
The Diverse Effects of Complex Chromosome Rearrangements and Chromothripsis in Cancer Development

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Abstract

In recent years, enormous progress has been made with respect to the identification of somatic mutations that contribute to cancer development. Mutation types range from small substitutions to large structural genomic rearrangements, including complex reshuffling of the genome. Sets of mutations in individual cancer genomes may show specific signatures, which can be provoked by both exogenous and endogenous forces. One of the most remarkable mutation patterns observed in human cancers involve massive rearrangement of just a few chromosomal regions. This phenomenon has been termed chromothripsis and appears widespread in a multitude of cancer types. Chromothripsis provides a way for cancer to rapidly evolve through a one-off massive change in genome structure as opposed to a gradual process of mutation and selection. This chapter focuses on the origin, prevalence and impact of chromothripsis and related complex genomic rearrangements during cancer development.

Keywords

Chromothripsis · Chromosomal rearrangement · Next-generation paired-end sequencing · Mutation signatures

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1 Signatures of Genetic Changes in Cancer Genomes

Genetic changes in cancer genomes can arise from a variety of mutational mechanisms that are enforcing specific mutation signatures (Alexandrov et al. 2013). Systematic evaluation of mutation signatures from large sets of cancer genomes has uncovered more than 20 distinct profiles, associated with age of cancer diagnosis, strand asymmetry in transcribed regions, defective homologous recombination and drug treatment (Alexandrov et al. 2013).

A very striking mutation pattern observed in a large array of cancer genomes involves local hypermutation characterized by $C \rightarrow T$ and $C \rightarrow G$ mutations at TpC sites (Alexandrov et al. 2013; Nik-Zainal et al. 2012). This phenomenon has been termed *kataegis*, which is Greek for thunderstorm. Kataegis occurs at sites of structural genomic rearrangements and is mediated through deamination of cytosine by APOBEC deaminases (Taylor et al. 2013). On the level of structural genomic rearrangements, a remarkably complex pattern of changes has been described, which involves reshuffling of tens to hundreds of genomic segments leading to massively rearranged chromosomes (Stephens et al. 2011). This phenomenon has been termed chromothripsis, Greek for chromosome (chromo) shattering (thripsis). Chromothripsis has now been found in many different cancer types and appears a major mutational force driving tumor development (Kloosterman et al. 2014). Here, we will discuss our current understanding of chromothripsis, its origin and prevalence in cancer, functional consequences and relation to other types of complex genome rearrangements identified in cancer genomes.

2 The Landscape of Structural Genomic Rearrangements in Cancer

Many cancer genomes harbor structural changes, ranging from small insertions/deletions (indels) to duplications, inversions, translocations and whole chromosome aneuploidies. The number of acquired structural changes in cancer genomes is typically smaller than for substitutions (Vogelstein et al. 2013). For

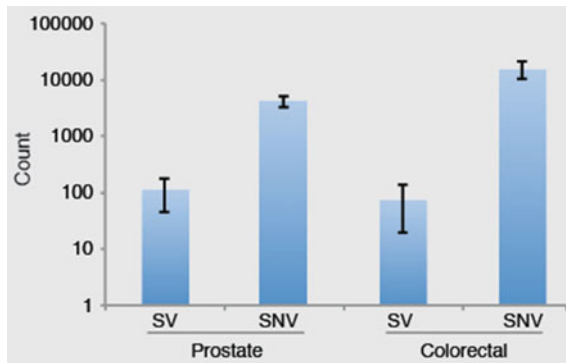


Fig. 1 Numbers of somatic structural variants (SV) and single nucleotide variants (SNVs) identified in prostate (Berger et al. 2011) and colorectal cancer (Bass et al. 2011)

example, prostate and colorectal tumors harbor 37 and 204-fold more single nucleotide changes than structural variants, respectively (Fig. 1).

Insight into structural genomic rearrangements in cancer has first been obtained through the identification of cytogenetically visible changes, such as whole-arm translocations. The Philadelphia translocation (t(9;22)) was the first structural variation observed in cancer (Rowley 1973). This translocation occurs in 95 % of chronic myelogenous leukemia (CML) cases and results in the formation of an oncogenic fusion gene between *BCR* and the tyrosine kinase gene *ABL1* (Shtivelman et al. 1985). These initial findings have triggered subsequent investigation of cytogenetic aberrations in cancer cells, culminating into the identification of hundreds of cancer gene fusions (Mitelman et al. 2007).

The emergence of array-based techniques such as comparative genomic hybridization (aCGH) and SNP-arrays (Box 1) has provided further crucial insight into the impact of chromosomal rearrangements in cancer genomes (Beroukhim et al. 2007). Pan-cancer analyses of thousands of datasets have uncovered genomic regions and genes that show significant change in copy number across different cancer types (Beroukhim et al. 2010; Zack et al. 2013), leading to the identification of novel cancer genes.

Patterns of structural rearrangements can be substantially refined by the use of next-generation sequencing technology. Paired-end sequencing strategies have been instrumental to detect both copy number changes and balanced rearrangements in the human genome simultaneously (Box 1) (Korbel et al. 2007). Application of this methodology to cancer genomes has led to the classification of somatic rearrangements across all size ranges and types at unprecedented resolution (Campbell et al. 2008) (Fig. 2). Sequencing data have shown that cancer genomes contain more somatic rearrangements than previously anticipated (Stephens et al. 2009). Also, rearrangement types may vary for different cancers, and even cancer subtypes. This is illustrated by the predominance of tandem duplications among the structural landscape of subsets of breast and ovarian cancers, whereas other subsets

