though initial reports suggested that only a small percentage of cancer cases were linked to chromothripsis,1 recent sequencing efforts detected rearrangements due to chromothripsis in more than 20% of cancer cases for a number of tumor entities.4,5 Importantly, chromothripsis is linked with aggressive tumor behavior and poor prognosis for cancer patients.2,7,8

Until today, the underlying mechanisms that lead to this mutational process are only partly understood. To gain a deeper understanding of the mechanistic basis of chromothripsis and to interfere with this process, robust assays and cell-based model systems are needed. Current models for chromothripsis vary in terms of how

Abbreviations: BFB, breakage-fusion-bridge; Cas9, CRISPR associated protein 9; CRISPR, clustered Regularly Interspaced Short Palindromic Repeats; DSB, double-strand break; dsDNA, double-stranded deoxyribonucleic acid; hTERT, telomerase reverse transcriptase; NE, nuclear envelope; RPE, retinal pigment epithelium; TREX1, three prime repair exonuclease 1; TRF2, telomeric repeat-binding factor 2; UV, ultraviolet.

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representative they are for events occurring in vivo and how well the observed alterations match a stringent chromothripsis scoring. In addition, the efficiency of the available systems differs, and this affects the potential to apply the various approaches in cell culture systems.

In this review, we present and discuss currently available approaches and recent developments to induce and analyze chromothripsis on a cellular level.

2 | MODELS BASED ON THE INDUCTION OF MICRONUCLEI

In recent years, one attractive model to explain how chromothripsis might occur has been the micronuclei hypothesis (Figure 2A). Micronuclei are small, DNA-containing compartments in the cytoplasm which are formed as a result of lagging chromosomes or chromosome missegregation during mitosis or from nuclear blebs during interphase. As these abnormal nuclear structures contain only one or a few chromosomes, the physical separation from the main nucleus would explain why the massive rearrangements characteristic of chromothripsis are restricted to one or a few chromosomes. Compared to the main nuclei, micronuclei show a distinct composition of factors responsible for DNA damage repair, proteasome activity, membrane composition as well as chromatin organization. As a result of asynchronous DNA replication and impaired checkpoint arrests, the micronuclear DNA can undergo pulverization. Due to the altered composition of micronuclei, the enclosed micronuclear DNA cannot be repaired with the fidelity of functional repair machinery. In the following mitosis, the pulverized genomic material can be reintegrated into the main nucleus and can be transmitted to the daughter cells, leading to genomic alterations.

By combining live-cell imaging and single-cell genome sequencing, Zang et al. showed in 2015, that the formation of micronuclei is sufficient to induce genomic rearrangements. The authors developed an assay based on human retinal pigment epithelial cells (RPE-1) lacking functional p53 that were nocodazole-treated to induce the generation of micronuclei. After identifying cells that reincorporated micronuclei into the main nucleus by live-cell imaging, these cells were subjected to single-cell sequencing, followed by copy-number analysis which revealed massive alterations in the missegregated chromosomes. Interestingly, apart from intrachromosomal rearrangements, the authors identified interchromosomal rearrangements when more than one chromosome was entrapped in the original micronucleus.

3 | MICRONUCLEI-BASED MODEL SYSTEM UNRAVEL FUNCTIONAL CONSEQUENCES OF CHROMOTHIRPSIS IN HUMAN CELLS

In 2019, Kneissig et al. demonstrated that microcell-mediated chromosome transfer can induce massive chromosomal rearrangements in human cells and that these rearrangements were the consequence of chromosome shattering followed by rejoicing. The authors used chimeric A9 mouse cells containing several human chromosomes marked with antibiotic resistance genes. By using colchicine to induce the formation of micronuclei, these chromosomes were packed into individual micronuclei and transferred into human recipient cells, which subsequently presented genomic rearrangements. The observed alterations might be a consequence of shattering and reassembly or alternatively caused by replicative rearrangement processes like chromoanasynthesis. In addition, the isolated micronuclei lacked functional lamin B1, which was previously described to cause nuclear envelope (NE) rupture. The authors concluded that the subsequent DNA damage and aberrant replication might be a direct consequence of the observed alterations in the nuclear lamina.

4 | MODELS BASED ON SELECTIVE CENTROMERE INACTIVATION

In 2017, another study investigating the role of micronuclei in the formation of genomic rearrangements was published. The authors utilized a model system allowing the selective inactivation of the Y-centromere. This approach allows for the quasispecific generation of micronuclei harboring the centromere-inactivated Y-chromosomes. Following the fate of the micronuclei over three cell cycles, a cascade of events was identified that induced various genomic alterations including chromosome missegregation and fragmentation. The eventual religation was shown to be driven by canonical nonhomologous end-joining, but not homology-dependent repair. The authors concluded that the generation of micronuclei can lead to further genomic instability over the course of several cell cycles. In 2019, the same group used the aforementioned approach for the systematic study of the structural genomic landscape resulting from missegregated chromosomes. By coupling the system with next-generation DNA sequencing, they showed that single missegregated chromosomes can directly trigger a broad spectrum of genomic rearrangements including deletions, inversions and translocations, resulting in oscillating copy-number states, which are hallmarks of chromothripsis.

