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Chromothripsis in cancer cells: An update

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Mini Review

In 2011, a novel form of genome instability was reported by Stephens *et al.*, characterized by tens to hundreds of locally clustered rearrangements affecting one or a few chromosome(s) in cancer cells. This phenomenon, termed chromothripsis, is likely due to a single catastrophic event leading to the simultaneous formation of multiple double-strand breaks, which are repaired by error-prone mechanisms. Since then, the occurrence of chromothripsis was detected in a wide range of tumor entities. In this review, we will discuss potential mechanisms of chromothripsis initiation in cancer and outline the prevalence of chromothripsis across entities. Furthermore, we will examine how chromothriptic events may promote cancer development and how they may affect cancer therapy.

Genomic aberrations in cancer cells are thought to arise incrementally through a gradual process of mutation and selection.[1,2] According to this multistep model of cancer development, the progressive accumulation of genomic changes leads to the activation of oncogenes and to the loss of tumor suppressor functions, which in turn result in changes in cell function and transformation into malignant cells (Fig. 1*a*). In addition to site-specific mutational events, other mechanisms imply genome-wide processes such as polyploidization.

The increasing resolution of high-throughput genomic sequencing technologies has recently enabled the discovery of a novel phenomenon of genomic instability that plays a role in particular in cancer formation, where tens to hundreds of chromosomal rearrangements localized to a limited number of genomic regions are acquired in a single catastrophic event.[3] This form of genome instability was initially discovered in a case of chronic lymphocytic leukemia and called chromothripsis (introduced by Stephens *et al.*[3]; *chromo* stands for "chromosome" and *thripsis* describes the shattering process). Chromothripsis affects single chromosomes and/or chromosome regions that are shattered into numerous pieces.[3] Subsequent imperfect repair mechanisms lead to the formation of highly derivative chromosomes. Cells that survive such a drastic event likely have gained a strong selection advantage owing to their vastly rearranged genome—a process that potentially transforms such a cell into a cancer cell (Fig. 1*b*).

Several features shared by chromothripsis-associated rearrangements distinguish this unique catastrophic event from other complex aberrations. First, chromothripsis leads to the generation of multiple rearrangements as a consequence of locally clustered DNA double-strand breaks (DSBs). These rearrangements are confined to one single or very few chromosome(s) or chromosome subregion(s), rather than being scattered throughout the entire genome. Second, the fragmented DNA pieces are repaired imperfectly and joined in a random fashion. The repair mechanism likely to be involved in this process is nonhomologous end-joining (NHEJ) as the DNA fragments do not display any or very few homologies within their breakpoints.[4] Third, copy-number profiles of the reassembled fragments show regularly oscillating states between typically only two (or occasionally three) states, relating to loss and retention of heterozygosity (Figs. 1*c* and 1*d*), which would be rather unlikely in a scenario of gradually acquired rearrangements.[3,4] However, it is worth mentioning that if chromothripsis occurs in polyploid cells, the lower copy-number state may not display loss of heterozygosity.[5] Finally, fragments produced by the chromosomal DNA shattering typically originate from a single parental chromosome. Taken together, these hallmarks defining the signature of chromothripsis are important for the proper classification of rearrangements observed in cancer cells.

Particular features of chromothripsis-associated sequences, *i.e.*, clustering of the breakpoints or regularity of oscillating copy-number states, make it very likely that the chromosome is breaking at a single time point.

Key words: chromothripsis, genome instability, catastrophic genomic rearrangement

Abbreviations: aCGH: array comparative genomic hybridization; BFB: breakage-fusion-bridge; CT: chromothripsis; DSB: double-strand break; iAMP21: intrachromosomal amplification of chromosome 21; IDH: isocitrate dehydrogenase; IR: ionizing radiation; MMBIR: microhomology-mediated break-induced replication; MMJ: microhomology-mediated joining; NHEJ: nonhomologous end-joining; PCC: premature chromosome condensation; SHH: Sonic Hedgehog; SNP: single-nucleotide polymorphism; WGS: whole-genome sequencing; WT: wild type

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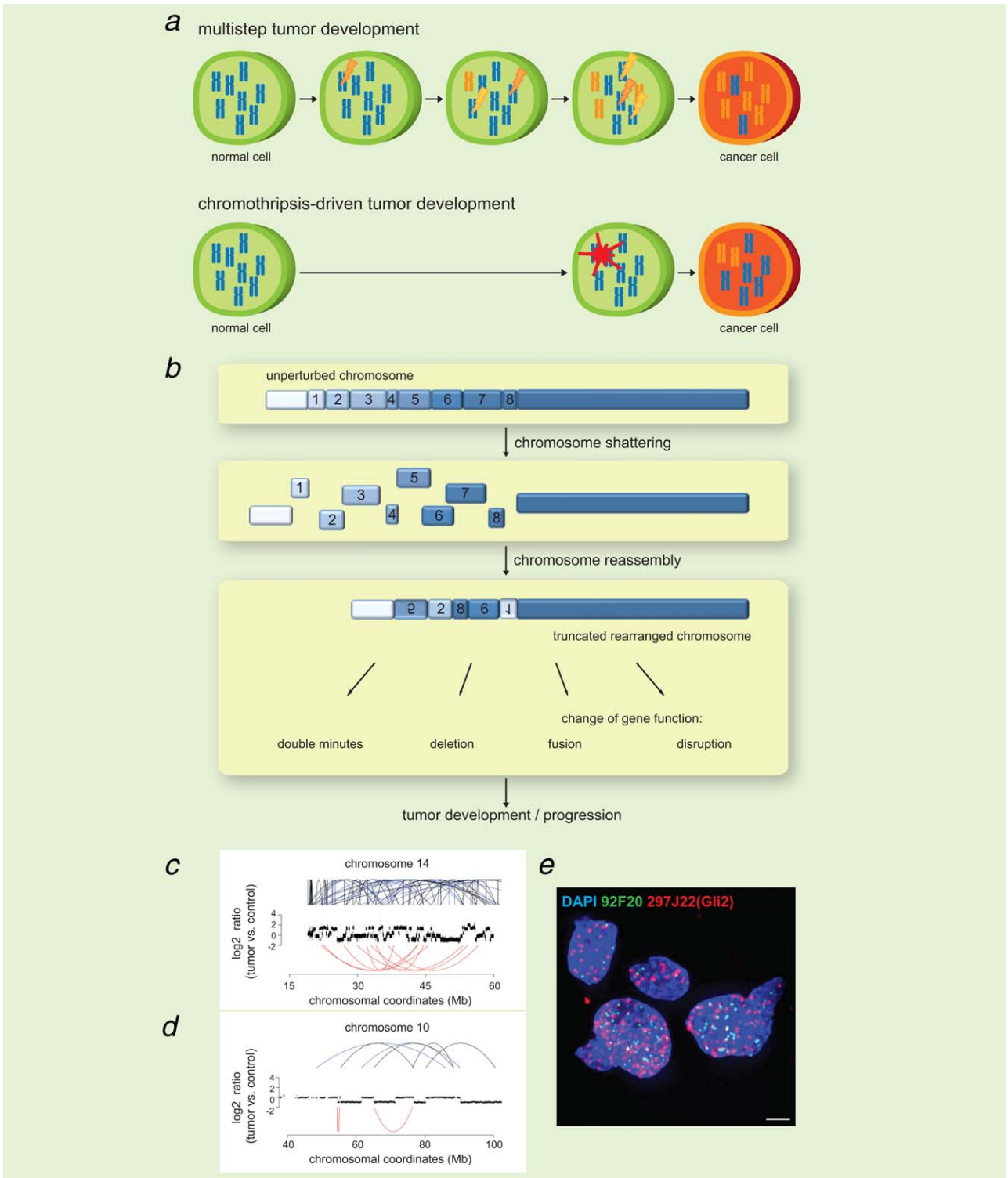

