OXFORD

CRISPRthripsis: The Risk of CRISPR/Cas9-induced Chromothripsis in Gene Therapy

Mario Amendola[1,2,*], Mégane Brusson[3], Annarita Miccio[3,*], iD

[1]Genethon, Evry, France
[2]Integrare Research Unit UMR_S951, Université Paris-Saclay, Univ Evry, Inserm, Genethon, Evry, France
[3]Laboratory of Chromatin and Gene Regulation during Development, INSERM UMR 1163, Université Paris Cité, Imagine Institute, Paris, France
*Corresponding authors: Mario Amendola, Genethon, 1 Rue de l'internationale, 91000, Evry, France. Email: mamendola@genethon.fr; Annarita Miccio, Imagine Institute, 24 Boulevard du Montparnasse, 75015 Paris, France. Email: annarita.miccio@institutimagine.org

Abstract

The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 nuclease system has allowed the generation of disease models and the development of therapeutic approaches for many genetic and non-genetic disorders. However, the generation of large genomic rearrangements has raised safety concerns for the clinical application of CRISPR/Cas9 nuclease approaches. Among these events, the formation of micronuclei and chromosome bridges due to chromosomal truncations can lead to massive genomic rearrangements localized to one or few chromosomes. This phenomenon, known as chromothripsis, was originally described in cancer cells, where it is believed to be caused by defective chromosome segregation during mitosis or DNA double-strand breaks. Here, we will discuss the factors influencing CRISPR/Cas9-induced chromothripsis, hereafter termed CRISPRthripsis, and its outcomes, the tools to characterize these events and strategies to minimize them.

Key words: genome editing; CRISPR/Cas9; chromothripsis; gene therapy; genotoxicity; micronuclei; chromosomal instability.

Graphical Abstract

CHROMOTHRIPSIS
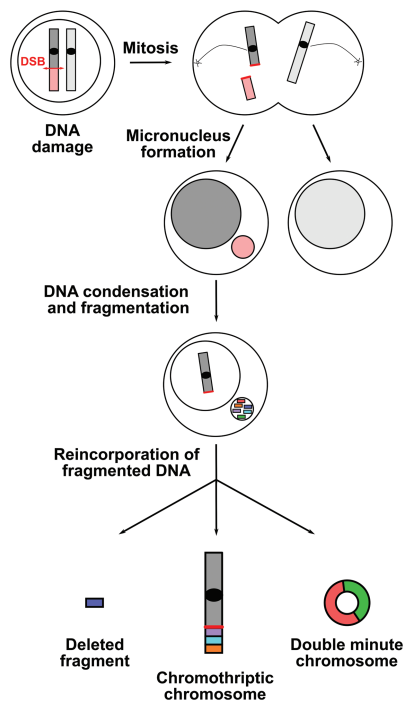
A DNA double-strand break can lead to the formation of acentric chromosome fragments and micronuclei. After DNA condensation, the chromosome fragment is shattered generating multiple DNA fragments, which are reincorporated in the nuclear genome forming a chromotriptic chromosome. If not reincorporated in the genome, these fragments can be lost (deleted fragments) or form double-minute chromosomes.

Significance Statement

The CRISPR/Cas9 system has revolutionized the field of gene therapy for genetic and non-genetic diseases allowing the generation of disease models and the development of effective treatments for numerous disorders. However, safety concerns on the use of CRISPR/Cas9 for clinical applications have emerged. Here, we will discuss recent findings on unanticipated catastrophic DNA rearrangements induced by CRISPR/Cas9 and their implications for gene therapy approaches.

Introduction

The CRISPR/Cas9 system has revolutionized the field of gene therapy for genetic and non-genetic diseases allowing the generation of disease models and the development of effective treatments for numerous disorders. However, safety concerns on the use of CRISPR/Cas9 for clinical applications have emerged. Here, we will discuss recent findings on unanticipated catastrophic DNA rearrangements induced by CRISPR/Cas9 and their implications for gene therapy approaches.

Gene Therapy Using Designer Nucleases

Gene therapy was originally devised as a therapeutic replacement approach for monogenic disorders based on the delivery to the cells of a functional gene capable of compensating for the defective gene. Nowadays, the uses and indications are much broader, with most clinical trials concerning cancer treatment. There has been considerable diversification in the techniques, which are based on various corrective strategies, vectors, and methods including genome editing. Genome editing approaches use designer nucleases, such as the CRISPR/Cas9 nuclease system to induce DNA double-strand breaks (DSBs) via a guide RNA (gRNA) complementary to the genomic target (Fig. 1). The DSB can be repaired via homology-directed repair (HDR) by providing a donor DNA template containing the wild type sequence, allowing direct gene correction of the mutation. However, in the absence of integration of the DNA donor template, the DSB is simply repaired by the non-homologous end joining (NHEJ) pathway that usually generates short insertions or deletions (InDel). Other genome editing approaches leverage the NHEJ pathway to inactivate genes or *cis*-regulatory regions, eg, the enhancer of the *BCL11A* gene encoding a master transcriptional repressor of fetal hemoglobin (HbF) expression with the aim of reactivating HbF expression in adulthood and cure diseases characterized by deficient adult hemoglobin expression (namely beta-hemoglobinopathies).