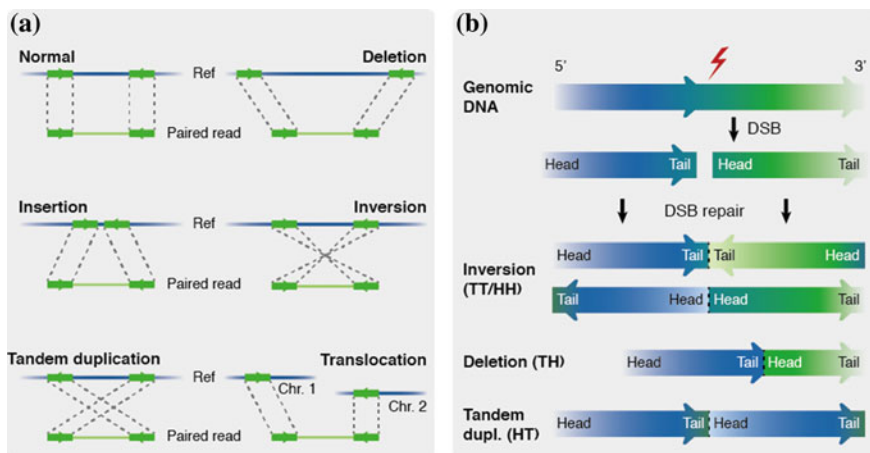


Fig. 2 The detection of genomic rearrangements from paired-sequencing data. **a** Individual reads are aligned to a reference genome and discordant reads indicate the presence of rearrangements (Box 1). Different SV types lead to different types of discordance; deletions show a larger distance between aligned reads compared to the original DNA fragment length, whereas insertions will be characterized by a smaller distance between aligned reads. In the case of an inversion and tandem duplication the order of the reads in the pair is swapped, for inversions the reads will map to the opposite DNA strand. In case of an inter-chromosomal translocation the reads of a read pair will map to different chromosomes. **b** Intrachromosomal rearrangements lead to a change in orientation of breakpoint junctions (Box 1). The orientation is indicated by using head (H), which indicates the 5' end of a fragment (i.e. the lowest coordinate), and tail (T), indicating the 3' end of a fragment (i.e. the highest coordinate). Different rearrangement types lead to different orientation conformations. The first letter corresponds to the fragment with the lowest coordinate and the second to the fragment with the highest coordinate. TH orientation of the breakpoint junction indicates the presence of a deletion, TT and HH indicate the presence of an inversion. HT (not TH) orientation indicates a tandem duplication, due to the fact that the first letter corresponds to the read with the lowest coordinate, which in the case of a tandem duplication is the second read in the pair

are mostly characterized by deletions (McBride et al. 2012). In addition, certain tumor types show a markedly increased number of structural changes (e.g., breast cancer and lung squamous cell cancer) when compared to others (kidney cancer) (Yang et al. 2013). Cancer genomes harbor an overrepresentation of complex changes, interchromosomal translocations and tandem duplications when compared to germline rearrangements (McBride et al. 2012; Campbell et al. 2010; Hillmer et al. 2011). Also, chromosomal rearrangements can arise throughout cancer development and metastasis, leading to the detection of both shared and lesion-specific rearrangements in several cancers (Campbell et al. 2010; Kloosterman et al. 2011a; Hoogstraat et al. 2014; Ding et al. 2010).

3 A Historical View on Complex Genome Rearrangements and Cancer Development

The complexity of some chromosomal aberrations in cancer was noted well before the introduction of high-resolution copy number and sequencing technologies. For example, the vast majority of leukemia's with a Philadelphia chromosome contain a simple translocation between chromosomes 9 and 22, but very complex variants involving up to five chromosomes have also been observed (Adhvaryu et al. 1988; Kadam et al. 1990; Rosson and Reddy 1988). In addition, BCR-ABL positive cases of leukemia without a karyotypically visible rearrangement have been described (Fitzgerald and Morris 1991). Careful analysis of the *BCR* and *ABL* loci in these cases has revealed complex exchanges of chromosome segments and it was suggested that this was not a result of serial changes, but rather a single concerted event, reminiscent of chromothripsis (Fitzgerald and Morris 1991). Furthermore, cytogenetic analysis already revealed that amplification of *MYCN* in neuroblastoma is a process involving complex genomic rearrangements (Nishi et al. 1992). Those studies suggested that the complex and heterogeneous rearrangements of *MYCN* in amplicons of neuroblastoma cell lines have preceded the amplification process. The amplification of oncogenes may involve the separate propagation of chromosome fragments by formation of episomes (double-minute chromosomes). Alternatively, oncogene-containing chromosome segments can form an array within a chromosome and appear as homogeneously staining regions (HSR) (Cowell 1982; Shimizu 2009). HSRs are thought to arise via breakage-fusion bridge (BFB) cycles, a process of chromosome fusion following telomere attrition (Shimizu et al. 2005; Cowell and Miller 1983). Sequencing of amplified regions in cancer genomes revealed a wide variety of configurations, including evidence for BFB cycles and integration of double minutes into the genome (Bignell et al. 2007).

Further evidence for the importance of complex genomic aberrations for cancer development was gained through cytogenetic studies that revealed recurrently affected regions, such as the identification of complex rearrangements on 12q13 in leiomyoma (Nilbert et al. 1989). The genes in this region are now known to be important drivers for this cancer type and the underlying complex rearrangements are frequently caused by chromothripsis. Osteosarcomas have a remarkably high frequency of complex chromosomal rearrangements, many of which are recurrent (Bayani et al. 2003). Notably, osteosarcomas form a cancer type with a very high frequency of chromothripsis (Stephens et al. 2011).

Altogether, early cytogenetic studies have shown the involvement of complex genomic rearrangements in cancer gene amplification, formation of cancer fusion genes and recurrent changes on specific chromosomal regions. It is very likely that at least some of these complex rearrangements resulted from chromothripsis. The precise structure of complex rearrangements, their differences and impact on cancer genes has only become apparent based on genome sequencing analysis (Box 1). This has led to the discovery of chromothripsis as a distinct and well-discernable entity among complex chromosome rearrangements.