Figure 1. Chromothripsis is a form of genome instability generating tens to hundreds of chromosome rearrangements in a single catastrophic event. (*a*) According to the textbook model of multistep tumor development, series of genetic alterations are acquired progressively (indicated by the orange lightenings). Aberrations can accumulate over decades before the identification of a malignant clone. In contrast, chromothripsis is a one-off catastrophic process leading to the simultaneous acquisition of multiple genomic aberrations affecting one or a few chromosomes. (*b*) Chromosomes that undergo chromothripsis shatter into many pieces. The DNA fragments resulting from this process are randomly rejoined by error-prone DNA repair processes, most likely nonhomologous end-joining (NHEJ). Many DNA fragments are not reincorporated into the derivative chromosome and are lost to the cell. End-joining-based repair can lead to the loss of tumor suppressor functions, to the formation of oncogenic fusions and to oncogene amplification *via* double-minute chromosomes. (*c*) Chromothripsis in a primary glioblastoma sample. (*d*, *e*) Chromothripsis in a primary medulloblastoma sample associated with the formation of circular double-minute chromosomes derived from chromosome 2 fragments. Read-depth plots show rearrangements resulting from chromothripsis, with a typical oscillating copy-number pattern. Links involving duplicated regions are displayed on the top in blue and links involving deleted regions are displayed at the bottom in red, inversions are displayed in black. (*e*) FISH validation of rearrangements contributing to chromothripsis-associated double-minute chromosomes derived from chromosome 2 segments, with amplification of the *GLI2* oncogene (red, clone 297J22) and another region of chromosome 2 (green, clone RP11-92F20). Scale bar: 5 μm.

Different subtypes of complex genome rearrangements deriving from single catastrophic events have been reported recently. Holland and Cleveland[6] suggested the term chromoanagenesis as a common description for chromothripsis and chromoanasynthesis (from *chromo* for chromosomes and *anasynthesis* for reconstitution). Unlike chromothripsis, chromoanasynthesis is characterized by copy-number gain and retention of heterozygosity. Although both rearrangement types likely occur in a single event affecting limited regions of the genome, the underlying mechanisms may be distinct. In addition, chromothripsis is mostly observed in cancer cells, whereas rearrangements due to chromoanasynthesis underlie congenital or developmental defects.[7] Thus, the authors suggest treating the common term chromoanagenesis with caution and prefer the distinction between chromothripsis and chromoanasynthesis. Beside these two subtypes of complex catastrophic chromosomal rearrangements, chromoplexy makes up the third class, characterized by chains of translocations, with little or no copy number alteration.[8]

The highly localized nature of the DSBs on single chromosomes affected by chromothripsis leads to the proposal that initiating mechanisms may involve condensed chromosomes in association with mitosis.[3] Alternatively, the nonrandom spatial organization of chromosomes into territories within the interphase nucleus[9–11] might provide the local basis for the restricted shattering of a few chromosomes.

In this review, we will focus on potential mechanisms leading to chromothripsis and on the prevalence of chromothripsis across different cancer entities. Furthermore, we will discuss how chromothriptic events can cause or promote cancer and how this impacts cancer therapy.

Chromothripsis Prevalence Across Different Tumor Entities

The prevalence of chromothripsis across tumor entities remains an open question. Studies reporting the occurrence of chromothripsis have used different methodologies and definitions, making a comparison between entities challenging. In addition, cancers with high genomic instability, which progressively acquire many DNA alterations per chromosome, can erroneously be suspected to have undergone chromothripsis.[5] Therefore, there is a need for reproducible and accurate inference of chromothripsis.

In Table 1, we summarize studies that reported chromothripsis in human cancer. Although the authors of these publications used different technologies and applied different criteria for the inference of chromothripsis, this comparison provides a rough estimation of the prevalence of chromothripsis.