Initial concerns on the use of the CRISPR/Cas9 system were focused on the potential off-target activity leading to unwanted generation of InDels in genomic regions other than the on-target site.[1] Fortunately, numerous studies to understand the underpinning mechanism and strategies to predict, detect and reduce this risk have been proposed.[2] Later, several groups have described that CRISPR/Cas9-induced DSB can be resolved in a complex and heterogeneous way, with the risk of inducing genomic rearrangements, such as large 1- to 50-kb deletions/inversions, translocations, chromosome loss, or chromosome truncations and combination of these rearrangements.[3,4] Overall, large deletions around the nuclease cutting site are the most common rearrangement and have been observed in mouse zygotes,[5,6] human embryos,[7,8] human and mouse embryonic stem cells[9] and human hematopoietic stem cells (HSCs).[10] Thanks to the use of long-read sequencing, in combination with long-range PCR and targeted sequencing,[11-13] this outcome can be quantified and characterized at the nucleotide level. Very recently, CRISPR/Cas9-induced chromothripsis (hereafter termed CRISPRthripsis; Fig. 1) has been described in cell lines.[14] Unfortunately, unlike off-targets, these on-target risks cannot be reduced by more specific DSB approaches.

Chromoanagenesis or Genomic Chaos

Chromothripsis was firstly described in cancer cells as a process characterized by the occurrence of massive chromosomal rearrangements usually clustered on one or few chromosomes.[15] In fact, although these events can lead to cell death, they can also have a role in malignant transformation. Chromothripsis is observed with a frequency of >50% in several cancers[16] and is emerging as a predictor of negative clinical outcome. The mechanisms underlying chromothripsis are not fully elucidated. It is believed that DNA fragmentation is followed by the formation of micronuclei containing an entire chromosome or part of a chromosome that are further fragmented, reassembled, and eventually incorporated into the genome of the nucleus in the following mitoses[17] (Fig. 2A). Chromothripsis can also occur as a consequence of the formation of chromosomal bridges during mitosis due to the fusion of the two sister chromatids with telomeric loss[18] (Fig. 2B). Finally, chromothripsis can generate double minute chromosomes, small circular acentric chromosomes that can be present at very high copy number and carry oncogenes, thus promoting tumor development.[19]

Nowadays, it is clear that the chromothripsis mechanism could not account for all the phenomena of rapid chromosomal rearrangements arising during single chaotic cellular events. In fact, these catastrophic phenomena include not only chromothripsis, but also chromoanasynthesis and chromplexis, and are grouped under the name of "chromoanagenesis" (chromosome regeneration/evolution).[20] Chromoanasynthesis (chromosome resynthesis) results in localized complex rearrangements with duplication and triplication of a single chromosome due to erroneous DNA replication,[21] and chromoplexy (chromosome restructuring) refers to the occurrence of multiple inter- and intra-chromosomal translocations and deletions with little or no copy number alterations.[22]

Safety Concerns of Nuclease-based Editing Approaches: Micronuclei, Chromosomal Bridges, and CRISPRthripsis

Recently, CRISPR/Cas9-induced chromothripsis (CRISPRthripsis) has been described in cell lines[14] (Fig. 1). In addition, the formation of micronuclei and chromosomal bridges due to the on-target cleavage has also been observed in
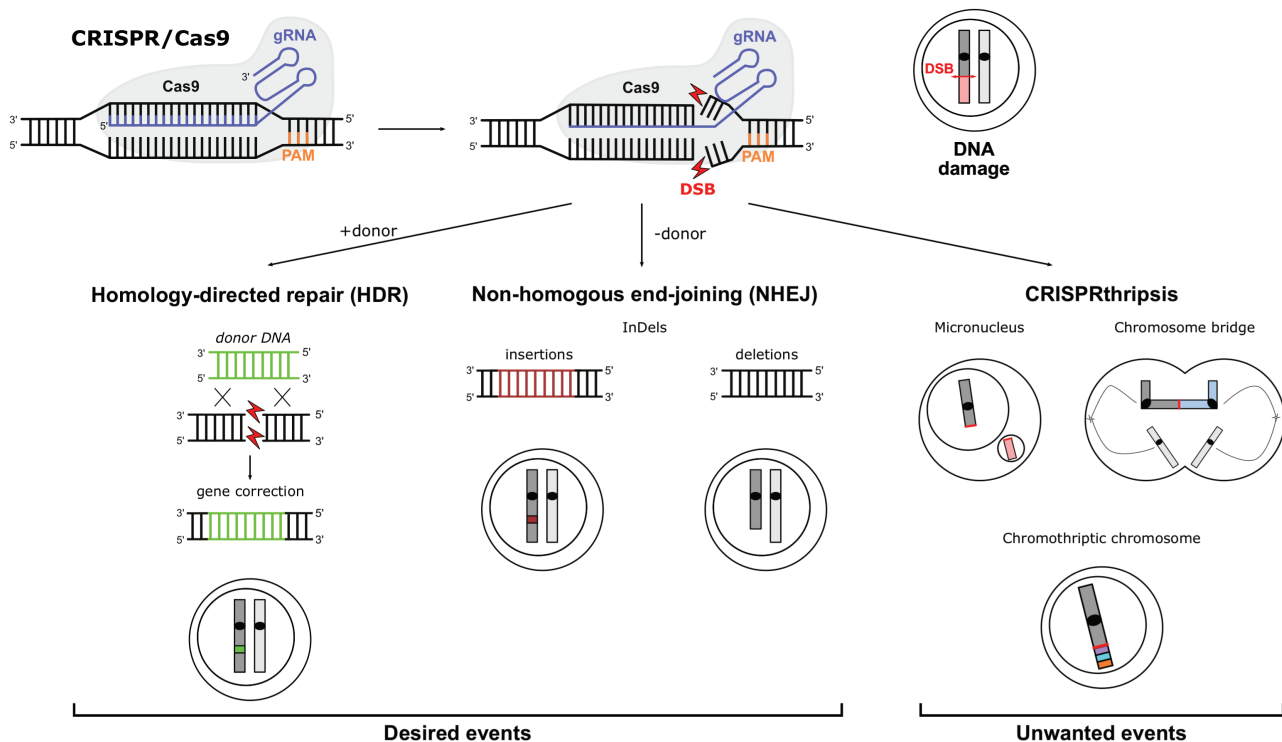
Figure 1. CRISPR/Cas9-induced events. The CRISPR/Cas9 complex is driven to a specific genomic site thanks to the complementarity of the gRNA to the target DNA region. Then, Cas9 induces a DNA double-strand break (DSB, red double arrow) 3-4 nucleotides upstream of the protospacer adjacent motif (PAM). This DSB can be repaired by the homology-directed repair (HDR) in the presence of a donor DNA or by non-homologous end joining (NHEJ) to generate InDels (insertion and deletions). However, if the DSB is not correctly repaired, one of the possible outcomes is CRISPRthripsis, with the formation of micronuclei containing an acentric chromosome fragment (light red box), chromosome bridges, and chromothriptic chromosomes (colored blocks represent shuffled DNA) (see Fig. 2).