4 Hallmarks of Chromothripsis in Cancer Genomes

A key feature of chromothripsis is the geographic localization of rearrangements within the genome and profound clustering of breakpoints on one or multiple parts of one or a few chromosomes. The highly localized character of chromothripsis is further emphasized by the fact that all rearrangements involve a single parental chromosome (Stephens et al. 2011; Korbelt and Campbell 2013). A second hallmark is the presence of frequent oscillations between two copy number states due to the loss of fragments to the cell, with loss-of-heterozygosity (LOH) in the lower copy number (deleted) regions, but retention of heterozygosity in regions with the

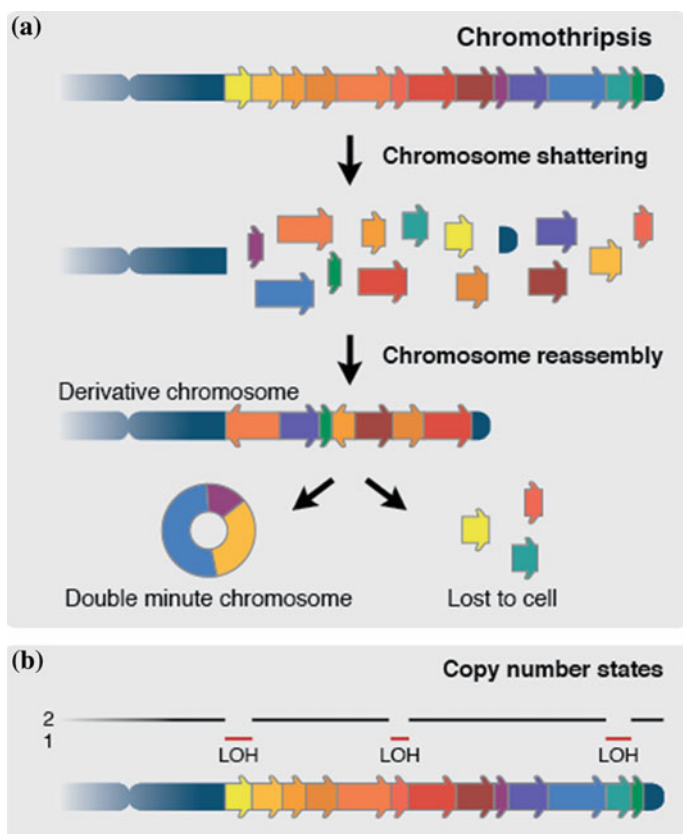


Fig. 3 Chromosome shattering and reassembly by chromothripsis. **a** One or multiple parts of one or a few chromosomes are shattered into pieces in a one-off catastrophic event. Chromosomes are subsequently stitched back together in a random order by NHEJ, with some fragments being incorporated into a double-minute chromosome and others getting lost to the cell. **b** Chromothripsis typically leads to frequent oscillations between two copy number states in which the lower copy state shows loss of heterozygosity (LOH)

higher copy number (Stephens et al. 2011). Alternatively, three or more copy-number states can be observed. Chromosome fragments can be incorporated into a double-minute chromosome (Fig. 3), which can subsequently become amplified if it contains an oncogene (Stephens et al. 2011; Rausch et al. 2012). Additionally, partial chromosome duplication preceding or following chromothripsis may occur (Zhang et al. 2013). In those instances, three or more copy number states can be observed: one for lost fragments, one for retained fragments and one or more, high copy-number states for duplicated fragments and fragments of amplified double-minute chromosomes (Stephens et al. 2011; Rausch et al. 2012). As a third hallmark of chromothripsis, regions with the lower copy number are not caused by simple tail-to-head deletions, but by a series of complex breakpoint junctions that span the involved chromosomal regions. Fourth, all four possible intrachromosomal breakpoint orientations (Fig. 2b) are represented in approximately equal numbers. Finally, the overall chromosome configuration resulting from chromothripsis involves joined fragments that are often not located in the proximity of each other in the reference genome (Stephens et al. 2011).

These hallmarks of chromothripsis have led to a model, whereby one or multiple parts of one or more chromosomes are shattered into pieces and randomly stitched back together, with some parts getting lost to the cell, and others incorporated into a double-minute chromosome (Fig. 3). This results in massively rearranged chromosomes involving tens to hundreds of rearrangements. A lower limit for the number of breaks constituting a chromothripsis event is not defined. It could be that complex rearrangements constituted by just a few or many local breakpoints are both caused by the same molecular trigger (Kloosterman and Cuppen 2013).

Chromothripsis is not restricted to cancer, but also occurs in the germline, leading to severe congenital phenotypes (Kloosterman et al. 2011b, 2012; Chiang et al 2012). The nature of chromosome breakage and reassembly appears similar for cancer and germline, although germline chromothripsis rearrangements have substantially less breakpoints and display a more balanced state (Kloosterman et al. 2011b, 2012; Chiang et al 2012). Both differences are likely due to strong selective pressure against catastrophic DNA damage during embryonic development. Alternatively, they could reflect differences in chromothripsis mechanisms (Zhang et al. 2013; Kloosterman and Cuppen 2013).

5 The Mechanism Behind Chromothripsis

Although chromothripsis has been found in many different cancer types and appears a major mutational force driving tumor development, the precise origin of chromothripsis is only beginning to be understood. The vast majority of chromothripsis breakpoint junctions are characterized by microhomology of 2–4 nt (Stephens et al. 2011; Rausch et al. 2012; Morrison et al. 2014). This is consistent with repair of the DNA fragments by nonhomologous end-joining (NHEJ) (Moore and Haber 1996).

Several hypotheses and experimental data have shed light on possible causes of chromothripsis (Stephens et al. 2011; Zhang et al. 2013; Kloosterman et al. 2012; Holland and Cleveland 2012; Forment et al. 2012; Maher and Wilson 2012). The most compelling explanation concerns the formation of micronuclei that capture (part of) one or multiple chromosomes following chromosome segregation errors (Crasta et al. 2012; Hatch et al. 2013). This model for chromothripsis formation provides an elegant explanation for the clustering of rearrangements to one or a few chromosomes, because these are physically separated during DNA damage formation and repair, but can rejoin the other chromosomes in the nucleus during subsequent cell divisions (Crasta et al. 2012; Hatch et al. 2013). It has been shown that repair of DNA damage is defective and/or delayed in micronuclei due to defects in DNA damage response signaling (Crasta et al. 2012; Terradas et al. 2009). Chromosomes in micronuclei showed γ -H2AX foci, but lacked efficient recruitment of its downstream components of the DNA damage response, leading to extended persistence of γ -H2AX foci in the micronuclei (Crasta et al. 2012). Furthermore, DNA replication in micronuclei is asynchronous with the primary nucleus as many micronuclei show DNA replication in G2-phase (Crasta et al. 2012). Premature chromosome compaction (PCC) is a well-known mechanism that can lead to pulverization of chromosomes (Johnson and Rao 1970; Sperling and Rao 1974). If micronuclei enter mitosis before completion of DNA replication PCC can occur, leading to the occurrence of massive amounts of DNA double-stranded breaks (DSBs) (Crasta et al. 2012; Donley and Thayer 2013). This damage occurs in the first cell cycle following micronuclei formation (Crasta et al. 2012). Indeed, chromosome paintings of micronucleated cells demonstrated small fragments from one or two chromosomes (Crasta et al. 2012). A study by Hatch et al. (2013) uncovered a possible explanation for DNA damage to chromosomes contained in micronuclei. The authors showed a strong correlation between micronuclear envelope breakdown and the occurrence of massive DNA damage, although it remains unknown how nuclear envelope breakdown exactly leads to this damage (Hatch et al. 2013). Recent work has now demonstrated that chromosomes captured in micronuclei can undergo massive genomic rearrangements, including all the known hallmarks of chromothripsis as observed in cancer genomes (Zhang et al. 2015), thus providing experimental proof for a mechanism causing chromothripsis.

