Chromoanagenesis and cancer: mechanisms and consequences of localized, complex chromosomal rearrangements

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Next-generation sequencing of DNA from human tumors or individuals with developmental abnormalities has led to the discovery of a process we term chromoanagenesis, in which large numbers of complex rearrangements occur at one or a few chromosomal loci in a single catastrophic event. Two mechanisms underlie these rearrangements, both of which can be facilitated by a mitotic chromosome segregation error to produce a micronucleus containing the chromosome to undergo rearrangement. In the first, chromosome shattering (chromothripsis) is produced by mitotic entry before completion of DNA replication within the micronucleus, with a failure to disassemble the micronuclear envelope encapsulating the chromosomal fragments for random reassembly in the subsequent interphase. Alternatively, locally defective DNA replication initiates serial, microhomology-mediated template switching (chromoanasynthesis) that produces local rearrangements with altered gene copy numbers. Complex rearrangements are present in a broad spectrum of tumors and in individuals with congenital or developmental defects, highlighting the impact of chromoanagenesis on human disease.

Karyotype abnormalities comprise numerical and structural alterations in chromosomes and are defining features of the cancer cell genome. Structural rearrangements in chromosomes are caused by erroneous repair of DNA double-strand breaks and include deletions, duplications, inversions and translocations. Recurrent translocations are common in hematological malignancies, in which they have been shown to drive tumorigenesis through the creation of fusion genes derived from portions of two normal genes joined together[1]. In addition, rearrangements also contribute to disruption of tumor suppressor genes and amplification of oncogenes.

The advent of high-throughput DNA sequencing has enabled the interrogation of the cancer genome in unprecedented detail. Catalogs of the somatic mutations present in cancer cells are rapidly appearing (for example, http://www.sanger.ac.uk/genetics/CGP/Census/). Sequencing of both ends of the same DNA fragment (known as paired-end sequencing) reduces alignment ambiguities when matching short sequence reads to the reference genome. Paired-end sequencing of millions of genomic fragments from a single tumor can map genome-wide chromosomal rearrangements. Its use has recently brought considerable attention to the effect of structural chromosomal changes on cancer development[2–4] and uncovered an unexpected phenomenon in which tens to hundreds of rearrangements occur within one or a handful of genomic regions[5]. Sequencing of DNA from individuals with developmental abnormalities has identified constitutive chromosomes with similarly complex, localized rearrangements[6–9].

Two mechanisms have been proposed to provoke such rearrangements in a single event. The first is a cellular crisis termed chromothripsis[5] (from the Greek 'chromo' for chromosomes and 'thripsis,' for shattering). The second is local rearrangements with altered gene copy numbers produced by serial, microhomology-mediated template switching during DNA replication, termed chromoanasynthesis[8] ('chromo' for chromosomes and 'anasynthesis,' for reconstitution). Evidence to date suggests that chromothripsis is the probable mechanism underlying most of the rearrangements identified in cancer. Nevertheless, recognizing that at least two mechanisms produce complex, localized rearrangements, we propose the word chromoanagenesis ('chromo' for chromosomes and 'anagenesis,' for rebirth) to describe this class of chromosomal rearrangement that is independent of the provoking mechanism.

Here we discuss the evidence supporting the view that chromoanagenesis occurs as a one-off cellular event that may contribute to the initiation and development of human cancer. We outline the mechanisms that have been proposed to create highly localized complex genomic rearrangements, including provocative recent work suggesting that chromoanagenesis is initiated by a chromosome mis-segregation error, producing a micronucleus in which the localized shattering and religation take place in two subsequent cell cycles. We also describe how similarly complex rearrangements with copy number changes can be driven by cellular stress during DNA replication, resulting in replication fork collapse which initiates microhomology-mediated template switching.

A one-off cellular cataclysm

Three primary lines of evidence indicate that many of the localized chromosomal rearrangements observed in cases of chromoanagenesis do not arise from a progressive series of independent rearrangements; rather, they occur in a single catastrophic event[5].

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First, in cancer cells the chromosome rearrangements primarily alternate between two copy number states. The lower copy number state represents heterozygous deletion of a DNA fragment and the higher copy state indicates retention of a DNA piece (**Fig. 1**). The higher copy number state does not always result from two copies of a DNA fragment, as tumors are often aneuploid (containing an abnormal number of chromosomes). Progressive models with sequential chromosomal translocations predict substantially more than two copy number states[5]. Second, heterozygosity is preserved in multiple separate regions with higher copy number states in which DNA fragments have been retained. Regions in which heterozygosity is maintained can be encompassed within an area spanned by multiple additional rearrangements that have the orientation of deletions, duplications and inversions[5]. If a deletion occurred early in a successive series of rearrangements, then heterozygosity would be permanently eliminated between the breakpoints. Thus, for a progressive model to explain chromoanagenesis, deletion events could only occur late in the sequence of rearrangements, a scenario that seems unlikely given the number of rearrangements involved in chromoanagenesis[5]. In contrast, alternating regions of heterozygosity (retention of a DNA fragment) and loss of heterozygosity (loss of a DNA fragment) inevitably result from rearrangements that are caused by a one-off cataclysmic event proposed to occur during chromothripsis (**Fig. 1**). Third and finally, in tumors in which chromoanagenesis has occurred, the chromosomal breakpoints cluster to a greater degree than expected from sequential independent rearrangements[5].

Overall, it is therefore likely that most of the rearrangements present in the chromoanagenesis found in cancer cells occur in a single catastrophic event arising from chromosome pulverization followed by the rejoining of chromosomal fragments in a random order (**Fig. 1**). The idea that cancer genomes evolve in rapid bursts is in line with the evolutionary theory of punctuated equilibrium originally proposed by Eldredge and Gould in 1972, which posits that species undergo little alteration for most of their evolutionary history, with rare events leading to rapid evolutionary shifts that can result in the creation of a new species[10]. Similarly, creating many alterations in a single genomic event increases the probability that large adaptive leaps can be achieved, which may be advantageous in the severe genetic or environmental pressures encountered in tumors.

Even if it is accepted that multiple complex rearrangements can occur in a single event, the high frequency of genome changes in cancer cells firmly suggests additional rearrangements can also be expected before or after chromoanagenesis, consistent with the widely held view that genomic changes in many cancers accumulate from a progressive series of errors. Indeed, some regions of rearranged chromosomes alternate between two and three copy number states, which suggests that a partial duplication of the rearranged chromosome occurs after chromoanagenesis takes place[5]. Alternatively, if an initiating event created massive DNA double-strand breaks simultaneously on both genetically identical sister chromatids of a replicated chromosome, then the random stitching together of chromosome fragments could lead to a duplication of specific chromosomal fragments in the rearranged sister chromatids[11].