To circumvent the lack of cross-entity comparability—due to varying technologies and definitions—several groups attempted to investigate the occurrence of chromothripsis in large datasets that are publically available. For instance, Cai *et al.* identified chromothripsis-like patterns in 918 cancer samples derived from a dataset of >22,000 cases covering 132 cancer types.[35] However, this study relies essentially on a model for the discovery of clustered genomic aberration patterns. The detection of a large number of locally clustered copy-number aberrations is the smallest consensus and does not correspond to a stringent definition of chromothripsis. Another study screened >8,000 human cancer copy-number profiles using an algorithm detecting regions in which the copy number oscillates rapidly between fixed levels indicating chromothripsis.[36] As the analysis was exclusively based on array comparative genomic hybridization, not all hallmarks of chromothripsis could be evaluated. Nevertheless, the reported prevalence rates are largely consistent with those described here.[36]

Because of the broad availability of copy number data, such approaches, restricted to operational definitions of chromothripsis (*i.e.*, that require fixed numbers of oscillating copy number changes), are widespread.[37] For an initial screening, such criteria may be sufficient, and many examples with multiple oscillations between two or three copy number states likely are chromothripsis cases. However, the field is currently moving toward more comprehensive definitions of chromothripsis to add criteria that include information from paired-end sequencing.[37] Detailed analysis of the structure of the derivative chromosomes allows for the differentiation of chromothripsis from progressively acquired rearrangements in tumors with high levels of genetic instability.[5,37] Implementation of software tools for detecting and quantifying chromothriptic events will address the multiple challenges in the scoring of chromothripsis in cancer genomes, in order to achieve a more reproducible and standardized analysis.[38] Ultimately, an accurate picture of the tumor entities affected by chromothripsis will help us to examine questions such as in which context does chromothripsis occur, which pathways are potentially involved and what makes certain cell types more prone to chromothripsis.

Mechanistic Models for Chromothripsis

What initiates the chromosome shattering, and which processes are involved in the repair of the DNA breaks? Some potential mechanisms underlying chromothripsis are now starting to emerge, but a detailed picture of the complete process remains elusive.

Generation of DNA breaks and rejoining of the DNA fragments

DNA DSBs have the most deleterious effects on the genome and can be caused by various stress factors such as ionizing radiation (IR), reactive oxygen species, replication errors, topoisomerase failure, mechanical stress, inaccurate enzyme action or pathogen infections. Therefore, cells have DNA repair machineries to rejoin misplaced or incorrect ends. Ideally, homology-based repair allows the rejoining of the original sequence with full inheritance of the genomic information by homologous recombination. In the absence of homologous sequences to serve as templates, DSBs are repaired by DNA end-joining mechanisms like NHEJ or microhomology-mediated joining (MMJ). These end-joining

Table 1. Prevalence of chromothripsis across tumor entities

Tumor entity	*n*[1]	Method	CT prevalence (%)[2]	Ref.
Hematopoietic malignancies				
Acute lymphoblastic leukemia with iAMP21	9	WGS	**88.9**	12
Acute myeloid leukemia with mutant TP53	17	SNP array	**47.1**	4
Acute myeloid leukemia with WT TP53	91	SNP array	1.1	4
Myelodysplastic syndrome with complex chromosome aberrations	157	SNP array	**47**	13
Chronic lymphocytic leukemia	10	WGS	10	3
Multiple myeloma	764	SNP array	1.3	14
Carcinomas				
Invasive bladder carcinoma	5	WGS	**60**	15
High-risk breast cancer	29	aCGH	**41**	16
Breast cancer (basal-like)	12	WGS	0	17
Ovarian cancer	11	WGS	0	17
High-risk prostate cancer	6	WGS	16.7	18
Lung adenocarcinoma	6	WGS	**33.3**	17
Lung squamous cell carcinoma	13	WGS	15.4	17
Esophageal adenocarcinoma	123	WGS and SNP array	**32**	19
Malignant melanoma	20	aCGH	10	20
Uveal melanoma	25	OncoScan assay	8	21
Hepatocellular carcinoma	88	WGS	5.7	22
Central nervous system tumors				
SHH medulloblastoma with mutant *TP53*	10	SNP array and WGS	**100**	4
Medulloblastoma, all subgroups	98	SNP array and WGS	13.3	4
Medulloblastoma, all subgroups	1,070	SNP array	11.4	23
Glioblastoma	18	WGS	**38.9**	17
Grade IV glioma, *IDH* mutant	24	OncoScan assay	**37.5**	24
Grade II–III glioma	45	OncoScan assay	11.1	24
Low-grade glioma	41	SNP array	2.4	25
Ependymoma	41	WGS	22	26
Neuroblastoma	87	WGS	11.4	27
Neuroblastoma	233	SNP array	4.3	28
Meningioma	11	WGS	9.1	29
Retinoblastoma	94	WGS and SNP array	3.2	30
Glioneuronal tumors	114	WGS	2.6	31
Soft tissue tumors				
Uterine leiomyoma	36	WGS	**41.7**	32
Uterine leiomyoma	5	SNP array	20	33
Osteosarcoma	9	SNP array	**33.3**	3
Chordoma	11	WGS	18.2	3
Phaeochromocytoma	36	SNP array	2.5	34

Bold values show prevalences higher than 30%.
Case studies and studies based on cell lines are not included in the table.
[1] *n*, total number of cases analyzed in the respective study.
[2] Please be aware that the assessment of chromothrispis in these studies is based on different definitions of chromothripsis and different technologies for analysis.
Abbreviations: WT: wild type; IDH: isocitrate dehydrogenase; SHH: Sonic Hedgehog; iAMP21: intrachromosomal amplification of chromosome 21; CT: chromothripsis; WGS: whole-genome sequencing; SNP: single-nucleotide polymorphism; aCGH: array comparative genomic hybridization.

processes are error prone and can have two possible outcomes. When the rearrangements are not compatible with cell survival, the affected cell dies. Alternatively, if the cell does not undergo apoptosis, the genomic remodeling induced by the inaccurate repair can become a major driver in cancer initiation and/or progression (Fig. 1*e*). Rejoining patterns of the chromothripsis-associated fragments show only short to no sequence homology, which points to NHEJ or MMJ. Several nonexclusive models for the initiation of chromothripsis have been proposed, which will be discussed below.

Chromothripsis through micronuclei

The micronuclei hypothesis is one likely model to explain how complex chromosomal rearrangements affect only certain chromosomal regions, single chromosomes or occasionally a few chromosomes (Fig. 2*a*). Micronuclei provide an enclosed environment with specific characteristics in terms of DNA damage and DNA repair.