Although the micronucleus model has now been tested experimentally, other possible causes of chromothripsis may exist, including the influence of exogenous sources of DNA damage during mitosis. It has been suggested that free radicals and ionizing radiation can trigger chromothripsis rearrangements during mitosis when the chromosomes are highly condensed and DNA damage signaling is suppressed (Stephens et al. 2011; Zhang et al. 2011, 2013; Kloosterman and Cuppen 2013; Holland and Cleveland 2012; Maher and Wilson 2012). External damage occurring during this state of the cell cycle could possibly slice through multiple, closely located, segments of one or a few chromosomes, thereby explaining the localized character of chromothripsis (Stephens et al. 2011; Zhang et al. 2013).

Finally, it has been suggested that the occurrence of repeated BFB-cycles associated with telomere attrition could lead to chromothripsis rearrangements (Stephens et al. 2011; Sorzano et al. 2013). Telomere attrition leads to dicentric chromosomes, which form an anaphase bridge when the centromeres are pulled to opposite daughter cells during anaphase. The dicentric chromosome subsequently breaks at a random location between the two centromeres and fragments of this break are inherited by the daughter cells (Sorzano et al. 2013). It has been suggested that the dicentric chromosome may acquire the massive DNA damage seen in chromothripsis at the cleavage furrow during cytokinesis in a one-off event (Holland and Cleveland 2012) or that successive BFB-cycles, over the course of multiple cell divisions, are responsible for chromothripsis rearrangements (Sorzano et al. 2013).

Repeated BFB-cycles can explain the clustering of rearrangements near telomeres. However, BFB-cycles characteristically lead to multiple amplified copy number states, which is inconsistent with the two copy number states typically detected in chromothripsis. Furthermore BFB-cycles lead to an enrichment of inverted-type rearrangements, while rearrangement orientations in chromothripsis are randomly distributed (Bignell et al. 2007; Korb and Campbell 2013; Zhang et al. 2013). A more plausible suggestion is the incorporation of lagging dicentric chromosomes into a micronucleus, rather than the nucleus of one of the daughter cells. In this way, telomere attrition may facilitate the occurrence of chromothripsis by inducing micronuclei formation (Holland and Cleveland 2012; Li et al. 2014). In acute lymphoblastic leukemia (ALL) the occurrence of dicentric chromosomes due to BFB-cycles or a germline Robertsonian translocation preceded chromothripsis, possibly through micronuclei formation after the formation of an anaphase bridge by the lagging chromosome (Li et al. 2014).

6 Other Types of Complex Genomic Rearrangements in Cancer Genomes

Prostate cancers exhibit patterns of complex rearrangements related to chromothripsis, characterized by chains of translocations and deletions, that occur in a highly interdependent manner (Fig. 4a). This phenomenon has been termed chromoplexy (from the Greek *pleko*, which means to weave or braid) (Baca et al. 2013). Chromoplexy chains typically contain three to over 40 rearrangements, involving one to up to ten chromosomes. A key characteristic is the occurrence of ‘deletion bridges’, small deletions between fusion junctions of translocation rearrangements in a subset of rearrangements, although chromoplexy in general seems to be a relatively copy-neutral process, when compared to chromothripsis (Zhang et al. 2013; Baca et al. 2013). Chromoplexy has been detected in 88 % of prostate tumors and frequently accounts for the dysregulation of prostate cancer genes, often disrupting multiple genes coordinately. Strikingly, two or more separate chromoplexy chains were detected in 63 % of prostate tumors (Baca et al. 2013). Where chromothripsis typically leads to a very high number of rearrangements in one event, multiple chromoplexy events can occur in successive cell cycles (Fig. 4b) (Baca et al. 2013).