primary cells including mouse embryos and human hematopoietic stem/progenitor cells (HSPCs), the target cell population in gene therapy approaches for many hematopoietic and non-hematopoietic disorders[14, 23] (Fig. 1). In particular, the formation of micronuclei was observed in 20%-30% of mouse embryos and 2.5% of human HSPCs edited with Cas9 targeting the *BCL11A* enhancer, a proposed clinical target for beta-hemoglobinopathies[14, 24] (NCT03655678 and NCT03745287). The analysis of micronuclei in the drug product used in these clinical trials[15] was not reported. However, CRISPRthripsis was observed neither in mouse embryos nor in human HSPCs, probably because only a small fraction of cells containing micronuclei undergo CRISPRthripsis and the number of cells analyzed in these studies was likely too low to observe this event. Furthermore, some cells can undergo cell death after CRISPRthripsis. However, even a rare event leading rather to a malignant transformation could be a concern in many gene therapy approaches where millions of cells are targeted. Fortunately, to date, no genotoxic events were reported in the NCT03655678 and NCT03745287 clinical trials.

Tools to Study Micronuclei and Chromosomal Bridges Formation and CRISPRthripsis

Micronuclei are defined as small-sized nuclei between 1/20th and 1/5th of the size of the main nucleus[25] containing one/few chromosomes or chromosome fragments.[26, 27] Micronuclei differ from nuclei in terms of chromatin condensation, nuclear envelope composition, and the absence of proteasomes.[28] Since micronuclei are considered a reliable readout of genomic instability, several approaches to quantify and characterize them have been described. *In primis*, nuclear and chromosomal alterations, such as micronuclei and chromothripsis, can be studied with classical and modern cytogenetics approaches, like GTG banding[29] and fluorescence in situ hybridization,[30] which enables a whole genomic view in a cost-efficient and single-cell oriented fashion. A special case of fluorescence hybridization is comparative genomic hybridization (CGH array), which allows genome copy number variation analysis.[31] However, the resolution of these methods is limited to kilo- to mega-base pair. An exception is molecular combing, which consists in performing FISH after combing high molecular weight DNA on a glass surface.[32] An overview of chromosomes' spatial organization and interactions can be obtained via high-throughput chromosome conformation capture approaches, which can provide both a multi-cell-based genomic view or at a single-cell level.[33-35]