Formation of micronuclei. Nuclear abnormalities such as micronuclei, nucleoplasmic bridges and nuclear buds are markers of genotoxicity and genomic instability.[39] Micronuclei can arise either from lagging chromosomes or chromatid fragments during mitosis, or from nuclear budding of DNA repair complexes or excess, amplified DNA in interphase cells. The functional organization of micronuclei is not well characterized, but there are some studies describing defects in micronuclear import[40] and the loss of integrity of the micronuclear envelope.[41,42]

DNA damage and repair in micronuclei. The DNA content of micronuclei appears to be prone to DNA damage.[43] Until now it is unclear whether DNA damage occurs more frequently in micronuclei when compared to primary nuclei or whether the DNA damage response fails to be efficiently activated and therefore more DNA damage can be detected in micronuclei. Early studies showed that the protein Rad51 involved in homologous recombination and the single-strand DNA-binding protein RPA are specifically recruited in micronuclei upon radiation.[44] Later observations showed that the DSB-specific histone variant γH2AX accumulates in irradiation-induced micronuclei (Fig. 3) and co-localizes with ATM, while ATM downstream targets such as 53BP1, Rad50, MRE11 and DNA-PKcs were not found to accumulate in micronuclei.[45,46] A study using γ-ray-induced micronuclei and subsequent, independent UV-light-induced DNA damage showed that micronuclei are generally unable to generate an effective DNA-damage response.[40] Crasta *et al.* showed for the first time that missegregated chromosomes are entrapped in micronuclei and that their DNA content can be pulverized and subsequently reintegrated into the genome. This study provides the first experimental rather than analytical evidence pointing to a mechanism for the generation of chromothripsis.[41] A recent work showed that in nocodazole-induced micronuclei, DNA damage is not only caused by a ruptured nuclear envelope but also requires entry into the S-phase.[47] The authors observed that micronuclear DNA is underreplicated in general. Furthermore, they describe microhomology of the junctions, which favors the involvement of microhomology-mediated break-induced replication (MMBIR) as a potential mechanism for DNA rearrangements.[47]

Reincorporation of micronuclei. The inheritance of shattered and rearranged chromosomes requires the reincorporation of the derivative chromosomes into the main nucleus. However, the fate of micronuclei remains unknown. There are three possible postmitotic fates for micronuclei. The micronucleus could be (*i*) expelled from the cell or (*ii*) degraded, with both scenarios resulting in a loss of the genetic material. However, two studies from the Pellman lab showed a third possibility, namely the re-engulfment of the micronuclear DNA content within the same cell cycle.[41,47]

To summarize the current knowledge, the inconsistent results with regard to replication, transcription, repair and fate of micronuclear DNA arise from the variability of micronuclei. Even if first mechanistic insights for the generation of chromothripsis strongly point to the micronuclei hypothesis, a number of relevant questions still need to be addressed to enlighten the process of chromothriptic cancer development.

Premature chromosome condensation

Premature chromosome condensation (PCC) can be observed when a mitotic cell fuses with a cell in S-phase, which mimics a mitotic environment for the latter and causes the chromatin to compact prematurely by cyclin-dependent kinase activity. A similar scenario can be pictured for an asynchronous cell cycle between the main nucleus and micronucleus of one cell (Fig. 2*b*). Dependent on the cell cycle discrepancy, the results are either discrete units with one or two chromatids for G1 and G2 nuclei, respectively, or a pulverized appearance for S-phase nuclei. The final genetic consequence can vary from whole or partial to nonincorporation into the main nucleus.[48] S-phase replicons might represent highly sensitive regions for PCC and yield a high degree of scattering of the micronuclei chromatin. In combination with a nucleotide deficiency in cancer cells,[49] the highly sensitive S-phase replicons may represent the templates for DNA breaks and fragmentation. The phenomenon of DNA loss as a consequence of failure to repair or reincorporate into the main nucleus, *e.g.*, tumor suppressor loss or a selection for oncogene-containing regions in the case of chromothripsis, was described for conventional PCC.[48,50]

Breakage-fusion-bridge cycle and telomere dysfunction

The breakage-fusion-bridge (BFB) cycle is an ongoing dynamic process leading to tumor heterogeneity, nowadays considered one of the main causes of structural instability in cancer.[51] A dicentric chromosome is formed by the fusion of two broken chromosomes or by the fusion of two unprotected telomeres (Fig. 2*c*). In the subsequent cell cycle, this highly unstable chromosome with two centromeres is pulled