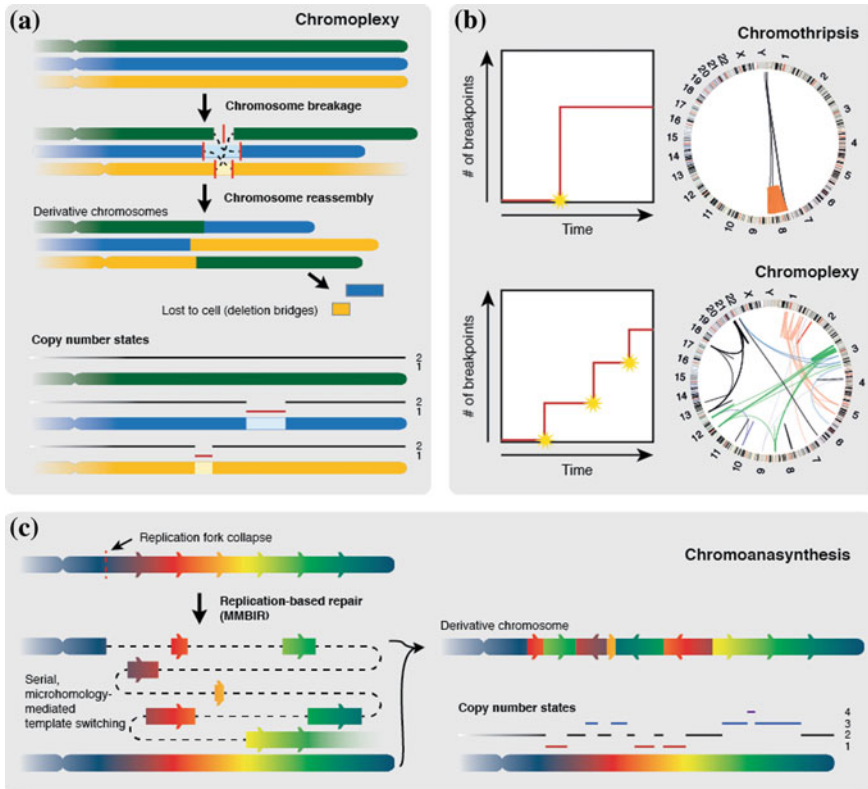


Fig. 4 Other mechanisms for complex rearrangements. **a** Chromoplexy. Multiple chromosomes undergo one or multiple DSBs (*solid lines* indicate breaks). Chained translocations are formed when chromosomes are reassembled with deletion bridges spanning translocations in some cases. In general, chromoplexy leads to fewer copy number alterations when compared to chromothripsis. **b** Differences between chromothripsis and chromoplexy. Chromothripsis generates a large number of breakpoints in a one-off catastrophic event. Chromoplexy leads to fewer breakpoints per event, but multiple chromoplexy chains can be formed in one cell in subsequent cell cycles. Due to this, chromothripsis usually leads to extreme clustering of breakpoints, whereas chromoplexy breakpoint clustering is less extreme. **c** Chromoanasythesis is a replication-dependent process. Replication fork collapse leads to serial microhomology-mediated template switching leading to the formation of a derivative chromosome showing inversions and deletions, but also duplications and triplications of genomic regions

Chromoplexy rearrangements are usually not as highly localized as chromothripsis, as chromothripsis typically involves only one or a few chromosomes, whereas chromoplexy can involve up to ten chromosomes (Stephens et al. 2011; Baca et al. 2013). Furthermore, the total number of rearrangements in chromoplexy is smaller than for chromothripsis. Also, the relatively high number of (large) deletions seen in chromothripsis is not found in chromoplexy (Baca et al. 2013). Thus, chromoplexy could be regarded as a phenomenon involving rearrangements of intermediate complexity. In ETS + prostate cancer, well-known ERG fusions (e.g. TMPRSS2-

ERG) frequently result from chromoplexy (58 %) (Baca et al. 2013). This clearly emphasized the oncogenic capability of chromoplexy in prostate tumors.

Like chromothripsis, each chromoplexy chain is suggested to form during a one-off catastrophic event, which is supported by the fact that both ends of each break are involved in chained translocations. A mechanism for the occurrence of this phenomenon remains to be established, but chromoplexy in ETS + tumors involves highly transcribed regions, which are colocalized in interphase nuclei, suggesting a mechanistic coupling of chromosome rearrangement and transcription (Baca et al. 2013).

An alternative category of complex genomic rearrangements was described in patients with developmental delay and cognitive anomalies (Liu et al. 2011). Chromoanasythesis (anasythesis for reconstruction) is characterized by interspersed changes in copy number, including duplications, triplications and deletions, combined with translocations and inversions (Fig. 4c). Like chromothripsis, chromoanasythesis rearrangements are highly localized, usually involving only a single chromosome. Breakpoint junctions of chromoanasythesis rearrangements frequently show microhomology or templated sequence insertions (54–1542 bp) (Liu et al. 2011). Despite the similarities between chromothripsis and chromoanasythesis there are marked differences concerning rearrangement clustering and copy number changes (Zhang et al. 2013; Kloosterman and Cuppen 2013; Holland and Cleveland 2012). Chromoanasythesis is characterized by multiple copy number states on a single chromosome due to a combination of deletions, duplications and triplications, as opposed to chromothripsis, where deletions are frequently observed, but typically no duplications or triplications are found (Zhang et al. 2013; Liu et al. 2011). Furthermore, detailed analysis of chromoanasythesis breakpoint junctions revealed that these lack the typical double-strand break signatures that are found in chromothripsis (Kloosterman and Cuppen 2013).

The templated insertions and microhomology found at breakpoint junctions in chromoanasythesis rearrangements are a signature of replication-based mechanisms, specifically microhomology-mediated breakage-induced repair (MMBIR) (Liu et al. 2011; Carvalho et al. 2009; Zhang et al. 2009). MMBIR is initiated by a broken DNA replication fork and leads to the incorporation of templated sequences of rearranged segments through serial, microhomology-mediated template switching, explaining the occurrence of multiple copy number states (Fig. 4c) (Liu et al. 2011; Hastings et al. 2009).

7 Methods for Identificaton of Complex Genomic Rearrangements in Cancer Genomes

Despite the major technical advances in the past decades (Box 1), the detection of genomic rearrangements remains complex due to both technical issues related to next-generation DNA sequencing, such as short sequence reads and small library insert size, and biological factors, such as the occurrence of (highly) repetitive regions, overlapping SVs and the complexity of breakpoints. Although different

Table 1 Variation in criteria and methods used for chromothripsis and chromoplexy detection

Study (type)	Criteria	Technique (sample size)	Detection tool
Stephens et al. (2011) (Chromothripsis)	<p>(1) Tens to hundreds of genomic rearrangements that show geographic localization within the genome</p> <p>(2) Pronounced clustering of breakpoints on chromosomes or chromosome arms</p> <p>(3) Rearranged regions show frequent oscillations between two and occasionally three copy number states</p> <p>(4) Regions with copy number 1 are caused by a series of complex rearrangements that span the involved region and not by simple deletions</p> <p>(5) Heterozygosity is retained in regions with higher copy number</p> <p>(6) The complex rearrangements represent all four intrachromosomal breakpoint orientations in approximately equal numbers</p> <p>(7) The two conjoined fragments of chromosomes at each breakpoint fusion are often not located in the proximity of each other on the reference genome</p>	Paired-end sequencing, SNP array (776)	In-house SV/CNA detection tools

(continued)

Table 1 (continued)