Numbers, shape, and size of micronuclei, and chromosome bridges can be studied via imaging using either microscopy[36, 37] or imaging flow cytometry.[38, 39] Beyond frequency, size of micronuclei represents an important feature since there is a strong correlation between the size of the micronucleus and the presence of centromere, with centromere-positive micronuclei being larger than centromere-negative micronuclei.[40] Recent development of flow imaging will allow enrichment of rare micronuclei-containing cells for subsequent analysis.[41] In addition, novel techniques
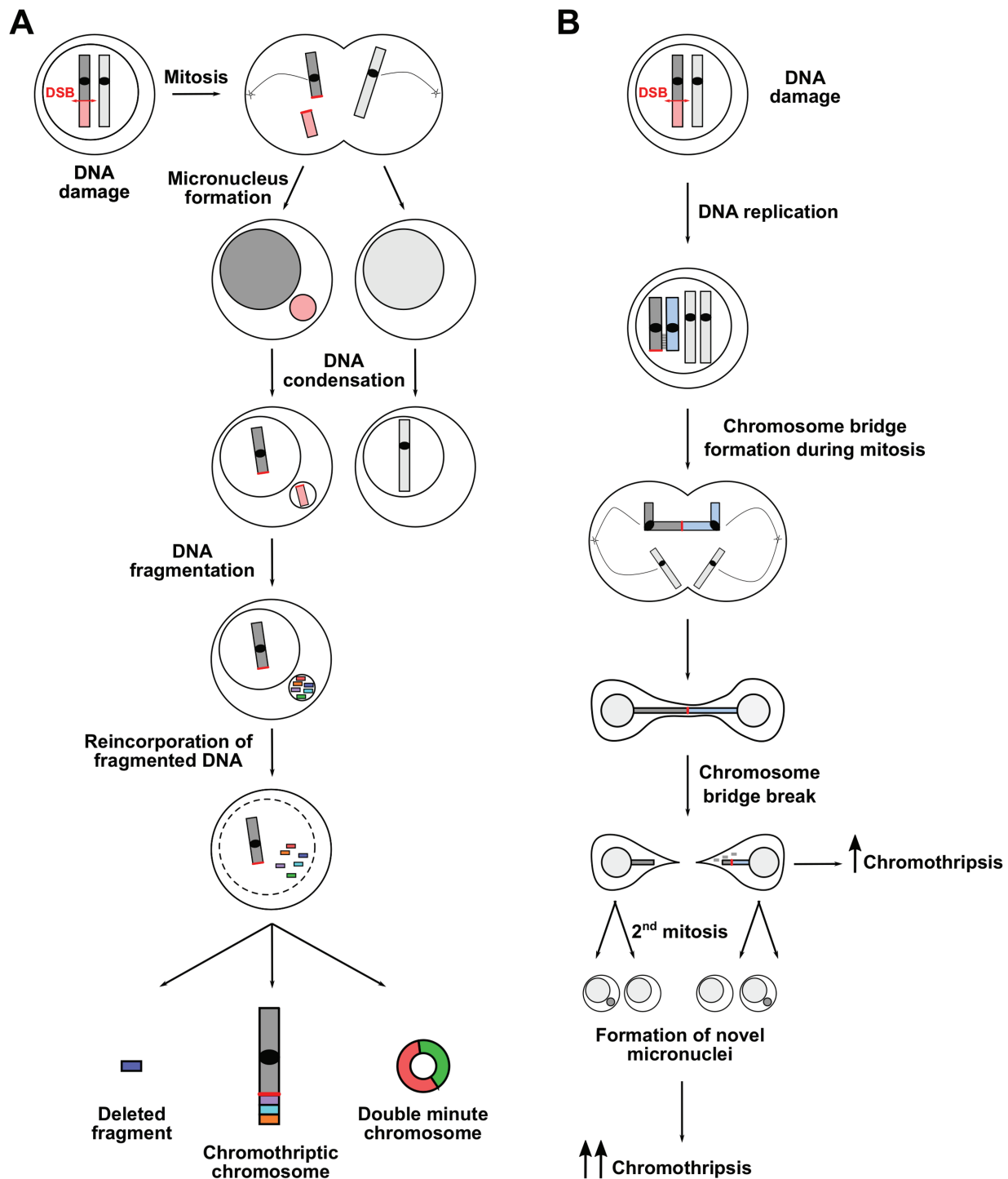
Figure 2. Chromothripsis leads to massive genomic rearrangements. (A) A DNA double-strand break (DSB, red double arrow) can lead to the formation of an acentric chromosome fragment (light red box). After mitosis, this fragment can be enwrapped by a lipid membrane forming a micronucleus (red circle). After DNA condensation, the chromosome fragment is shattered generating multiple DNA fragments, which are eventually reassembled and reincorporated into the nuclear genome forming a chromotriptic chromosome. If not reincorporated in the genome, these fragments can be lost (deleted fragments) or form double-minute chromosomes. (B) A DNA DSB can lead to the formation of sister chromatids with shortened or absent telomeres that form a chromosomal bridge. During the first mitosis, cell division leads to breakage of the chromosomal bridge, which can induce local DNA fragmentation and chromothripsis. During the second mitosis, the broken chromosome missegregates, potentially leading to the formation of micronuclei, which also trigger chromothripsis (see panel A).

allowing purification of micronuclei and sequencing, at both population and single-cell levels, will provide a systematic approach to study genomic instability and reveal novel molecular details in the process.[42]

Finally, "Look-Seq" is a powerful technique for tracking cells containing micronuclei and subjecting their progeny to single-cell sequencing in order to understand the evolution and fate of micronuclei and cells bearing them.[14]

Factors Influencing Micronuclei and Chromosomal Bridges Formation and CRISPRthripsis and Their Outcomes

There are several factors that can influence the formation of micronuclei and chromosome bridges, and CRISPRthripsis and determine their consequences.

First, these events occur in dividing cells, therefore the genotoxic risk is theoretically minimized or abolished in quiescent and non-dividing cells. By way of example, HSPCs are a mixed population of short-term progenitors and HSCs, which will sustain in the long-term the hematopoietic system in the treated patients. HSCs are mostly quiescent and therefore the risk of such chromosomal rearrangements is low. HDR-based approaches for gene correction require a long stimulation of the cell cycle (72-96 hours) as HDR occurs only in dividing cells. Therefore, when possible, NHEJ-based approaches should be preferred as NHEJ occurs in all the phases of the cell cycle. Nevertheless, it is difficult to assess the cell cycle in *bona fide* HSCs at the time of transplantation as they are difficult to identify, and we cannot exclude that even a short-term treatment (typically 48 hours for NHEJ-based approaches) can induce the cell cycle in HSCs. Finally, HSPC-based gene therapy involves the transplantation of proliferating hematopoietic progenitors together with HSCs. If such chromosomal rearrangements occur in progenitor cells, we cannot exclude the development of malignant clones from this cell population.