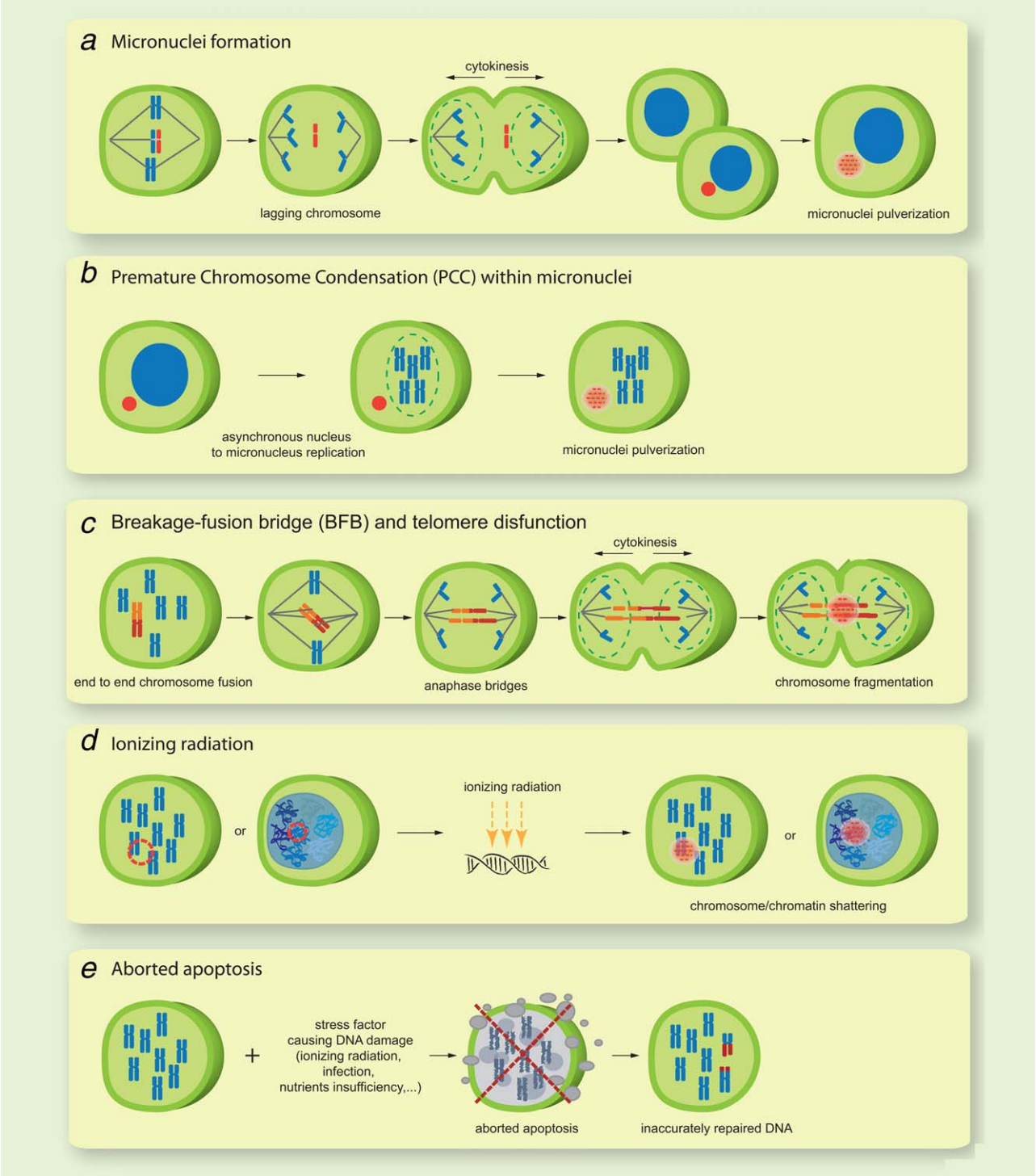

Figure 2. Mechanistic models of chromothripsis initiation. The mechanistic basis of the chromothriptic process is unknown, but several nonexclusive models have been proposed. (*a*) The micronuclei hypothesis explains the locally restricted DNA damage. According to this model, chromosomes contained within micronuclei suffer aberrant DNA replication and can undergo extensive DNA fragmentation. Subsequent rejoining of the DNA segments leads to derivative chromosomes that can be reincorporated into the primary nucleus. The timing of the reincorporation into the primary nucleus of micronuclear DNA content is still under debate. (*b*) Premature chromosome condensation (PCC). Asynchronous cell-cycle progression between primary nucleus and micronucleus could induce the premature condensation of replicating DNA and instability of the condensed DNA fragments. (*c*) Breakage-fusion-bridge (BFB) cycles and telomere dysfunction. Unprotected chromosome ends can be joined to form an instable derivative chromosome, which gets ruptured in the subsequent cell cycle. By this mechanism, multiple rearrangements can occur before the derivative chromosome becomes stable and is inherited. (*d*) Ionizing radiation (IR). External factors such as IR can induce multiple DNA DSBs, which could be repaired inaccurately and thereby initiate chromothripsis. (*e*) Aborted apoptosis. The initiation of apoptosis leads to DNA fragmentation. In rare cases, apoptosis may be initiated and then aborted, the cell escaping complete DNA fragmentation.