5 | NUCLEAR ENVELOPE ASSEMBLY DEFECTS LINK MITOTIC ERRORS TO CHROMOTHIRPSIS

In 2018, one of the potential mechanisms that could explain the induction of genomic rearrangements, including chromothripsis through micronuclei, was experimentally assessed by Liu et al. By using light and electron microscopy combined with the specific staining of a large set of components of the NE, the authors demonstrated that the composition of the NE formed around lagging chromosomes massively differed from the NE of the main nuclei and that this difference was largely dependent on the location of the lagging chromosomes during mitosis. Due to the lack of nuclear pore complexes and other nuclear membrane proteins including the lamin B receptor, protein import was heavily altered in the resulting micronuclei. As a consequence, the import of proteins that are
necessary for a proper DNA damage response and genome integrity was distorted.19

6 | MODELS BASED ON TELOMERE DYSFUNCTION

In addition to models based on micronuclei formation and mitotic errors, other nonexclusive models have been suggested to explain the mechanistic basis of chromothripsis and have been used for in vitro modeling (Figure 2B). Chromosome breakage-fusion-bridge (BFB) cycles contribute to a process that leads to genome instability by telomeric lesions or loss of telomerase activity.20 Genomic alterations resulting from BFB cycles are frequently detected in cancer genomes. BFB cycles are initiated by the loss of telomere ends. When the resulting unstable chromosomes replicate, their centromeres are pulled in opposite directions and form anaphase bridges in the subsequent mitosis. These bridges eventually break, leading to defective chromosomes in the resulting daughter cells.
In 2015, Maciejowski et al published a method generating chromothriptic cells by inducing telomere dysfunction in hTERT expressing RPE-1 cells. By disrupting telomeric repeat-binding factor 2 (TRF2) using a dominant-negative allele of TRF2, telomere fusions were induced. The resulting anaphase bridges persisted and developed into chromatin bridges. These bridges eventually break, and the resulting chromosome fragments are reincorporated into the interphase nucleus. As a consequence, the subsequent rejoining of the DNA fragments can lead to derivative chromosome(s). Cell model systems based on external factors: DNA-toxic factors like radiation or certain chemicals can induce multiple DNA DSBs, which may lead to DNA fragmentation in one or several chromosomes, followed by error-prone DNA repair [Color figure can be viewed at wileyonlinelibrary.com]

**FIGURE 2**  Cell-based model systems for chromothripsis. A, Cell-based model systems relying on the formation of micronuclei: A lagging chromosome is entrapped into a micronucleus and exposed to extensive DNA fragmentation. Subsequent rejoining of the DNA fragments results in a derivative chromosome that can be reincorporated into the main nucleus. B, Cell model systems based on the formation of anaphase bridges: Chromosome fusions can give rise to anaphase bridges that develop into long persisting chromatin bridges. These bridges eventually break, and the resulting chromosome fragments are reincorporated into the interphase nucleus. As a consequence, the subsequent rejoining of the DNA fragments can lead to derivative chromosome(s). C, Cell model systems based on external factors: DNA-toxic factors like radiation or certain chemicals can induce multiple DNA DSBs, which may lead to DNA fragmentation in one or several chromosomes, followed by error-prone DNA repair [Color figure can be viewed at wileyonlinelibrary.com]

7 | CHROMOTHRIPSIS AND KATAEGIS INDUCED BY TELOMERE CRISIS

In 2015, Maciejowski et al published a method generating chromothriptic cells by inducing telomere dysfunction in hTERT expressing RPE-1 cells. By disrupting telomeric repeat-binding factor 2 (TRF2) using a dominant-negative allele of TRF2, telomere fusions were induced. The resulting anaphase bridges persisted and developed into chromatin bridges. These bridges broke up after cytokinesis in the subsequent G1 phase. The authors showed that NE rupture leads to the release of the cytoplasmic 3' exonuclease TREX1 which most likely induced the formation of single-stranded DNA in the chromatin bridges. The remaining fragile and fragmented bridge DNA was eventually taken up by the main nuclei, undergoing repair and reintegration. By sequencing the resulting monoclonal lines, signatures typical for chromothripsis and kataegis were observed.

In 2020, Umbreit and colleagues demonstrated that a mutational cascade can be triggered by a single mitotic error leading to the aberrant formation of a chromosome bridge. Importantly, this process was sufficient to rapidly increase the genomic complexity of the resulting cell population.

8 | MODELS BASED ON THE PHYSICAL OR CHEMICAL INDUCTION OF DNA DOUBLE-STRAND BREAKS

Since its first description and definition, chromothripsis has been viewed as a result of chromosome shattering triggered by DNA...
double-strand breaks (DSBs). Therefore, early studies proposed ionizing radiation, a well-known source of DNA DSBs, as a potential mechanism to induce chromothripsis (Figure 2C).