Furthermore, an important consideration is that the occurrence and frequency of these rearrangements can vary across tissues and cell types according to their response to DNA DSBs,[43] eg, between HSCs and hematopoietic progenitors.[44]

The probability of these events is also specific to each CRISPR/Cas9-based therapeutic strategy and the number of DSB that are generated per cell, eg, editing of multiple genes (gamma-[45] or alpha-globin[46] genes) or performing genomic deletions (beta-[47] or alpha-globin[48] genes) will introduce more than one DSB per chromosome.

Moreover, the chromatin context of the target site can also influence the choice of DNA repair pathway and thus the occurrence of these massive genomic rearrangements. By way of example, locus-specific differences in the chromatin status or in DNA repair efficiency[49] can determine if DSBs are correctly repaired or if they lead to loss of part of a chromosome, and as a consequence formation of micronuclei, chromosome bridges, and CRISPRthripsis. Similarly, the chromosomal location of the target site can also affect the outcome of these events in terms of cell death or clonal expansion, depending on the number and nature of genes present in the target chromosomes (eg, oncogenes and tumor suppressor genes).

Finally, another important factor is the presence of TP53. In fact, while TP53 does not influence the formation of micronuclei, it inhibits the division of around 50% of micronucleated cells, thus potentially avoiding the occurrence of CRISPRthripsis in the following mitosis.[14, 50] Therefore, transient TP53 inhibition proposed to increase HDR efficiency by minimizing apoptosis of edited cells[51] should be considered in view of a possible increase in the occurrence of the CRISPRthripsis.[52]

Overall, the occurrence of these catastrophic events should be closely monitored in pre-clinical gene therapy studies. The recent studies described the occurrence of these events in an HSC-based gene therapy product potentially used for many different diseases.[52] However, long-term in vitro and in vivo experiments in the specific target cell types using highly sensitive tools are required to assess the safety of CRISPR/Cas9 nuclease-based gene therapy approaches.

Strategies to Minimize Micronuclei and Chromosomal Bridges Formation and CRISPRthripsis

To reduce the possibility of such complex genomic rearrangements, gene therapy approaches should: (i) reduce the number of edited cells by stringent selection of target cells (eg, only real HSCs[53]); (ii) reduce cell cycling or restrict editing to the G1 phase of the cell cycle[54-56]; (iii) reduce additional stress associated with cell manipulation[57, 58]; (iv) target the "right" genomic harbor for each application, eg, to achieve high transgene expression with a limited number of edited cells[59] or to avoid chromosomal regions enriched in oncogenes or oncosuppressors; (v) reduce large genomic rearrangements due to repeated DNA cleavage of seamless repaired DSB, by modulating DNA repair[60] or by reducing exposure time to nucleases using protein delivery.[61]

In addition, approaches based on the use of dead Cas9 or Cas9 nickase (eg, base editing, prime editing, and epigenome editing) minimize the formation of DSB and, likely, the generation of chromosomal bridges and the occurrence of CRISPRthripsis. However, there is still a risk of DSB when using technologies using Cas9 nickase (eg, base and prime editing), therefore the occurrence of these rearrangements should be investigated when there is evidence of DSB occurring after Cas9 nickase treatment.

Summary/Conclusion

Chromotripsis is a major driver of extrachromosomal DNA,[62] thus representing an important safety issue for gene and cell therapy that needs to be carefully addressed. Although occurring at low frequency, it is a dangerous event considering the large number of cells that need to be modified to achieve a clinical benefit. In addition, chromothripsis is not the only genome catastrophe that can happen. According to in vitro models, chromothripsis makes up roughly <10% of all different types of chaotic genomes identified.[63, 64] Besides the occurrence of chromoanasynthesis and chromoplexy, various types of cell death, including mitotic cell death,[65-67] apoptosis,[68, 69] necroptosis—a programmed version of necrosis[70] and entosis[71] can all reverse their own process, causing genomic alterations in surviving cells. The newly available technologies and the sudden interest brought to the domain by the CRISPRthripsis phenomenon promise to elucidate in the near future the mechanism causing chromothripsis, micronuclei, and genomic chaos, and their consequences on the surviving cell population, the end product that really matters for gene therapy.

Acknowledgments

Funding

Conflict of Interest

The authors declare no potential conflicts of interest.

Author Contributions

M.A., M.B., A.M.: conception and design, manuscript writing, final approval of manuscript.

Data Availability

No new data were generated or analyzed in support of this research.