Solitary confinement: locked away in a micronucleus

Since its discovery, the most perplexing feature of chromoanagenesis is how chromosomal rearrangements can be limited to a very small subset of chromosomes, often a single chromosome or chromosome arm. What event(s) causes this massive damage and how can it be highly localized to distinct genomic regions? A very surprising

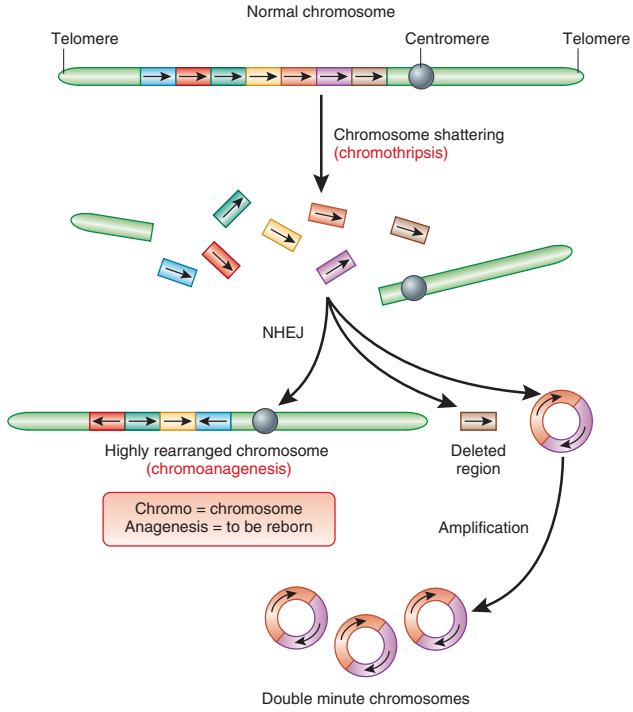

Figure 1 Mechanism for the creation of complex chromosomal rearrangements by NHEJ after chromosome shattering. Chromothripsis results in the shattering of one or a few chromosomes (or a chromosome arm), leading to the simultaneous creation of many double-strand breaks. Most of the shattered fragments are stitched back together through NHEJ, leading to chromoanagenesis: the creation of a chromosome with complex, highly localized chromosomal rearrangements. The rearranged chromosome contains two copy number states: a high copy number state for each religated fragment and a low copy number state for fragments that were not reincorporated and therefore lost. Broken DNA fragments may also be joined together to form circular, extrachromosomal double minute chromosomes that often harbor oncogenes and are frequently amplified, resulting in a dramatically increased copy number of DNA fragments on these chromosomes.

mechanism was identified in early 2012: chromosome shattering may arise from an error in mitotic chromosome segregation that leads to the production of a micronucleus[12].

During normal mitosis, the replicated genetic information is divided equally into the two new daughter nuclei such that each cell receives a single copy of each duplicated chromosome. Errors in chromosome segregation during mitosis result in the production of aneuploid cells. Aneuploidy is a hallmark of cancer and has been widely proposed to have a role in the initiation and development of tumors[13,14]. Although aneuploidy and structural alterations in chromosomes have often been thought to arise independently of one another, recent evidence has shown that these two chromosomal aberrations can be mechanistically linked.

Most tumor cells do not possess a stably aneuploid genome; rather, they have a continually changing karyotype driven by high rates of chromosome gain and loss during division, a phenomenon known as chromosomal instability[15]. Live-cell imaging experiments have revealed that chromosomally unstable tumor cells show an increase in the number of chromosomes that lag in the middle of the spindle during anaphase[16,17]. One or both copies of such lagging chromosomes often do not reach the two major chromosome masses at the poles of the cells before nuclear envelope reassembly, and consequently