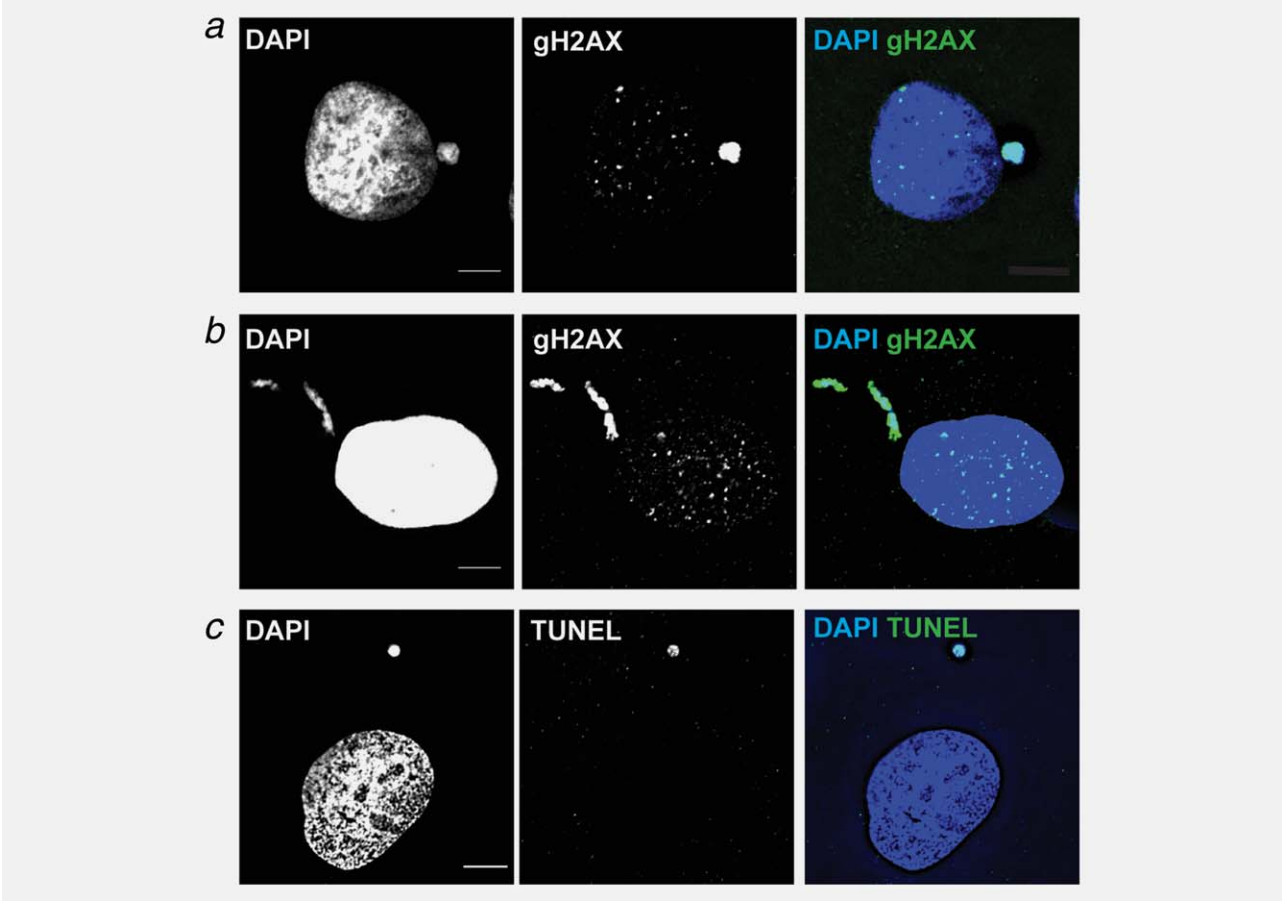

Figure 3. Immunostaining of micronuclei. Characteristics of micronuclei in U2OS cells were visualized by specific antibody staining or by TUNEL assay. (*a*) Enrichment of γH2AX signal in the micronuclear compartment. (*b*) Anaphase bridge with accumulated γH2AX signal. (*c*) TUNEL-positive micronucleus with corresponding TUNEL-negative main nucleus. Scale bar: 5 μm.

to opposite spindle poles forming a chromosomal bridge between the two daughter cells. This structure called anaphase bridge might harbor the same risks for fragmentation of the entrapped chromosomal structure as described for micronuclei before. The rupturing bridge generates two new unprotected chromosomal ends and initiates a new round of BFB cycle. In acute lymphoblastic leukemia with a germline Robertsonian translocation, chromothriptic patterns with involvement of both sister chromatids of the Robertsonian chromosome were described as a consequence of BFB cycles.[12] The BFB cycle could explain the translocation between fragments with different copy numbers that originate from one chromosome. Only this hypothesis can explain the discrepancy between one chromosome template and copy-number variations and observed sample heterogeneity by multiple rounds of breakage and fusion.[52] The extensive analysis of the architecture and evolution of cancer-associated neochromosomes from liposarcomas described chromosome corrosion by hundreds of BFB cycles to stabilize rearranged neochromosomes initially arising from chromothripsis.[53] Through evolution of the neochromosome, which implies an ongoing process, oncogenes are overexpressed while passenger genes might be silenced epigenetically. With the formation or capture of a centromeric structure and telomere capture the neochromosomes reach a metastable status.[53]

Ionizing radiation

IR can induce a wide range of DNA lesions, among them DSBs on DNA. It is difficult to assume that single chromosomes may be affected by radiation-induced damage, except if separate compartments such as micronuclei are involved. There are certain characteristics described for IR-induced lesions, which have to be taken under consideration for a potential enrichment of these regions in chromothriptic patterns. First, the damage to an individual chromosome can be expected to be proportional to the whole genome with the assumption that the deposition of irradiation energy is random along the DNA molecule. Second, there is evidence that telomeric DNA is excessively damaged by IR.[54] Third, it is well described that there is heterogeneity in the repair of lesions along the DNA molecule with preferential repair of actively transcribed to nontranscribed regions.[55] Taken

together, this might explain how certain chromosomes or chromosome regions might be more affected than others (Fig. 2*d*). Studies of the dynamics of independent IR-induced lesions suggest that distant locations can be recruited into preexisting or newly formed repair centers. This breakage-first theory may explain the nature of chromosomal translocations and the rearrangement of fragments from different chromosomes.[56] These centers might facilitate the repair but on the other hand harbor a high risk of illegitimate end-joining between chromosomes in spatial proximity.[57] Notably, IR-induced chromosomal aberrations can also result in the formation of micronuclei.

Abortive apoptosis

Another potential source of generation of a high number of DSBs in a single event is apoptotic DNA fragmentation (Fig. 2*e*). The tightly regulated molecular and biochemical events eventually leading to the death of a cell can be triggered by stress stimuli such as nutrient deprivation, oxygen shortage, exposure to IR, chemotherapeutic drugs or an infection. The initiation of higher order fragmentation of chromatin results in apoptosis for most cells, but the survival of a subpopulation of cells after inaccurate repair of the massively cleaved DNA can lead to chromosomal rearrangements. This so-called abortive apoptosis was described in reproducing programs as in spermatogenesis[58] and in a disease background of acute myeloid leukemia with a rearrangement of breakpoint cluster regions.[59,60] As an external factor interfering with the apoptosis program, the family of Y-herpes viruses could cause cancer by inhibiting apoptosis.[61] With this scenario, a random shattering and rearrangement of fragments cannot be explained. Furthermore, one would expect to observe either the DSBs in scaffold-associated domains of early apoptosis or the characteristic internucleosomal fragments of late apoptosis. However, this model cannot be excluded on the basis of existing data. Inactivation of p53 might support the survival of chromothripsis-positive clones by protecting cells with high levels of DSBs from elimination *via* p53-mediated cell cycle arrest, apoptosis or senescence.[62]

Role of p53

Depending on the tumor entity, an involvement of the tumor suppressor p53 in chromothripsis is highly indicated.[4] In diploid human cells, chromosome missegregation causes a cell cycle delay with accumulation of p53 and the cyclin kinase inhibitor p21 in the nucleus, whereas massive chromosome missegregation induces p53-mediated apoptosis.[63] Therefore, mutations in *TP53* create a permissive environment for the initiation and propagation of chromosomal rearrangements. But not only delayed chromosomes, also micronuclei trigger a p53 response by stabilization and therefore accumulation followed by cell-cycle arrest preventing the accumulation of aneuploid cells. These cells with micronuclei exhibit a decreased ability to enter S-phase.[64] p53 accumulates in micronuclei, which might be a consequence of DNA degradation occurring in aging of micronuclei and which might be a delayed form of cellular response to the presence of micronuclei in the cytoplasm.[65] A reduced p53 activity increases the frequency of micronucleated cells. Last, cells with *TP53* mutations show a preference to low fidelity repair mechanisms such as NHEJ.[66] However, one should keep in mind that there are also many cases of chromothripsis-positive tumors without *TP53* mutation. Additional mutational or biochemical signaling contexts, such as activation of the SHH pathway in medulloblastoma, have been associated with chromothripsis,[4] but in many cases the context that may have favored the occurrence of chromothripsis (and strong evidence as to whether the association is cause, consequence or mere correlation) remains unexplained.