References

1. Fu Y, Foden JA, Khayter C, et al. High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells. *Nat Biotechnol*. 2013;31:822-826. https://doi.org/10.1038/nbt.2623

2. Tsai SQ, Joung JK. Defining and improving the genome-wide specificities of CRISPR-Cas9 nucleases. *Nat Rev Genet*. 2016;17:300-312. https://doi.org/10.1038/nrg.2016.28

3. Boutin J, Cappellen D, Rosier J, et al. ON-target adverse events of CRISPR-Cas9 nuclease: more chaotic than expected. *CRISPR J*. 2022;5:19-30. https://doi.org/10.1089/crispr.2021.0120

4. Teboul L, Herault Y, Wells S, et al. Variability in genome editing outcomes: challenges for research reproducibility and clinical safety. *Mol Ther*. 2020;28:1422-1431. https://doi.org/10.1016/j.ymthe.2020.03.015

5. Parikh BA, Beckman DL, Patel SJ, et al. Detailed phenotypic and molecular analyses of genetically modified mice generated by CRISPR-Cas9-mediated editing. *PLoS One*. 2015;10:e0116484. https://doi.org/10.1371/journal.pone.0116484

6. Shin HY, Wang C, Lee HK, et al. CRISPR/Cas9 targeting events cause complex deletions and insertions at 17 sites in the mouse genome. *Nat Commun*. 2017;8:15464. https://doi.org/10.1038/ncomms15464

7. Alanis-Lobato G, Zohren J, McCarthy A, et al. Frequent loss of heterozygosity in CRISPR-Cas9-edited early human embryos. *Proc Natl Acad Sci USA*. 2021;118:e2004832117. https://www.pnas.org/doi/epdf/10.1073/pnas.2004832117

8. Zuccaro MV, Xu J, Mitchell C, et al. Allele-specific chromosome removal after Cas9 cleavage in human embryos. *Cell*. 2020;183:1650-1664.e15. https://doi.org/10.1016/j.cell.2020.10.025

9. Kosicki M, Tomberg K, Bradley A. Repair of double-strand breaks induced by CRISPR-Cas9 leads to large deletions and complex rearrangements. *Nat Biotechnol*. 2018;36:765-771. https://doi.org/10.1038/nbt.4192

10. Wen W, Quan ZJ, Li SA, et al. Effective control of large deletions after double-strand breaks by homology-directed repair and dsODN insertion. *Genome Biol*. 2021;22:236. https://doi.org/10.1186/s13059-021-02462-4

11. Gilpatrick T, Lee I, Graham JE, et al. Targeted nanopore sequencing with Cas9-guided adapter ligation. *Nat Biotechnol*. 2020;38:433-438. https://doi.org/10.1038/s41587-020-0407-5

12. Wallace AD, Sasani TA, Swanier J, et al. CaBagE: a Cas9-based background elimination strategy for targeted, long-read DNA sequencing. *PLoS One*. 2021;16:e0241253. https://doi.org/10.1371/journal.pone.0241253

13. Iyer SV, Kramer M, Goodwin S, et al. ACME: an affinity-based Cas9 mediated enrichment method for targeted nanopore sequencing. *bioRxiv*, doi:10.1101/2022.02.03.478550, February 5, 2022, preprint: not peer reviewed.

14. Leibowitz ML, Papathanasiou S, Doerfler PA, et al. Chromothripsis as an on-target consequence of CRISPR-Cas9 genome editing. *Nat Genet*. 2021;53:895-905. https://doi.org/10.1038/s41588-021-00838-7

15. Stephens PJ, Greenman CD, Fu B, et al. Massive genomic rearrangement acquired in a single catastrophic event during cancer development. *Cell*. 2011;144:27-40. https://doi.org/10.1016/j.cell.2010.11.055

16. Cortes-Ciriano I, Lee JJ, Xi R, et al. Comprehensive analysis of chromothripsis in 2,658 human cancers using whole-genome sequencing. *Nat Genet*. 2020;52:331-341. https://doi.org/10.1038/s41588-019-0576-7

17. Reimann H, Stopper H, Hintzsche H. Long-term fate of etoposide-induced micronuclei and micronucleated cells in Hela-H2B-GFP cells. *Arch Toxicol*. 2020;94:3553-3561. https://doi.org/10.1007/s00204-020-02840-0

18. Umbreit NT, Zhang CZ, Lynch LD, et al. Mechanisms generating cancer genome complexity from a single cell division error. *Science*. 2020;368:eaba0712. https://doi.org/10.1126/science.aba0712

19. Storlazzi CT, Lonoce A, Guastadisegni MC, et al. Gene amplification as double minutes or homogeneously staining regions in solid tumors: origin and structure. *Genome Res*. 2010;20:1198-1206. https://doi.org/10.1101/gr.106252.110

20. Ostapinska K, Styka B, Lejman M. Insight into the molecular basis underlying chromothripsis. *Int J Mol Sci*. 2022;23:3318.

21. Liu P, Erez A, Nagamani SC, et al. Chromosome catastrophes involve replication mechanisms generating complex genomic rearrangements. *Cell*. 2011;146:889-903. https://doi.org/10.1016/j.cell.2011.07.042

22. Baca SC, Prandi D, Lawrence MS, et al. Punctuated evolution of prostate cancer genomes. *Cell*. 2013;153:666-677. https://doi.org/10.1016/j.cell.2013.03.021

23. Papathanasiou S, Markoulaki S, Blaine LJ, et al. Whole chromosome loss and genomic instability in mouse embryos after CRISPR-Cas9 genome editing. *Nat Commun*. 2021;12:5855. https://doi.org/10.1038/s41467-021-26097-y

24. Frangoul H, Altshuler D, Cappellini MD, et al. CRISPR-Cas9 gene editing for sickle cell disease and beta-thalassemia. *N Engl J Med*. 2021;384:252-260. https://doi.org/10.1056/NEJMoa2031054

25. Schmid W. The micronucleus test. *Mutat Res*. 1975;31:9-15. https://doi.org/10.1016/0165-1161(75)90058-8

26. Heng HH, Liu G, Stevens JB, et al. Karyotype heterogeneity and unclassified chromosomal abnormalities. *Cytogenet Genome Res*. 2013;139:144-157. https://doi.org/10.1159/000348682

27. Ye CJ, Sharpe Z, Alemara S, et al. Micronuclei and genome chaos: changing the system inheritance. *Genes (Basel)*. 2019;10:366.