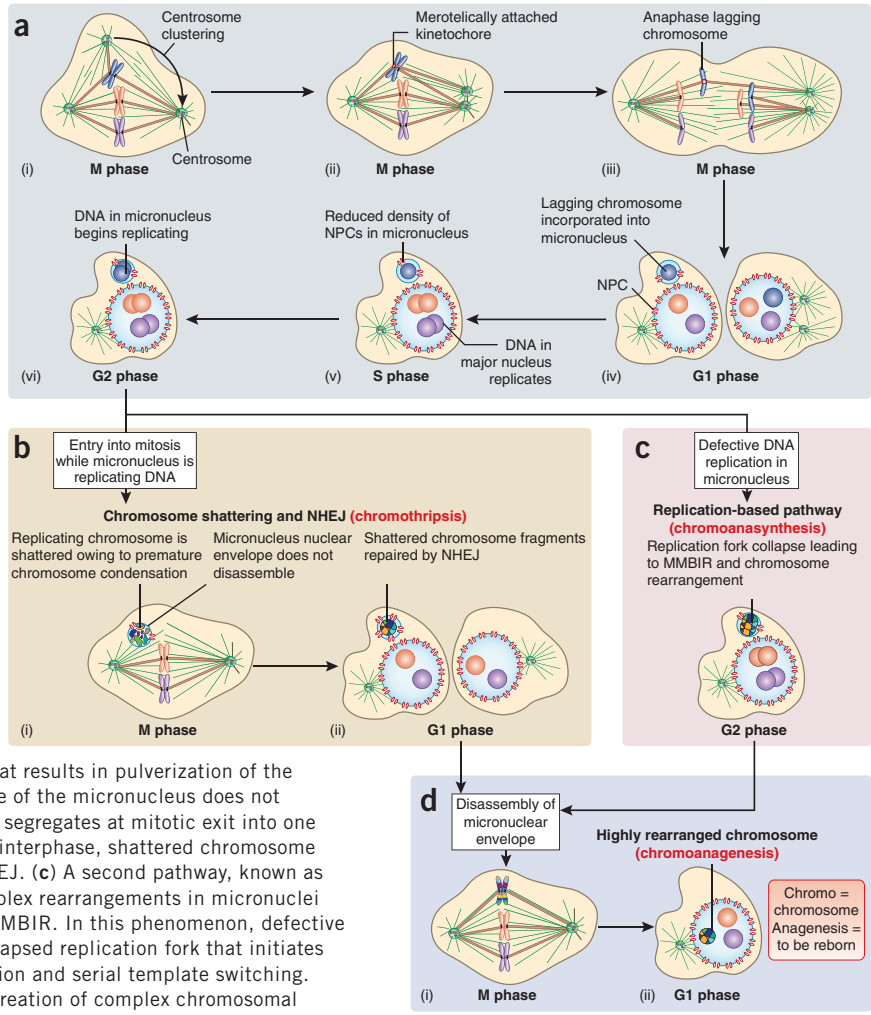

Figure 2 Mitotic errors produce micronuclei and subsequent chromoanagenesis. (**a**,i) Cells with extra centrosomes form multi-polar mitotic spindles. In many instances centrosomes coalesce into two groups before anaphase. (**a**,ii) Centrosome clustering increases the frequency of merotelic attachments, in which a duplicated chromosome attaches through its kinetochore to microtubules arising from both mitotic spindle poles[51,52]. (**a**, iii) If not corrected before anaphase, merotelically attached chromosomes may lag in the middle of the mitotic spindle. (**a**, iv) Lagging chromosomes are sometimes excluded from both daughter nuclei and instead form a micronucleus in one of the daughter cells in the subsequent interphase. (**a**) Micronuclei often contain fewer nuclear pore complexes (NPCs) (v), impairing nuclear import and delaying DNA replication of chromosome(s) in the micronucleus (vi). Chromoanagenesis (the creation of complex, localized chromosomal rearrangements) can arise in micronuclei through two distinct mechanisms. (**b**,i) The predominant pathway, known as chromothripsis[5], involves chromosome shattering after mitotic entry while the micronucleus is still replicating its DNA. The incompletely replicated micronuclear DNA undergoes premature chromosome condensation that results in pulverization of the trapped chromosome(s). Often the nuclear envelope of the micronucleus does not disassemble and the intact micronucleus randomly segregates at mitotic exit into one of the daughter cells. (**b**,ii) During the subsequent interphase, shattered chromosome pieces within the micronucleus are repaired by NHEJ. (**c**) A second pathway, known as chromoanasynthesis[8], leads to the creation of complex rearrangements in micronuclei through a replication-based mechanism, such as MMBIR. In this phenomenon, defective DNA replication in the micronucleus leads to a collapsed replication fork that initiates microhomology-dependent priming of DNA replication and serial template switching. MMBIR can result in chromoanagenesis, with the creation of complex chromosomal rearrangements at genomic regions surrounding the collapsed replication fork. (**d**,i) The micronucleus nuclear envelope eventually disassembles during a subsequent mitosis, releasing the rearranged chromosome. (**d**,ii) The rearranged chromosome is segregated on the mitotic spindle and reincorporated into the major nucleus of the cell.

they form a self-contained micronucleus (**Fig. 2**). Notably, newly formed micronuclei frequently possess an inadequate number of nuclear pores and consequently show defects in the nuclear import of some components in the subsequent interphase[12,18].

Reduced nuclear import has several consequences for the chromatin sequestered inside micronuclei. First, micronuclei show defective DNA damage response signaling, resulting in defective and/or delayed repair of induced DNA damage[12,19,20]. Second, DNA replication in micronuclei is delayed compared with that in the major nucleus, with some micronuclei still replicating DNA when the major nucleus is in G2 phase (**Fig. 2a**)[12]. Third, entry into mitosis while the micronucleus is undergoing DNA replication produces massive DNA double-strand breaks in the micronuclear DNA[12].

Pulverizing chromosomes within a micronucleus

The most plausible mechanism for the observed chromosomal pulverization that characterizes chromothripsis is entry into mitosis before the completion of DNA replication within a micronucleus, resulting in breaks in the incompletely replicated micronuclear DNA during premature chromosome condensation (PCC). PCC was originally described in classic cell fusion experiments and occurs when cyclin-dependent kinase activity in a mitotic cell induces incompletely replicated chromosomes in S-phase nuclei to undergo chromosome condensation and shattering[21–23]. PCC of an incompletely replicated micronucleus is expected to create catastrophic damage of the DNA trapped inside (**Fig. 2b**)[24].

Although it has been widely assumed that the micronuclear envelope will dissemble in mitosis, allowing the chromosome(s) contained within to spill into the cytoplasm of the mitotic cell, this is not what happens for many micronuclei. Indeed, disassembly of the micronuclear envelope frequently fails at the onset of the subsequent mitosis, with the intact micronucleus randomly segregating at mitotic exit into one of the daughter cells[12]. Persistence of a micronucleus into interphase of the second cell cycle after its initial formation provides a plausible mechanism for isolating the chromosomal fragments generated as a result of PCC so that they may subsequently be repaired by ligation (in a random order) (**Fig. 2b**). For the subset of micronuclei for which the nuclear membrane does disassemble, the fragments of pulverized chromosomes (nearly all of which lack centromeres and microtubule attachment sites) will be unable to be segregated and may be lost or may form *de novo* micronuclei at mitotic exit.

How a micronucleus escapes nuclear envelope disassembly during mitosis is unresolved, but the reduced density of nuclear pores may result in reduced incorporation of several key envelope constituents

that are phosphorylated by mitotic cyclin-dependent kinases to promote nuclear envelope breakdown. Eventually, however, further cycling of the cell will yield micronuclear envelope disassembly when it enters a subsequent mitosis, releasing the rearranged chromosome into the mitotic cytoplasm and allowing its conventional mitotic segregation with the main mitotic chromosome mass (**Fig. 2d**). The mis-segregation of chromosomes into micronuclei provides a plausible route through which whole chromosome mis-segregation can promote chromosome breaks and subsequent rearrangement, thereby mechanistically coupling events leading to the acquisition of numerical and structural chromosomal alterations. In addition, this pathway also offers an explanation for how the DNA breaks acquired during chromothripsis may be circumscribed to one or a small number of chromosomes—those trapped within a micronucleus[12]. Thus, an initial error in chromosome segregation during mitosis is likely to be one key event in the initiation of chromoanagenesis. As such it will now be of interest to look for evidence of chromothripsis in the tumors formed in mice that have been genetically manipulated to exhibit chromosomal instability and aneuploidy[25].

Alternative proposals for chromosome shattering

It should be noted that at present the mechanisms responsible for chromothripsis remain controversial. In addition to chromosome shattering caused by mitotic entry with incompletely replicated DNA, three additional proposals have been put forward to explain how localized chromosome shattering may result in complex, localized rearrangements. We propose that each of these mechanisms is made more plausible if one imparts the formation of micronuclei as a means to either spatially localize DNA damage or contain the chromosome fragments created by this damage so that they may be religated to produce chromosomal rearrangements.

To explain the confined nature of the DNA damage created during chromothripsis, an initial proposal was that localized double-strand DNA breaks were induced by free radicals or ionizing radiation during mitosis[5], when chromosomes are highly compacted and DNA damage signaling is suppressed[26]. The formation at mitotic exit of a micronucleus containing the damaged chromosome fragments would provide a means to constrain the fragments produced so as to facilitate their religation into a rearranged chromosome characteristic of chromoanagenesis.

Telomere dysfunction has also been proposed as a cause of chromothripsis[5]. Continued proliferation of somatic cells in the absence of telomerase activity leads to the progressive attrition of telomeres. Eventually, telomere-shortening exposes uncapped chromosome ends that are prone to fusion, which creates a dicentric chromosome with two microtubule attachment sites on each sister chromatid. If these sites attach to opposite spindle poles during mitosis, the resulting chromosome will become highly stretched during anaphase. As chromothripsis seems to occur in a single catastrophic cellular event, one possibility is that the bridging chromosome undergoes massive localized genomic damage at the cleavage furrow during cytokinesis[27]. A more attractive explanation, however, is that the lagging dicentric chromosome does not incorporate into the major nucleus of either daughter cell and instead forms a micronucleus. Therefore, telomerase deficiency could promote chromothripsis indirectly, by disrupting chromosome segregation and leading to the production of micronuclei. The examination of telomerase-deficient mouse models for evidence of extensive localized genomic rearrangements will be an important test of whether telomere dysfunction can promote chromothripsis.