How Can Chromothripsis Promote or Cause Cancer Development?

Complex genomic rearrangements, such as chromothripsis, can affect gene function in several ways, and thereby cause or promote cancer development. The structure and dosage of multiple genes are disrupted at once. Therefore, chromothripsis potentially offers a shortcut to the acquisition of numerous cancer-causing mutations. However, in the vast majority of cases, catastrophic events of this magnitude likely have extremely deleterious consequences eventually leading to apoptosis (*e.g.*, due to disruption of essential or dosage-sensitive genes) and thus result in elimination by clonal outgrowth (Fig. 4*a*). In addition, the high number of locally confined DSBs required to produce such rearrangements can initiate the activation of the DNA damage response machinery and thereby inhibit cell proliferation.[67] These negative selection pressures may explain why chromothripsis so rarely gives rise to a detectable clone. In clones surviving chromosome shattering, three types of beneficial driver alterations—in addition to a more general gene-dosage effect—can be detected: the formation of fusion genes (Fig. 4*b*), the loss of tumor suppressor functions (Fig. 4*c*) and/or the amplification of oncogenes (Fig. 4*d*).

Formation of fusion genes

The random joining of chromosome fragments can produce gene fusions, which can activate oncogenes or generate proteins with new cellular/oncogenic functions. Fusion gene transcripts arising through chromothripsis may consist of multiple genomic fragments and even multiple coding fragments, which together make polygene fusions.[23,68] For instance, recurrent fusions involving the noncoding gene *PVT1* were found as a result of chromothripsis in medulloblastoma, including *PVT1-MYC* and *PVT1-NDRG1*.[23] Also, more than two-thirds of supratentorial ependymomas contain oncogenic fusions resulting from chromothripsis involving *RELA*, the principal effector of canonical NF-kB signaling, and an uncharacterized gene, *C11orf95*.[26]

Loss of tumor suppressor functions

Chromothripsis can also exert cancer-promoting effects through loss or disruption of tumor suppressor genes, because many