Study (type)	Criteria	Technique (sample size)	Detection tool
	(8) Rearrangements involve a single parental copy of the chromosome (9) Double minute chromosome may form		
Magrangeas et al. (2011) (Chromothripsis)	Identical to criteria set by Stephens et al. (2011)	SNP array (764)	Partek Genomic Suite
Rausch et al. (2012) (Chromothripsis)	At least 10 changes in segmental copy number involving two or three copy-number states per chromosome	Paired-end/long-mate-pair sequencing, SNP array (605)	PEMer tool and in-house tools
Kim et al. (2013) (Chromothripsis)	(1) At least 10 CNAs localized to one chromosome (2) Occurrence of breakpoints is random e.g. the size of neighboring segment is similar	aCGH, SNP array (8227)	In-house statistic tool
Malhotra et al. (2013) (Chromothripsis)	Breakpoints are assigned to the same breakpoint cluster if: (1) Breakpoints are located within 100 kb of each other in the reference genome (2) Different loci share breakpoint calls, indicating interconnection between breakpoints Breakpoint clusters are defined as complex and resulting from a one-off event when: (3) No more than three copy number states are detected	Paired-end sequencing, de novo assembly (64)	HYDRA-MULTI and in-house tools

(continued)

Table 1 (continued)

Study (type)	Criteria	Technique (sample size)	Detection tool
	(4) A maximum of one amplified copy number state exceeding four predicted copies is present in the region and this is not a focal amplification of a contiguous amplified region. Chromothripsis: (5) At least 10 or more clustered breakpoints and copy number profiles are present that meet all four criteria described above.		
Zack et al. (2013) (Chromothripsis)	(1) Unexpectedly large number of CNAs on one chromosome compared to the CNA frequency of the sample (2) CNAs on a single chromosome are highly localized, discordant with what is expected by chance (3) CNAs lead to copy number changes of +1 or -1 and are non-overlapping	SNP array (4934)	In-house method, to be published
Cai et al. (2014) (Chromothripsis)	(1) Significant clustering of breakpoints (2) Segmental copy number changes	Paired-end/mate-pair sequencing, whole-genome sequencing, SNP array, aCGH (18,394)	Scan-statistic based algorithm
Govind et al. (2014) (Chromothripsis)	(1) Genome localization: low number of chromosomes involved in	Whole-genome sequencing (21)	Various tools for SV detection, ShatterProof for detection of chromothripsis

(continued)

Table 1 (continued)

Study (type)	Criteria	Technique (sample size)	Detection tool
	<p>complex rearrangements</p> <p>(2) Chromosome localization: highly localized SV clustering on chromosome</p> <p>(3) Low number of copy number states, but high number of copy number oscillations</p> <p>(4) Translocation clustering: high number of localized translocations</p> <p>(5) Insertions at translocation breakpoints: high fraction of translocations where short insertions are found</p> <p>(6) High retained heterozygosity in areas between CNVs</p> <p>(7) Presence of a non-synonymous TP53 mutation (does currently not influence chromothripsis score)</p>		
Baca et al. (2013) (Chromoplexy)	<p>ChainFinder determines whether genomic rearrangements where formed in an interdependent manner and assigns breakpoints that where formed interdependently to the same (rearrangement) chain (e.g. cycle)</p> <p>(1) Two breakpoints are</p>	NGS, paired-end sequencing (57)	dRanger algorithm for detection of SVs, ChainFinder for detection of chromoplexy

(continued)

Table 1 (continued)

Study (type)	Criteria	Technique (sample size)	Detection tool
	<p>assigned to the same chain if the p-value for the independent generation of the breakpoints is rejected with a false-discovery rate $>10^{-2}$.</p> <p>(2) All scenarios are considered for one or more rearrangements in a cycle to occur independently. Only if each scenario is rejected with a family-wise error rate $<10^{-2}$, all rearrangements in a cycle are assigned to the same chain</p>		

types and sizes of simple SVs can be detected relatively straightforward, the distinction of chromothripsis from other complex rearrangement types is challenging. Several bioinformatics tools have been developed to specifically detect chromothripsis or chromoplexy rearrangements (Baca et al. 2013; Cai et al. 2014; Govind et al. 2014; Malhotra et al. 2013; Kim et al. 2013). An essential part of these tools is their definition of criteria to distinguish each type of complex genomic rearrangement. These criteria are not entirely fixed across different studies, leading to differences in sensitivity for detection of complex rearrangement types (Table 1).

A new software tool, termed ShatterProof, was developed to standardize detection and quantification of chromothripsis (Govind et al. 2014). ShatterProof identifies highly mutated regions and subsequently scans these for chromothripsis based on seven variables (Table 1): (1) Genome localization: a high score indicates a much higher SV density on the investigated chromosome than on other chromosomes; (2) Chromosome localization: a high score indicates a high SV density in the region compared to the SV density of the total chromosome; (3) Aberrant CNVs: The presence of a low number of copy number states but a high number of copy number oscillations leads to a high score; (4) Translocation clustering: regions that show a high number of localized translocations are given a high score; (5) Insertions of translocation breakpoints: regions with a high fraction of translocations where short insertions are found receive a high score; (6) Amount of retained heterozygosity: regions that retained heterozygosity in areas between CNVs receive a high score; (7) TP53 mutation; the presence of non-synonymous

TP53 mutations is reported back, but does not influence the chromothripsis score. An overall chromothripsis score is determined per region and reported back to the user. The higher the overall score, the more chromothriptic the region is. Although this tool provides a standardized pipeline for detection of chromothripsis, ShatterProof uses SV calls generated by other tools that have varying outcomes, thereby also influencing the outcome of ShatterProof, affecting the robustness of the tool.

Other tools for chromothripsis detection required either genome sequencing data (Malhotra et al. 2013), or copy number profiles from arrayCGH or SNParray (Cai et al. 2014; Kim et al. 2013). The differences between each of these tools mainly concern assumptions on the numbers of breaks involved, the numbers of copy number states allowed and the clustering of breakpoints within chromosomes (Table 1).

Baca et al. (2013) developed an algorithm specifically for detection of chromoplexy chains, termed ChainFinder. This algorithm identifies genomic rearrangements and their associated deletions that appear to have arisen interdependently from combined analysis of somatic breakpoints junctions and segmented copy number profiles. In order to do so, ChainFinder first identifies potential deletion bridges and subsequently performs a statistical analysis of all closely neighboring breakpoint pair distances to identify chain-like rearrangement patterns. Next, ChainFinder determines the probability of observing the two detected fusion breakpoints independently (i.e., not formed in the interdependent manner as breakpoints formed in a chain). Only breakpoints fulfilling the criteria for interdependent formation are assigned to chains (Table 1). It has been suggested that ChainFinder may be used in a broader sense to distinguish chromoplexy and chromothripsis events from other non-associated rearrangements on the same chromosome or its other parental counterpart (Zhang et al. 2013).