Finally, chromothriptic chromosome shattering has been suggested to result from an aborted attempt at apoptosis[28]. Whereas apoptosis has traditionally been considered as an irreversible cascade that, once initiated, irrevocably leads to cell death, recent evidence has clearly shown that initial apoptotic events can be reversed if the initiating stimulus is removed[29]. Reversal of apoptosis has been termed anastasis (Greek for 'rising to life'), and it can occur after measurable DNA damage, allowing cells to acquire permanent genetic changes that facilitate transformation[29]. Anastasis promotes an increase in numerical and structural chromosomal alterations as well as an increase in micronuclei formation[29]. Reversal of apoptosis after the initiation of DNA damage and chromosome fragmentation may lead to the religation of chromosome fragments and the production of chromosomal rearrangements[30,31]. In the tumor microenvironment, apoptosis can be initiated by various stresses, including chemotherapy, ionizing radiation, hypoxia and nutrient deprivation. This raises the possibility that these transient stress stimuli could induce an aborted apoptosis that initiates the DNA damage responsible for chromothripsis. As in the other proposed mechanisms, if anastasis occurred specifically within a micronucleus, DNA damage would be confined to the chromosome(s) trapped inside[32]. It will be of interest to establish whether anastasis can initiate chromothripsis and the development of complex chromosomal rearrangements in cultured cells.

After shattering: weaving together chromosomal fragments

Most of the breakpoints of the reassembled chromosomes created by chromothripsis in human cancers show either a lack of homology or areas of microhomology, pointing toward nonhomologous end joining (NHEJ) as the predominant mechanism that stitches the shattered chromosomes back together after extensive double-strand breaks (**Fig. 1**)[5,33,34]. NHEJ can occur at any point in the cell cycle and often occurs at regions of microhomology that are 1–4 nucleotides in length[35]. NHEJ occurs in a series of steps[35]. First, the Ku protein heterodimer (Ku70/Ku80) is recruited to both ends of the DNA at the site of a double-strand break. Ku recruits a complex of the protein artemis and the DNA-dependent protein kinase catalytic subunit involved in processing the ends of DNA breaks. As a final step, the ligase IV complex (comprising DNA ligase IV and its cofactor XRCC4) is recruited by Ku and ligates the adjacent DNA ends, thereby repairing the double-strand break. Ligation of incorrect ends though NHEJ can lead to chromosomal translocations. The random reassembly by NHEJ of many simultaneously created chromosome fragments can account for the majority of the chromosomal translocations created during chromoanagenesis in cancer cells.

Constitutional rearrangements from a slip-up in DNA replication

In contrast to the chromosome shattering and NHEJ found in cancer cells, some instances of chromoanagenesis contain complex constitutional chromosomal rearrangements carrying a signature with microhomology at the ends of rearranged segments indicative of a DNA replication-based mechanism as the causative agent[8,9] (**Box 1**). These rearrangements are associated with congenital or developmental abnormalities and contain multiple duplications and triplications, neither of which can be readily explained by a mechanism involving the NHEJ-mediated repair of many simultaneously created double-strand breaks.

The most persuasive evidence for rearrangements from DNA replication-based mechanisms, including fork-stalling and template switching (FoSTes)[36] and microhomology-mediated break-induced

BOX 1 Germline chromothripsis contributes to human disease

Complex genomic rearrangements consist of at least two breakpoint junctions and are associated with various congenital or developmental abnormalities. In contrast to rearrangements generated by cancer chromoanagenesis, which arise in differentiated somatic cells, constitutional rearrangements occur in the germ line or very early in embryonic development. Several recent studies have revealed that some people with inherited genetic defects have complex constitutional chromosomal rearrangements that strongly resemble the somatic rearrangements found in cancer chromoanagenesis. Analysis of a family trio identified a complex series of *de novo* chromosomal rearrangements occurring in a child with congenital abnormalities[6]. These rearrangements clustered in small genomic regions on three chromosomes (chromosomes 1, 4 and 10) and bore the hallmarks of chromoanagenesis. An additional study of 17 individuals with developmental and congenital abnormalities revealed four with inherited chromoanagenesis-like rearrangements in a single chromosome (involving chromosomes 1 or 9 in one person each and chromosome 22 in two additional people)[8].

High-resolution analysis of the breakpoints in 52 patients with cytogenetically defined chromosomal abnormalities identified at least two additional cases of constitutional complex genomic rearrangements that share similarities with the rearrangements identified in cancer chromoanagenesis[7]. The genomic rearrangements involved two or three chromosomes (chromosome 5 and X or chromosomes 3, 5 and 7) and showed few losses and gains of DNA segments. The largely dosage-balanced state (in which genes or DNA sequences are present in the correct copy number) observed in these multichromosome constitutional rearrangements is distinct from the more extensive copy number changes frequently observed in cancer chromoanagenesis[5] and from some other individuals with constitutional chromoanagenesis involving only a single chromosome[8]. Dosage alterations may be favored in cancer cells owing to a loss of tumor suppressor genes, whereas the more balanced chromosomal translocations observed in complex genomic rearrangements may reflect a selection for rearrangements that are compatible with organismal viability. Indeed, less-complex rearrangements are expected for heritable disorders, because massive constitutional rearrangements would be expected to be lethal during development.

In an effort to determine the mechanism responsible for the creation of constitutional complex chromosomal rearrangements, a recent study analyzed the breakpoints in ten individuals with congenital abnormalities[9]. The rearrangements consisted of between 3 and 24 inter- and intrachromosomal breakpoints with features similar to those observed in cancer chromoanagenesis. Eight of the individuals had rearrangements that probably arose through the creation of multiple simultaneous double-strand DNA breaks followed by non-homologous repair (as originally proposed for chromothripsis in cancer cells[5]). However, two other individuals had a genetic signature at the junctions of rearrangements that was most consistent with the idea that these arrangements arose through defective DNA replication, which led to serial template switching (chromoanasynthesis)[9] (**Box 2**).

replication (MMBIR)[37], is from sequencing of breakpoint junctions, which reveals areas of microhomologies and templated insertions (54–1542 bps)[8]. MMBIR may occur when a replication fork collapses after encountering a nick in the template strand (**Box 2**). Breakage of a replication fork then promotes microhomology-dependent priming of DNA replication and serial template switching, resulting in complex chromosomal rearrangements surrounding the site of the collapsed fork[8] (**Fig. 3**). Oncogene-induced DNA replication stress can cause cellular senescence and may provide an additional source of collapsed replication forks that trigger MMBIR[38,39]. In FoSTes, rearrangements seem to arise as a result of a stalled replication fork coupled with a consecutive series of long-range replication fork template switches (**Fig. 3**).