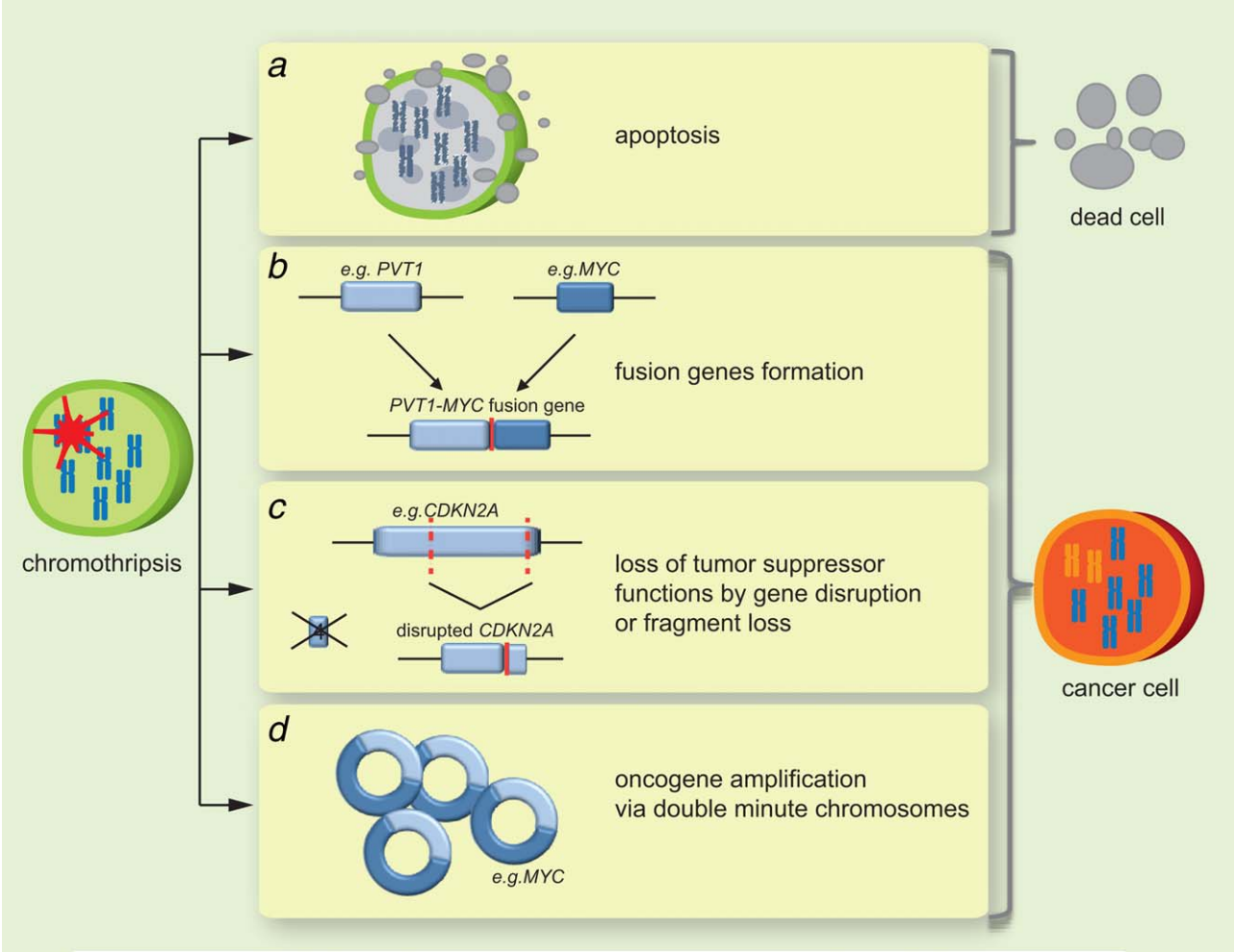

Figure 4. Mechanisms of cancer promotion *via* chromothripsis. Chromothripsis deranges the structure and dosage of a large number of genes at one stroke, through the induction of extensive rearrangements. (*a*) In most cases, such a massive disruption of chromosome integrity leads to cell death. Hence, the vast majority of clones undergoing chromothripsis are probably eliminated by apoptosis or clonal outgrowth. (*b–d*) In rare cases—probably at the upper limit of what a cell can tolerate in terms of damage—such random changes may provide a driving force in cancer development by disrupting haploinsufficient tumor suppressor genes and/or activating proto-oncogenes without affecting cell-essential genes. (*b*) The random rejoining of shattered chromosome fragments produces gene fusions, such as *PVT1-MYC* in medulloblastoma. Gene fusions can lead to the activation of oncogenes or the generation of proteins with new cellular/oncogenic functions. (*c*) Chromothripsis events result in massive DNA loss. The lost fragments might include one or more tumor suppressor genes. Loci containing tumor suppressor genes can also be disrupted by the shattering process itself. (*d*) Shattered chromosomal segments that fail to get reincorporated into the derivative chromosome can be linked to form a double-minute chromosome. These double-minute chromosomes are readily amplified, which frequently occurs if they harbor oncogenes such as *MYC*.

DNA fragments are lost to the cell, and the remaining pieces bear the scar of DSBs. For example, the cell-cycle regulator *CDKN2A* is frequently affected by chromothripsis; complex rearrangement breakpoints affecting this gene have been found in chordoma and diffuse large B-cell lymphoma.[3,69] Simultaneous loss of tumor suppressor genes located on different chromosomes has also been observed, such as loss of *CDKN2A*, *FBXW7* and *WRN* in chordoma and loss of *CDKN2A* and miR15a/16 in chronic lymphocytic leukemia.[3]

Oncogene amplification

Genomic fragments produced by chromosome shattering can undergo three distinct fates: (*i*) the shattered pieces may become parts of newly assembled mosaic chromosomes, (*ii*) they may be lost to the cell or (*iii*) they may be rejoined into double minutes—circular fragments of extrachromosomal DNA that frequently harbor amplified oncogenes.[3,4] For instance, amplification of the *MYC* oncogene resulting from chromothripsis was described in small cell lung cancer, neuroblastoma, esophageal adenocarcinoma and medulloblastoma.[3,19,23,27] In the subgroup of Sonic Hedgehog (SHH) medulloblastoma, chromothripsis-associated amplified regions typically contain medulloblastoma oncogenes, such as SHH pathway members *MYCN*, *GLI2* and *BOC*.[4] In neuroblastoma, *MYCN* is also frequently amplified in association with chromothripsis.[27,36]

In summary, genomic rearrangements arising from chromothripsis can exert cancer-promoting effects in different ways. Exceptionally, outgrowth of a recurrent clone lacking the derivative pieces might occur, as shown by longitudinal genomic analysis of a chronic lymphocytic leukemia case, for which chromothripsis was not detectable anymore at the later time points.[70] However, FISH experiments showed rearrangement outcomes of chromothripsis to be detectable in nearly all cells in a tumor, and not only in certain subclones.[4] This finding argues for the fact that the shattering is probably—at least in many cases—an early event in tumorigenesis, which acts as a driving force in cancer development and progression. Therefore, a better understanding of the mechanistic basis of chromothripsis will allow the development of strategies to interfere with the process in a therapeutic setup.

Impact for Therapy

Currently, cancer patients for whom chromothripsis-associated rearrangements have been detected do not receive any specific therapeutic treatment.

Chromothripsis appears to be strongly linked to poor prognosis in some entities, such as multiple myeloma,[14] melanoma,[20] neuroblastoma[27] and acute myeloid leukemia.[4] The poor outcome of the disease could be explained by the fact that the function of many important genes becomes affected,[71] cells do not respond with an apoptotic signal to DNA breaks and can adapt to the treatment or escape cell death due to the numerous genomic aberrations. Targeting oncogenes present in chromothripsis-associated amplified regions might offer a potential therapeutic option. As an additional strategy, the large number of rearrangements detectable in chromothripsis-positive tumors might make these tumors more sensitive to immune therapies, similarly to successful immune checkpoint blockade to treat tumors with a large number of somatic mutations due to mismatch-repair defects.[72]

It has also been noticed that Fanconi anemia patients carrying *TP53* mutations frequently develop secondary malignancies when they are treated with IR.[73] Also, patients with Li-Fraumeni syndrome are affected by chromothripsis at much higher rates than the overall incidence (36% *versus* 2–3%).[4] Those patients might benefit from a regular screening and the use of treatments without DNA damaging agents and/or radiotherapy to reduce the potential of therapy resistance. Finally, it is also possible that chromothripsis could make tumors vulnerable to therapeutic strategies that specifically target regions of genomic loss. All of the above mentioned points show that the identification of chromothripsis is important because it can help with prognostication. Those tumors could be directed to rationally targeted therapies or treated by drugs targeting the DNA damage response pathway.

Finally, one recently described positive impact of chromothripsis should be mentioned. McDermott *et al.*[74] reported the case of a patient suffering from an inherited immunodeficiency syndrome caused by overactivity of a receptor called CXCR4. The patient was cured by chromothripsis, which deleted the abnormal copy of the *CXCR4* gene in a single hematopoietic stem cell; the latter took over the bone marrow and restored normal immune function. Even though it is hard to envision the induction of chromothripsis as a therapeutic approach, this "experiment of nature" demonstrated for the first time a beneficial aspect of chromothripsis that may even confer a cure for certain inherited diseases in rare cases. This finding opens new avenues relative to the potential role of chromothripsis in evolution.

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