In 2016, Morishita et al. reported the induction of chromosomal rearrangements by utilizing a proton microbeam irradiation system. This focused vertical microbeam system was able to irradiate areas in the micrometer range within individual nuclei. The authors used this system to induce genomic aberrations in oral squamous cell carcinoma cells and established monoclonal sublines after irradiation. After analyzing the resulting cell lines, one subline showed a “chromothripsis-like” complex chromosomal alteration pattern. Even though the use of stable diploid cells would increase the impact of these findings and the relatively loose criteria applied for a chromothripsis scoring may be discussed, it will be critical to understand the potential contribution of radiation in chromothripsis initiation. Despite major therapeutic implications, no conclusive evidence for a causative role has been provided so far.

In 2015, an alternative approach utilizing chemical induction of DNA DSBs was followed by Mardin et al. To induce DNA damage in cells, the authors used doxorubicin, which induces DNA damage by intercalating into dsDNA and inhibiting topoisomerase II. Surviving cells were selected using anchorage-independent growth in soft agar substrate, as resulting chromothriptic cells were expected to gain a strong selective advantage from the massive rearrangements. Consequently, the authors named their method “complex alterations after selection and transformation”. Besides hyperploidy, the genomic analysis of the resulting cell lines that survived the DNA damage induction and subsequent selection procedure implicates telomere attrition as a cause for the induction of chromothriptic patterns in the selected cells. Conceptually, it seems interesting that a chemical-inducing genome-wide DNA DSBs generated chromothriptic clones with clustered rearrangements. As for radiation-induced DNA DSBs, this approach has clinical implications due to potential chromothriptic events induced by chemotherapeutic agents. Longitudinal analyses of matched tumor pairs occasionally identify primary tumors without chromothripsis and relapsed tumors with chromothripsis which suggests that putative therapy-induced chromothriptic events should be considered.

9 | A VERSATILE SYSTEM TO INTRODUCE CLUSTERS OF GENOMIC DOUBLE-STRAND BREAKS IN LARGE CELL POPULATIONS

One of the main characteristics of chromothripsis is that the typical DNA lesions appear in a clustered pattern. In 2020, our laboratory developed a “versatile system” to induce DNA DSBs in different cell types. This method allows for a tight control of the exact area of irradiation by utilizing a photomask in combination with high-energy UV light. By restricting the transmission of light to specific areas, we can induce DNA DSBs in DNA damage foci of the size of one or a few chromosomes. By irradiating RPE-1 cells that lack functional p53, we were able to detect in four out of seven irradiated clones copy-number alterations that affected one or two chromosomes per clone. In addition, the irradiated clones harbored significantly higher loads of single nucleotide variants.

10 | MODELS BASED ON THE ENZYMATIC INDUCTION OF DOUBLE-STRAND BREAKS

In addition to ionizing radiation, DNA DSBs can be efficiently induced by using targeted nucleases such as Cas9. In 2016, Lekomtsev et al. were able to introduce genomic rearrangements including intra- and interchromosomal translocations by using two guide RNAs targeting different genomic loci. Even though it was not the main objective of this study, this work showed that the CRISPR/Cas9 system is in general capable of introducing genomic rearrangements that are hallmarks of cancer cells and possibly match the criteria defining chromothripsis.

More recently, in 2020, Leibowitz et al. demonstrated that chromothripsis can occur as an “on-target consequence” of CRISPR/Cas9-mediated genome editing. Their work demonstrates that genetic modifications using the CRISPR/Cas9 system can induce the formation of micronuclei and chromosome bridges in dividing cells. As a result, changes in the number of copies of large chromosome segments can occur, which are hallmarks of chromothripsis. While their study mainly focused on potential risks for therapeutic genome manipulation strategies which require DSB formation, they demonstrated that the CRISPR/Cas9 technology, in principle, can be used to generate cell model systems that develop complex genome rearrangements including chromothripsis. In addition, these studies raise the question of the putative role of enzymes in chromothripsis in vivo.

11 | CONCLUSIONS

Despite almost 10 years of intensive research in the field of chromothripsis, the underlying mechanisms that trigger this phenomenon are only partly understood and remain a subject for discussion. A number of different approaches have been used to study the possible origins and consequences of rearrangements due to chromothripsis. Within the cell-based model models, several strategies have been followed. Most systems use an initial DNA-damaging event by either DNA-toxic chemicals, irradiation or the incorporation of external chromosomes, utilizing the compromised DNA repair machinery in micronuclei to induce genomic rearrangements. These systems, however, differ substantially in terms of efficiency and most likely regarding their physiological relevance. While systems that alter the entire genome by irradiation or DNA-damaging substances are relatively straightforward to implement, they lack the ability to induce DNA damage in localized and clustered DNA regions, one of the hallmarks of chromothripsis. A better understanding of the mechanisms triggering chromothripsis in vivo and the development of representative in vitro models will be essential to pave the way for future strategies to interfere with this process.
CONFLICT OF INTEREST
The authors declare no conflict of interest.

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