Replication-based mechanisms do not necessarily require micronuclei to explain the formation of complex localized chromosomal rearrangements. Indeed, the organization of chromosomes into distinct territories within a cell's nucleus may help bias microhomology-dependent template switching to sequences on a single or small subset of chromosomes that are in close proximity in three-dimensional space. Nevertheless, micronuclei often show defective DNA replication[12] and the partitioning of a chromosome into a micronucleus would provide an elegant explanation for how aberrant DNA replication could be restricted to one or a few spatially isolated chromosomes (**Fig. 2c**).

Although FoSTes and MMBIR offer a plausible route for the generation of complex copy number variations and an explanation for how multiple copy number changes may arise in some germline cases of chromoanagenesis[8,9] (**Boxes 1** and **2**), it should also be noted that inspection of breakpoints in several additional cases of constitutional structural rearrangements bear similarity to what is predicted by local chromosome shattering followed by NHEJ[6,9]. Thus, although the weight of evidence supports a mechanism of massive chromosomal breakage followed by NHEJ to explain the complex genomic rearrangements observed in many cancers[5,33,34], there seem to be at least two distinct mechanisms responsible for the chromoanagenesis-like rearrangements associated with genomic disorders[6,8,9].

Chromoanagenesis in human cancer

The multiple, localized, chromosome rearrangements characteristic of chromoanagenesis occur in many different types of cancers with an overall frequency of ~2–3% (refs. 5,33,34,40–44). The frequency of chromoanagenesis is elevated in specific tumor types, including ~25% of bone cancers[5] and ~18% of late-stage neuroblastomas[44]. Moreover, chromoanagenesis is widespread in primary and metastatic colorectal cancer[34] and there is a striking association between mutations in *TP53* (encoding the p53 protein) and chromoanagenesis in sonic hedgehog–induced medulloblastoma (SHH-MB) and acute myeloid leukemia (AML)[33]. Notably, chromoanagenesis has been associated with poor survival in a variety of tumor types[33,43,44], but further studies will be required to define the incidence and consequence of chromoanagenesis across a larger set of human cancers.

In the vast majority of cases it is probable that chromoanagenesis creates genomic alterations that do not produce any advantages or lead to a substantial reduction in cellular fitness. As a result, the occurrence of chromoanagenesis in cancer is likely to be considerably higher than the ~3% that has been observed, with most cases of chromoanagenesis expected not to provide a selectable advantage and to escape clinical detection. However, in rare circumstances chromoanagenesis may lead to the creation of one or more

BOX 2 Replication-based mechanisms that lead to complex chromosomal rearrangements

The insertion of short sequences at breakpoint junctions that are 'templated' from nearby genomic regions provides evidence of a DNA replication–associated mechanism for chromosomal rearrangements. Such a mechanism (chromoanasynthesis) has been proposed to account for some examples of complex constitutional chromosomal rearrangements[8,9] (**Box 1**). Two related mechanisms have been proposed for these genomic alterations. The first, known as fork-stalling and template switching (FoSTes), occurs when a replication fork stalls at a DNA lesion, allowing the lagging strand of the replication fork to disengage and switch to an area of microhomology on a neighboring replication fork[36]. The two replication forks will be in physical proximity, but they may be separated by large stretches of DNA sequence. DNA synthesis would initiate temporarily at this second site before the nascent strand disengages again and invades an additional replication fork. This process may repeat multiple times, leading to serial template switching before completion of DNA synthesis on the original template.

The second mechanism is known as microhomology-mediated break-induced replication (MMBIR) and is initiated when a replication fork collapses upon encountering a nick in the template strand[37]. This process creates a DNA double-strand break in one arm of the replication fork; however, as there is not an additional DNA end to be used in double-strand break repair, the 5′ end of the broken arm is resected to leave a 3′ single-stranded DNA overhang, which invades a DNA sequence with microhomology to the single stranded 3′ end. The 3′ end primes DNA synthesis and establishes a replication fork. The extended arm eventually separates from the template and the 3′ end re-invades an additional region to repeat the process. Eventually, a switch occurs to the original genomic region and replication continues to the chromosome end.

FoSTes and MMBIR can result in complex genomic rearrangements surrounding the site of the original defective replication fork. Serial template switching can lead to the insertion of specific DNA sequences from distinct genomic regions that lie in close proximity in three-dimensional space and can also explain the increases in copy number (duplications and triplications) observed in some cases of complex constitutional chromosomal rearrangements[8]. For example, duplication can occur when a template switch occurs to a DNA sequence that lies behind (relative to the direction of the fork) the location where the replication fork collapsed[37].

cancer-causing lesions in a single catastrophic event, thereby providing an advantage for cellular growth.

One key route through which chromosome shattering and religation by NHEJ could promote aberrant cellular proliferation is by facilitating oncogene amplification through the creation of small, circular fragments of DNA that lack centromeres and telomeres and frequently harbor oncogenes (**Fig. 4a**). These extrachromosomal fragments are known as double minute chromosomes and are often present at many copies per cell[45]. During chromothripsis it is postulated that individual chromosomes (or portions of them) are initially broken into many pieces and randomly reassembled by NHEJ. Although many of the pieces are stitched back together in random order to produce a highly rearranged chromosome, some fragments may also be joined together to create a circular double minute (**Fig. 4a**), the amplification of which can be selected for if it confers a growth advantage[5,33]. One example of such a rearrangement is exemplified in one small-cell lung cancer cell line that was found to contain a double minute chromosome (carrying the *MYC* oncogene) created by the fusion of several segments of chromosome 8 that were absent from a rearranged copy of chromosome 8 generated by chromoanagenesis[5].