8 Prevalence of Chromothripsis in Human Cancer

Since its initial discovery in 2011, chromothripsis has been observed in many cancer types. Current pan-cancer estimates of the frequency of chromothripsis range from 1 to 2 % (Stephens et al. 2011; Kim et al. 2013) to 5 % (Zack et al. 2013). However, at the level of individual cancer types marked differences have been observed (Fig. 5). These estimates are dependent on three main factors. First, the detection methods used in several studies have variable capacity to detect chromothripsis (Box 1). High-resolution sequencing will provide insights into copy-neutral rearrangements and smaller changes, whereas array-based methods only provide copy number measurements. As a result, a higher estimate for the frequency of chromothripsis is obtained from whole-genome sequencing data (Kloosterman et al. 2014). Second, since its initial discovery in 2011, several studies have used different criteria to define chromothripsis (Table 1). Comprehensive criteria for inference of chromothripsis have only recently been outlined in detail, but are applicable to next-generation sequencing data only (Korbel and

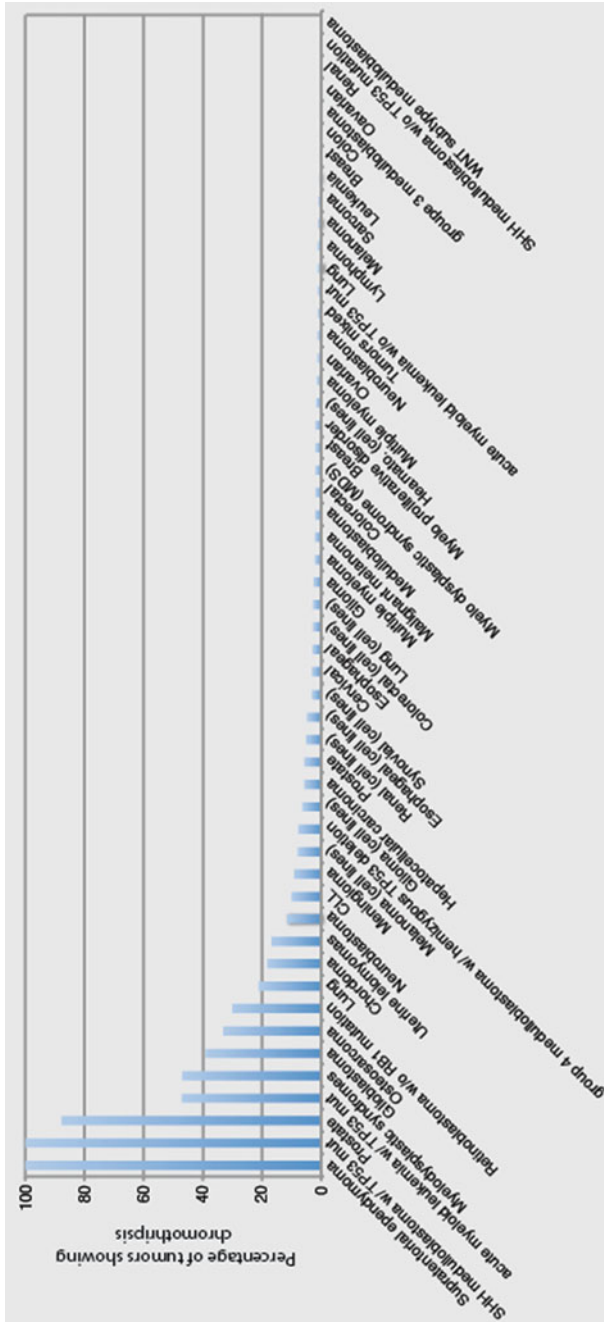


Fig. 5 Occurrence of chromothripsis in different tumor types

Campbell 2013). Third, biases with respect to sample selection have a strong influence on reported rates for chromothripsis and should be taken into account. Retinoblastoma tumors with a lack of mutations in *RBI* display a chromothripsis frequency of 27 % (3/11) (McEvoy et al. 2014), whereas, the fraction of chromothripsis in retinoblastoma in general is much lower (3/94) (McEvoy et al. 2014). Also, tumor cell lines show an overrepresentation of chromothripsis when compared to primary tumors (Cai et al. 2014).

A consistently high frequency of chromothripsis is displayed by genomes of glioblastoma as defined by copy number profiling [16 %, (Zack et al. 2013)] and genome sequencing [39 %, (Malhotra et al. 2013)]. In another study complex genomic changes in glioblastoma were also noted at a high frequency (Yang et al. 2013). In most tumors the complex changes were concentrated on genes known to drive glioblastoma formation, including gain of *EGFR* and loss of *CDKN2A*. High rates of chromothripsis were also found in bone cancers (25 %), and in most of these tumors multiple chromosomes are involved (Stephens et al. 2011).

Chromothripsis further appears to occur at high frequency in specific subtypes or selected groups of samples. A strong association has been found between chromothripsis and mutations in *TP53* in medulloblastoma (Rausch et al. 2012). Virtually all medulloblastoma of sonic-hedgehog subtype (SHH-MB) that are *TP53* mutated display chromothripsis, whereas SHH-MB without *TP53* mutations show no chromothripsis (Rausch et al. 2012). Several patients carried a germline *TP53* mutation, leading to Li-Fraumeni syndrome, characterized by a high risk for cancer development. Various other subtypes of medulloblastoma show much lower frequencies of chromothripsis (Rausch et al. 2012), but the association of *TP53* with chromothripsis has been confirmed in group 3 medulloblastoma as well (Northcott et al. 2012). The association between *TP53* mutation and chromothripsis has been further substantiated in acute myeloid leukemia, where 47 % of cases with *TP53* mutation harbor chromothripsis versus 1 % of cases without *TP53* mutation (Rausch et al. 2012). Chromothripsis in urinary bladder cancer also seems correlated with *TP53* mutation (Morrison et al. 2014). These findings suggest that the presence of *TP53* mutations in specific cancer types makes the cell more permissive for the occurrence of chromothripsis or provides a selective advantage for cells carrying chromothripsis rearrangements (Rausch et al. 2012; Zhang et al. 2013). However, the association between *TP53* mutation and chromothripsis is certainly not prominent in all cancers. Uterine leiomyomas contain chromothripsis with more than 20 breaks in 17 % of the cases (Mehine et al. 2013), while none of these carry *TP53* mutations. In fact, some of the leiomyomas harboring chromothripsis were benign tumors.