28. Maass KK, Rosing F, Ronchi P, et al. Altered nuclear envelope structure and proteasome function of micronuclei. *Exp Cell Res*. 2018;371:353-363. https://doi.org/10.1016/j.yexcr.2018.08.029

29. Seabright MA. rapid banding technique for human chromosomes. *Lancet*. 1971;2:971-972.

30. Lee C, Rens W, Yang F. Multicolor fluorescence in situ hybridization (FISH) approaches for simultaneous analysis of the entire human genome. *Curr Protoc Hum Genet*. 2001;Chapter 4:Unit4.9. https://doi.org/10.1002/0471142905.hg0409s24

31. Kallioniemi A, Kallioniemi OP, Sudar D, et al. Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. *Science*. 1992;258:818-821.

32. Schurra C, Bensimon A. Combing genomic DNA for structural and functional studies. *Methods Mol Biol*. 2009;464:71-90. https://doi.org/10.1007/978-1-60327-461-6_5

33. Dekker J. Mapping the 3D genome: aiming for consilience. *Nat Rev Mol Cell Biol*. 2016;17:741-742. https://doi.org/10.1038/nrm.2016.151

34. Nagano T, Lubling Y, Stevens TJ, et al. Single-cell Hi-C reveals cell-to-cell variability in chromosome structure. *Nature*. 2013;502:59-64. https://doi.org/10.1038/nature12593

35. Mallard C, Johnston MJ, Bobyn A, et al. Hi-C detects genomic structural variants in peripheral blood of pediatric leukemia patients. *Cold Spring Harb Mol Case Stud*. 2022;8:a006157.

36. Lepage CC, Thompson LL, Larson B, et al. An automated, single cell quantitative imaging microscopy approach to assess micronucleus formation, genotoxicity and chromosome instability. *Cells*. 2020;9:344. https://doi.org/10.3390/cells9020344

37. Janssen AFJ, Breusegem SY, Larrieu D. Current methods and pipelines for image-based quantitation of nuclear shape and nuclear envelope abnormalities. *Cells*. 2022:11:347.

38. Wills JW, Verma JR, Rees BJ, et al. Inter-laboratory automation of the in vitro micronucleus assay using imaging flow cytometry and deep learning. *Arch Toxicol*. 2021;95:3101-3115. https://doi.org/10.1007/s00204-021-03113-0

39. Rodrigues MA, Probst CE, Zayats A, et al. The in vitro micronucleus assay using imaging flow cytometry and deep learning. *NPJ Syst Biol Appl*. 2021;7:20. https://doi.org/10.1038/s41540-021-00179-5

40. Hashimoto K, Nakajima Y, Matsumura S, et al. An in vitro micronucleus assay with size-classified micronucleus counting to discriminate aneugens from clastogens. *Toxicol In Vitro*. 2010;24:208-216. https://doi.org/10.1016/j.tiv.2009.09.006

41. Schraivogel D, Kuhn TM, Rauscher B, et al. High-speed fluorescence image-enabled cell sorting. *Science*. 2022;375:315-320.

42. Pereira C, Rebelo AR, Massey DJ, et al. Sequencing micronuclei reveals the landscape of chromosomal instability. *bioRxiv*, doi:10.1101/2021.10.28.466311, October 29, 2021, preprint: not peer reviewed.

43. Meyenberg M, Ferreira da Silva J, Loizou JI. Tissue specific DNA repair outcomes shape the landscape of genome editing. *Front Genet*. 2021;12:728520. https://doi.org/10.3389/fgene.2021.728520

44. Milyavsky M, Gan OI, Trottier M, et al. A distinctive DNA damage response in human hematopoietic stem cells reveals an apoptosis-independent role for p53 in self-renewal. *Cell Stem Cell*. 2010;7:186-197. https://doi.org/10.1016/j.stem.2010.05.016

45. Weber L, Frati G, Felix T, et al. Editing a γ-globin repressor binding site restores fetal hemoglobin synthesis and corrects the sickle cell disease phenotype. *Sci Adv*. 2020;6:eaay9392.

46. Pavani G, Laurent M, Fabiano A, et al. Ex vivo editing of human hematopoietic stem cells for erythroid expression of therapeutic proteins. *Nat Commun*. 2020;11:3778. https://doi.org/10.1038/s41467-020-17552-3

47. Antoniani C, Meneghini V, Lattanzi A, et al. Induction of fetal hemoglobin synthesis by CRISPR/Cas9-mediated editing of the human β-globin locus. *Blood*. 2018;131:1960-1973. https://doi.org/10.1182/blood-2017-10-811505

48. Pavani G, Fabiano A, Laurent M, et al. Correction of β-thalassemia by CRISPR/Cas9 editing of the α-globin locus in human hematopoietic stem cells. *Blood Adv*. 2021;5:1137-1153. https://doi.org/10.1182/bloodadvances.2020001996

49. Schep R, Brinkman EK, Leemans C, et al. Impact of chromatin context on Cas9-induced DNA double-strand break repair pathway balance. *Mol Cell*. 2021;81:2216-2230.e10. https://doi.org/10.1016/j.molcel.2021.03.032