A second potential route by which chromoanagenesis could create cancer-causing mutations is through the loss or disruption of tumor suppressor genes (**Fig. 4b**). For example, in one chordoma, chromosome shattering facilitated the loss of chromosomal fragments that contained, or led to rearrangements that directly disrupted, each of three tumor suppressor genes (F-box and WD repeat-containing 7 *(FBXW7)*, Werner Syndrome, RecQ helicase-like *(WRN)* and cyclin-dependent kinase inhibitor 2A *(CDKN2A))*[5]. In addition, in colorectal cancer the breakpoints generated by chromothripsis have been found to affect several known cancer-causing genes (notch 2, *(NOTCH2)*, exonuclease 1 *(EXO1)* and myeloid/lymphoid or mixed-lineage leukemia 3 *(MLL3))*[34]. The rearrangements created by chromoanagenesis

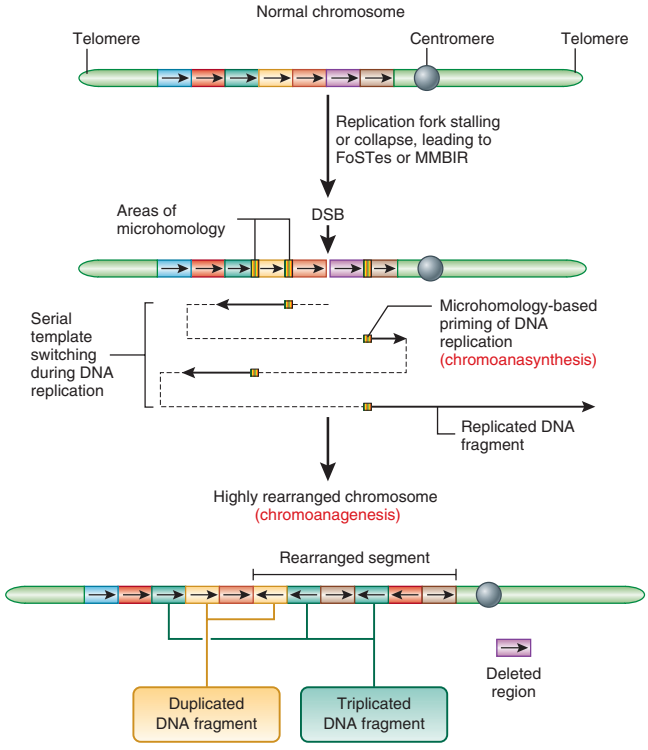

Figure 3 Mechanism for complex chromosomal rearrangements as a result of FoSTes and MMBIR. FoSTes occurs when a replication fork stalls at a DNA lesion, whereas MMBIR is initiated after replication fork collapse. FoSTes and MMBIR lead to microhomology-dependent priming of DNA replication and serial template switching, which can lead to chromoanagenesis. In addition to the deletion and retention of DNA fragments, FoSTes and MMBIR can also lead to duplication and triplication of DNA sequences. Therefore, FoSTes and MMBIR can result in more than two copy number states on the rearranged chromosome. Modified from ref. 53. DSB, double-strand break.

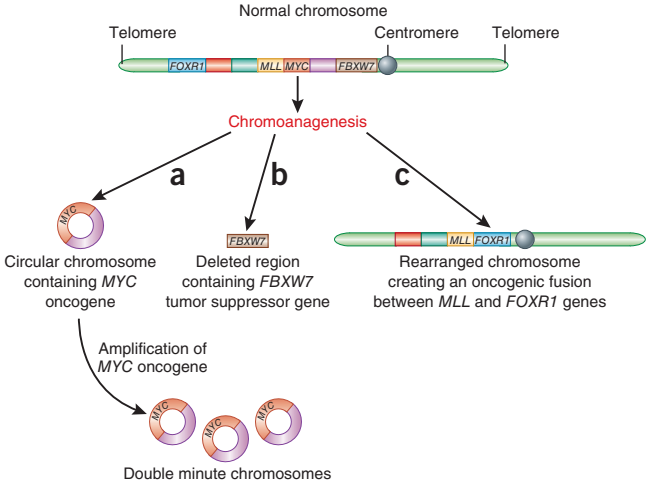

Figure 4 Chromoanagenesis may create oncogenic lesions. The complex chromosomal rearrangements created by chromoanagenesis can be oncogenic. (**a**) Initial chromosomal shattering followed by rejoining by NHEJ may create circular fragments of DNA harboring oncogenes such as *MYC*. Amplification of these extrachromosomal double minute chromosomes can provide a growth advantage. Other pieces of a shattered chromosome may be joined together to create a highly rearranged chromosome. (**b**) Chromoanagenesis can lead to the loss or disruption of regions containing tumor suppressor genes such as *FBXW7*. (**c**) Rearrangements may also create oncogenic fusion genes by joining the coding sequence two normal genes together, for example, the fusion of the *MLL* and the forkhead box R1 (*FOXR1*) genes.

often affect only a single allele of a tumor suppressor gene and the other copy of the gene is retained, implying that the second intact allele may be inactivated epigenetically. Alternatively, the affected gene may act as a haploinsufficient tumor suppressor, as has been shown for the tumor suppressor *FBXW7* (refs. 46,47).

Finally, the chromosome shattering and religation characteristic of chromoanagenesis can generate oncogenic fusion genes by joining the coding portions of two genes in the same orientation (**Fig. 4c**). For example, chromoanagenesis in medulloblastoma tumors leads to recurrent translocations that fuse *PVT1* (a non–protein coding gene) to the *MYC* proto-oncogene, resulting in *MYC* amplification[42].

Chromoanagenesis: an early or late event in human tumors?

Although chromoanagenesis can sculpt the cancer genome, leading to the creation of potentially oncogenic lesions, it is notable that it has yet to be formally shown that the genetic abnormalities that arise as a consequence of chromoanagenesis act, either individually or in combination, to drive tumorigenesis. The handful of studies reported so far have analyzed chromoanagenesis by sampling a single and relatively late stage in tumor development; thus, it remains unsettled at which stage during tumor evolution chromoanagenesis occurs. Chromoanagenesis often occurs after *TP53* mutations in patients with AML or SHH-MB[33], whereas in neuroblastoma chromoanagenesis has been identified in 18% of late-stage cancers but is absent in early stage tumors[44]. These observations argue that chromoanagenesis may be a relatively late event, at least in the development of these types of cancer. In the future it will be important to do longitudinal studies in animal models or human cancer patients to establish whether chromoanagenesis is an early initiating event or a later event that only occurs after additional defects, such as *TP53* mutations, have been acquired.

Stayin' alive

It is remarkable that a cell can survive the catastrophic events of chromoanagenesis that arise either after replication fork collapse in chromoanasynthesis or after tens to hundreds of DNA breaks accompanying chromothripsis. This suggests that that acquired defects in DNA damaging signaling cascades may set the stage for tolerating the massive DNA damage that can trigger chromoanagenesis. Whole-genome sequencing coupled with microarray analysis has uncovered a striking association between chromoanagenesis and both germline and somatic inactivation of the *TP53* tumor suppressor gene[33,41,42]. There is a considerable enrichment for chromoanagenesis in samples from people affected by AML with *TP53* mutations[33]. In one study, chromoanagenesis was observed in all ten of the SHH-MB samples with *TP53* mutations, but was not observed in SHH-MBs with an intact *TP53* gene[33]. An independent study found that chromoanagenesis in Group 3 medulloblastomas (one of the four main medulloblastoma subgroups) is associated with loss of the *TP53* gene[42]. Other medulloblastoma subtypes rarely show chromoanagenesis (including WNT-subtype medulloblastomas harboring mutated *TP53*), indicating that the link between p53 mutation and chromoanagenesis is dependent on the tumor type[33,42].

Germline mutations of *TP53* in people with SHH-MB occur before chromoanagenesis, suggesting that *TP53* mutations may either predispose cells to DNA damage or allow cellular survival after it. Indeed, p53 has an important role in promoting cell cycle arrest, apoptosis or senescence in response to DNA damage[48]. Analysis of early T cell precursor acute lymphoblastic leukemia has also hinted at a link between mutations in genes involved in DNA repair and chromoanagenesis[40]. Two neuroblastoma tumors with evidence of chromoanagenesis were also found to contain mutations in genes functioning in the Fanconi anemia–DNA damage response pathway, indicating that lesions that attenuate DNA damage signaling pathways may have a general role in facilitating the survival of cells that undergo events that initiate chromosome shattering[44].

Clinical implications of chromoanagenesis

It is clear that chromoanagenesis has the capacity to create novel genetic alterations that can potentially drive tumor progression. Indeed, chromoanagenesis has been associated with poor survival in AML[33], neuroblastoma[44] and multiple myeloma[43]. In AML the association between chromoanagenesis and poor survival is independent of patient age and leukemia karyotype classification, raising the possibility that this distinctive genetic alteration may be a useful prognostic marker for predicting disease outcome or therapeutic responsiveness[33]. However, in neuroblastoma, chromoanagenesis has only been observed in late-stage patients (stage 3–4) who have a poorer outcome than those with early-stage tumors[44]. Therefore, the prognostic value of chromoanagenesis will probably depend upon the cancer type and will be sensitive to when during tumor evolution chromoanagenesis occurs as well as to what additional genetic events (such as *TP53* mutations) predispose to chromoanagenesis. In addition to its role in shaping cancer genomes, chromoanagenesis has also been reported to create complex constitutional genomic rearrangements, which probably contribute to congenital or developmental defects (**Box 1**). Clearly, more work will be needed in larger patient cohorts to determine the full clinical implications arising from chromoanagenesis.

Li-Fraumeni syndrome patients with heterozygous germline mutations in *TP53* show an increased incidence of chromoanagenesis in SHH-MB and possibly also in other Li-Fraumeni syndrome–associated malignancies[33]. The use of DNA-damaging

agents and ionizing radiation in cancer therapy may induce chromosome shattering, especially through an initial mis-segregation of a damaged chromosome. Comparing the genomes of primary tumors with those that relapse after therapy will provide important insights into whether chromoanagenesis can be induced by specific therapeutic regimes and whether this may contribute to the emergence of resistance in the primary tumor.

Looking forward

Given the large number of genomic alterations occurring in a single event, chromoanagenesis could allow the rapid development of new phenotypes that facilitate tumor initiation, progression and the evolution of resistance to drug therapy. For a more complete understanding of the role of chromoanagenesis in tumorigenesis, it will now be necessary to establish the point at which chromoanagenesis occurs during the clonal evolution of a tumor. In addition, establishing which types of tumors show the highest incidence of chromoanagenesis will aid in the discovery of additional cooperating genetic alterations that facilitate the initiation of—or response to—chromoanagenesis. Such studies will also need to determine whether chromoanagenesis is more common in tumors with specific DNA damage signaling defects and to establish whether there is tissue-type or tumor-type context specificity in the mechanisms leading to chromoanagenesis.

Along with variation in the genetic makeup of individual tumors, emerging evidence points to the existence of considerable intratumoral genetic heterogeneity[49,50]. Establishing what fraction of cells in a given tumor possess complex chromosomal rearrangements and how this subpopulation evolves over time is now an important next step in understanding subclonal tumor architecture and the context-specific factors that determine tumor development after chromoanagenesis.