50. Soto M, Raaijmakers JA, Bakker B, et al. p53 prohibits propagation of chromosome segregation errors that produce structural aneuploidies. *Cell Rep*. 2017;19:2423-2431. https://doi.org/10.1016/j.celrep.2017.05.055

51. Schiroli G, Conti A, Ferrari S, et al. Precise gene editing preserves hematopoietic stem cell function following transient p53-mediated DNA damage response. *Cell Stem Cell*. 2019;24:551-565.e8. https://doi.org/10.1016/j.stem.2019.02.019

52. Kloosterman WP, Cuppen E. Chromothripsis in congenital disorders and cancer: similarities and differences. *Curr Opin Cell Biol*. 2013;25:341-348. https://doi.org/10.1016/j.ceb.2013.02.008

53. Zonari E, Desantis G, Petrillo C, et al. Efficient ex vivo engineering and expansion of highly purified human hematopoietic stem and progenitor cell populations for gene therapy. *Stem Cell Rep*. 2017;8:977-990. https://doi.org/10.1016/j.stemcr.2017.02.010

54. Lomova A, Clark DN, Campo-Fernandez B, et al. Improving gene editing outcomes in human hematopoietic stem and progenitor cells by temporal control of DNA repair. *Stem Cells*. 2019;37:284-294. https://doi.org/10.1002/stem.2935

55. Gutschner T, Haemmerle M, Genovese G, et al. Post-translational regulation of Cas9 during G1 enhances homology-directed repair. *Cell Rep*. 2016;14:1555-1566. https://doi.org/10.1016/j.celrep.2016.01.019

56. Matsumoto D, Tamamura H, Nomura W. A cell cycle-dependent CRISPR-Cas9 activation system based on an anti-CRISPR protein shows improved genome editing accuracy. *Commun Biol*. 2020;3:601. https://doi.org/10.1038/s42003-020-01340-2

57. Mantel CR, O'Leary HA, Chitteti BR, et al. Enhancing hematopoietic stem cell transplantation efficacy by mitigating oxygen shock. *Cell*. 2015;161:1553-1565. https://doi.org/10.1016/j.cell.2015.04.054

58. Aerts-Kaya F. Strategies to protect hematopoietic stem cells from culture-induced stress conditions. *Curr Stem Cell Res Ther*. 2021;16:755-770. https://doi.org/10.2174/1574888X15666200225091339

59. Pavani G, Amendola M. Corrigendum: targeted gene delivery: where to land. *Front Genome Ed*. 2021;3:682171. https://doi.org/10.3389/fgeed.2021.682171

60. Yin J, Lu R, Xin C, et al. Cas9 exo-endonuclease eliminates chromosomal translocations during genome editing. *Nat Commun*. 2022;13:1204. https://doi.org/10.1038/s41467-022-28900-w

61. Lattanzi A, Meneghini V, Pavani G, et al. Optimization of CRISPR/Cas9 delivery to human hematopoietic stem and progenitor cells for therapeutic genomic rearrangements. *Mol Ther*. 2019;27:137-150. https://doi.org/10.1016/j.ymthe.2018.10.008

62. Shoshani O, Brunner SF, Yaeger R, et al. Chromothripsis drives the evolution of gene amplification in cancer. *Nature*. 2021;591:137-141. https://doi.org/10.1038/s41586-020-03064-z

63. Liu G, Stevens JB, Horne SD, et al. Genome chaos: survival strategy during crisis. *Cell Cycle*. 2014;13:528-537. https://doi.org/10.4161/cc.27378

64. Ye CJ, Liu G, Heng HH. Experimental induction of genome chaos. *Methods Mol Biol*. 2018;1769:337-352. https://doi.org/10.1007/978-1-4939-7780-2_21

65. Stevens JB, Liu G, Bremer SW, et al. Mitotic cell death by chromosome fragmentation. *Cancer Res*. 2007;67:7686-7694. https://doi.org/10.1158/0008-5472.CAN-07-0472

66. Stevens JB, Abdallah BY, Liu G, et al. Diverse system stresses: common mechanisms of chromosome fragmentation. *Cell Death Dis*. 2011;2:e178. https://doi.org/10.1038/cddis.2011.60

67. Heng HH, Stevens JB, Liu G, et al. Imaging genome abnormalities in cancer research. *Cell Chromosome*. 2004;3:1. https://doi.org/10.1186/1475-9268-3-1

68. Tang HL, Yuen KL, Tang HM, et al. Reversibility of apoptosis in cancer cells. *Br J Cancer*. 2009;100:118-122. https://doi.org/10.1038/sj.bjc.6604802

69. Tang HM, Talbot CC Jr., Fung MC, et al. Molecular signature of anastasis for reversal of apoptosis. *F1000Res*. 2017;6:43. https://doi.org/10.12688/f1000research.10568.2

70. Gong YN, Guy C, Olauson H, et al. ESCRT-III acts downstream of MLKL to regulate necroptotic cell death and its consequences. *Cell*. 2017;169:286-300.e16. https://doi.org/10.1016/j.cell.2017.03.020

71. Hamann JC, Surcel A, Chen R, et al. Entosis is induced by glucose starvation. *Cell Rep*. 2017;20:201-210. https://doi.org/10.1016/j.celrep.2017.06.037