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1. Mitelman, F., Johansson, B. & Mertens, F. The impact of translocations and gene fusions on cancer causation. *Nat. Rev. Cancer* **7**, 233–245 (2007).
2. Stephens, P.J. *et al.* Complex landscapes of somatic rearrangement in human breast cancer genomes. *Nature* **462**, 1005–1010 (2009).
3. Campbell, P.J. *et al.* Identification of somatically acquired rearrangements in cancer using genome-wide massively parallel paired-end sequencing. *Nat. Genet.* **40**, 722–729 (2008).
4. Campbell, P.J. *et al.* The patterns and dynamics of genomic instability in metastatic pancreatic cancer. *Nature* **467**, 1109–1113 (2010).
5. Stephens, P.J. *et al.* Massive genomic rearrangement acquired in a single catastrophic event during cancer development. *Cell* **144**, 27–40 (2011).
6. Kloosterman, W.P. *et al.* Chromothripsis as a mechanism driving complex *de novo* structural rearrangements in the germline. *Hum. Mol. Genet.* **20**, 1916–1924 (2011).
7. Chiang, C. *et al.* Complex reorganization and predominant non-homologous repair following chromosomal breakage in karyotypically balanced germline rearrangements and transgenic integration. *Nat. Genet.* **44**, 390–397 (2012).
8. Liu, P. *et al.* Chromosome catastrophes involve replication mechanisms generating complex genomic rearrangements. *Cell* **146**, 889–903 (2011).
9. Kloosterman, W.P. *et al.* Constitutional chromothripsis rearrangements involve clustered double-stranded DNA breaks and nonhomologous repair mechanisms. *Cell Rep.* **1**, 648–655 (2012).
10. Eldredge, N. & Gould, S.J. Punctuated equilibria: an alternative to phyletic gradualism. *Models in Paleobiol.* **82**, 82–115 (1972).
11. Chen, J.M., Ferec, C. & Cooper, D.N. Transient hypermutability, chromothripsis and replication-based mechanisms in the generation of concurrent clustered mutations. *Mutat. Res.* **750**, 52–59 (2012).
12. Crasta, K. *et al.* DNA breaks and chromosome pulverization from errors in mitosis. *Nature* **482**, 53–58 (2012).
13. Gordon, D.J., Resio, B. & Pellman, D. Causes and consequences of aneuploidy in cancer. *Nat. Rev. Genet.* **13**, 189–203 (2012).
14. Holland, A.J. & Cleveland, D.W. Losing balance: the origin and impact of aneuploidy in cancer. *EMBO Rep.* **13**, 501–514 (2012).
15. Lengauer, C., Kinzler, K.W. & Vogelstein, B. Genetic instability in colorectal cancers. *Nature* **386**, 623–627 (1997).
16. Thompson, S.L. & Compton, D.A. Examining the link between chromosomal instability and aneuploidy in human cells. *J. Cell Biol.* **180**, 665–672 (2008).
17. Gascoigne, K.E. & Taylor, S.S. Cancer cells display profound intra- and interline variation following prolonged exposure to antimitotic drugs. *Cancer Cell* **14**, 111–122 (2008).
18. Hoffelder, D.R. *et al.* Resolution of anaphase bridges in cancer cells. *Chromosoma* **112**, 389–397 (2004).
19. Terradas, M., Martin, M., Hernandez, L., Tusell, L. & Genesca, A. Nuclear envelope defects impede a proper response to micronuclear DNA lesions. *Mutat. Res.* **729**, 35–40 (2012).
20. Terradas, M., Martin, M., Tusell, L. & Genesca, A. DNA lesions sequestered in micronuclei induce a local defective-damage response. *DNA Repair (Amst.)* **8**, 1225–1234 (2009).
21. Rao, P.N. & Johnson, R.T. Mammalian cell fusion: studies on the regulation of DNA synthesis and mitosis. *Nature* **225**, 159–164 (1970).
22. Johnson, R.T. & Rao, P.N. Mammalian cell fusion: induction of premature chromosome condensation in interphase nuclei. *Nature* **226**, 717–722 (1970).
23. Sperling, K. & Rao, P.N. The phenomenon of premature chromosome condensation: its relevance to basic and applied research. *Humangenetik* **23**, 235–258 (1974).
24. Meyerson, M. & Pellman, D. Cancer genomes evolve by pulverizing single chromosomes. *Cell* **144**, 9–10 (2011).
25. Holland, A.J. & Cleveland, D.W. Boveri revisited: chromosomal instability, aneuploidy and tumorigenesis. *Nat. Rev. Mol. Cell Biol.* **10**, 478–487 (2009).
26. Zhang, W., Peng, G., Lin, S.Y. & Zhang, P. DNA damage response is suppressed by the high cyclin-dependent kinase 1 activity in mitotic mammalian cells. *J. Biol. Chem.* **286**, 35899–35905 (2011).
27. Janssen, A., van der Burg, M., Szuhai, K., Kops, G.J. & Medema, R.H. Chromosome segregation errors as a cause of DNA damage and structural chromosome aberrations. *Science* **333**, 1895–1898 (2011).
28. Tubio, J.M. & Estivill, X. Cancer: When catastrophe strikes a cell. *Nature* **470**, 476–477 (2011).
29. Tang, H.L. *et al.* Cell survival, DNA damage and oncogenic transformation following a transient and reversible apoptotic response. *Mol. Biol. Cell* **23**, 2240–2252 (2012).
30. Stanulla, M., Wang, J., Chervinsky, D.S., Thandla, S. & Aplan, P.D. DNA cleavage within the MLL breakpoint cluster region is a specific event which occurs as part of higher-order chromatin fragmentation during the initial stages of apoptosis. *Mol. Cell. Biol.* **17**, 4070–4079 (1997).
31. Stanulla, M., Chhalliyil, P., Wang, J., Jani-Sait, S.N. & Aplan, P.D. Mechanisms of MLL gene rearrangement: site-specific DNA cleavage within the breakpoint cluster region is independent of chromosomal context. *Hum. Mol. Genet.* **10**, 2481–2491 (2001).
32. Terradas, M., Martin, M., Tusell, L. & Genesca, A. Genetic activities in micronuclei: is the DNA entrapped in micronuclei lost for the cell? *Mutat. Res.* **705**, 60–67 (2010).
33. Rausch, T. *et al.* Genome sequencing of pediatric medulloblastoma links catastrophic DNA rearrangements with *TP53* mutations. *Cell* **148**, 59–71 (2012).
34. Kloosterman, W.P. *et al.* Chromothripsis is a common mechanism driving genomic rearrangements in primary and metastatic colorectal cancer. *Genome Biol.* **12**, R103 (2011).
35. Lieber, M.R. The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. *Annu. Rev. Biochem.* **79**, 181–211 (2010).
36. Lee, J.A., Carvalho, C.M. & Lupski, J.R.A. DNA replication mechanism for generating nonrecurrent rearrangements associated with genomic disorders. *Cell* **131**, 1235–1247 (2007).
37. Hastings, P.J., Ira, G. & Lupski, J.R. A microhomology-mediated break-induced replication model for the origin of human copy number variation. *PLoS Genet.* **5**, e1000327 (2009).
38. Bartkova, J. *et al.* Oncogene-induced senescence is part of the tumorigenesis barrier imposed by DNA damage checkpoints. *Nature* **444**, 633–637 (2006).
39. Di Micco, R. *et al.* Oncogene-induced senescence is a DNA damage response triggered by DNA hyper-replication. *Nature* **444**, 638–642 (2006).
40. Zhang, J. *et al.* The genetic basis of early T-cell precursor acute lymphoblastic leukaemia. *Nature* **481**, 157–163 (2012).
41. Wu, C. *et al.* Poly-gene fusion transcripts and chromothripsis in prostate cancer. *Genes Chromosom. Cancer* doi:10.1002/gcc.21999 (2012).

42. Northcott, P.A. *et al.* Subgroup-specific structural variation across 1,000 medulloblastoma genomes. *Nature* **488**, 49–56 (2012).
43. Magrangeas, F., Avet-Loiseau, H., Munshi, N.C. & Minvielle, S. Chromothripsis identifies a rare and aggressive entity among newly diagnosed multiple myeloma patients. *Blood* **118**, 675–678 (2011).
44. Molenaar, J.J. *et al.* Sequencing of neuroblastoma identifies chromothripsis and defects in neuritogenesis genes. *Nature* **483**, 589–593 (2012).
45. Wahl, G.M. The importance of circular DNA in mammalian gene amplification. *Cancer Res.* **49**, 1333–1340 (1989).
46. Mao, J.H. *et al. Fbxw7/Cdc4* is a p53-dependent, haploinsufficient tumour suppressor gene. *Nature* **432**, 775–779 (2004).
47. Kemp, Z. *et al. CDC4* mutations occur in a subset of colorectal cancers but are not predicted to cause loss of function and are not associated with chromosomal instability. *Cancer Res.* **65**, 11361–11366 (2005).
48. Kastan, M.B. & Bartek, J. Cell-cycle checkpoints and cancer. *Nature* **432**, 316–323 (2004).
49. Gerlinger, M. *et al.* Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *N. Engl. J. Med.* **366**, 883–892 (2012).
50. Yap, T.A., Gerlinger, M., Futreal, P.A., Pusztai, L. & Swanton, C. Intratumor heterogeneity: seeing the wood for the trees. *Sci. Transl. Med.* **4**, 127ps110 (2012).
51. Ganem, N.J., Godinho, S.A. & Pellman, D. A mechanism linking extra centrosomes to chromosomal instability. *Nature* **460**, 278–282 (2009).
52. Silkworth, W.T., Nardi, I.K., Scholl, L.M. & Cimini, D. Multipolar spindle pole coalescence is a major source of kinetochore mis-attachment and chromosome mis-segregation in cancer cells. *PLoS ONE* **4**, e6564 (2009).
53. Maher, C.A. & Wilson, R.K. Chromothripsis and human disease: piecing together the shattering process. *Cell* **148**, 29–32 (2012).