Genetic predisposition for chromothripsis was also demonstrated by the genomic analysis of childhood acute lymphoblastic leukemia with amplifications on chromosome 21 (iAMP21 ALL) (Li et al. 2014). Around 3 % of the patients with this type of ALL, carry a constitutional Robertsonian translocation, involving chromosome 15 and 21 (rob(15;21)c). Patients with rob(15;21)c have a 2700-fold increased risk for developing iAMP21 ALL. This is attributed to the dicentric Robertsonian chromosome, which may undergo aberrant chromosome segregation

during mitosis. Consequently, chromothripsis may occur following pulverization of the Robertsonian lagging chromosome in micronuclei (Crasta et al. 2012; Li et al. 2014). Interestingly, the creation of a dicentric chromosome by BFB-cycles in ALL iAMP21 in sporadic cases was followed chromothripsis by four out of five patients, indicating a predisposition to chromothripsis by the presence of a dicentric chromosome in this specific subtype of ALL (Li et al. 2014).

A final remarkable difference in chromothripsis rates in different subtypes of tumors is highlighted by a recent study on ependymoma, a tumor of the central nervous system (Parker et al. 2014). The authors show that virtually all ependymoma's of supratentorial origin contain chromothripsis leading to C11orf95-RELA fusions. In contrast, none of the posterior fossa ependymoma's show chromothripsis. These differences between the two types of ependymoma's are unexplained, but may suggest chromothripsis-predisposing cellular conditions for the supratentorial type.

9 Chromothripsis Can Drive Cancer Development by Establishing Oncogenic Lesions

The destructive nature of chromothripsis implies that it generally is not an advantageous event for cell survival. Yet, chromothripsis has been identified in a multitude of cancers suggesting that the maintenance of chromothripsis is due to a positive effect of the event on cancer cell survival.

Investigation of chromothripsis breakpoints has revealed clear effects on cancer genes through a variety of mechanisms. First, tumor suppressor genes are commonly disrupted. The *TP53* tumor suppressor gene is located on the p arm of chromosome 17 and is a frequent target of chromothripsis in a variety of tumors (Cai et al. 2014). Loss of *CDKN2A* is another recurrent aberration resulting from chromothripsis in glioblastoma (Yang et al. 2013), diffuse large B-cell lymphoma (Morin et al. 2013) and chronic lymphocytic leukemia (Stephens et al. 2011). Similar tumor-driving changes have been observed for chromoplexy in prostate cancer, where 46 % of the tumor specimens showed disruption of at least one tumor suppressor, among which *TP53*, *PTEN*, *CDKN1B*, *NKX3-1* and *RBI* (Baca et al. 2013). *RBI* has also been discovered as a recurrent target of chromothripsis in retinoblastoma samples without *RBI* point mutations (McEvoy et al. 2014).

A second mechanism by which chromothripsis may promote tumor development concerns the amplification of oncogenes. Although chromosomal regions affected by chromothripsis typically contain only two copy number states, some regions display high-level amplification within a chromothripsis context (Stephens et al. 2011; Rausch et al. 2012). This observation explained formation of double-minutes containing oncogenes, which are beneficial to the cancer cell if present in high numbers. Both *MYC* and *MYCN* are frequent targets of double-minute amplification as a result of chromothripsis in neuroblastoma and medulloblastoma (Rausch et al. 2012; Kim et al. 2013; Molenaar et al. 2012). Other targets of amplification by

chromothripsis involve the cell division kinase *CDK4* (neuroblastoma), the Sonic-Hedgehog signaling genes *GLI2* and *BOC* (Sonic-Hedgehog subtype of medulloblastoma) and *MDM2* (glioblastoma) (Rausch et al. 2012; Kim et al. 2013; Molenaar et al. 2012). Complex genomic rearrangements involving EGFR amplification in glioblastoma were also attributed to double-minute chromosome formation (Yang et al. 2013).

Third, chromothripsis involves the connection of multiple remote chromosomal fragments into a newly formed derivative chromosome. By chance each of the resulting junctions may result in the formation of novel fusion genes. Indeed, in-frame fusion genes are frequently formed due to chromothripsis. Those fusions may simply be a coincidence of the joining of two chromosome segments and lead to fusion genes without any role in tumor development. For example, the VCaP prostate cancer cell line contains an extreme example of chromothripsis involving 573 breakpoints on chromosome 5q (Alves et al. 2013). Eighteen of the breakpoints resulted in potential in-frame fusions, of which only five could be confirmed at the mRNA level. In general, the number of fusion genes created by chromothripsis does not appear to be higher than what is expected by chance. Furthermore, chromothripsis does not lead to a higher number of fusion genes than simple structural genomic rearrangements (Stephens et al. 2011; Alves et al. 2013). Several studies put forward strong evidence for the oncogenic capacity of some fusion genes caused by complex genomic rearrangements. An interesting example involves fusions of the non-coding gene *PTVI* in medulloblastoma (Northcott et al. 2012). *PTVI* is frequently co-amplified together with *MYC* and a large proportion of *MYC*-amplified medulloblastoma display *PTVI-MYC* fusions resulting from chromothripsis. The *PTVI* gene is a host for *miR-1204*, which is highly expressed in samples with *PTVI-MYC* fusions and antagonizing expression of *miR-1204* in a medulloblastoma cell line abrogates cell growth. In a recent study, whole genome sequencing was performed for ependymoma (Parker et al. 2014). A large fraction of the supratentorial subtype of ependymoma contains novel fusions between *C11orf95* and *RELA*. The latter gene is a transcription factor in the NF- κ B pathway and translocates spontaneously to the nucleus as a result of fusion to *C11orf95*. Chromothripsis caused the *C11orf95-RELA* fusion in all examined tumors in this study.

A fourth effect of chromothripsis rearrangements may be the disturbance of gene expression either through direct gene deletion or by interference with gene regulation (Stephens et al. 2011). Such examples have been reported in uterine leiomyomas, where expression of *HMGA1* and *HMGA2* is upregulated as a result of chromothripsis breakpoints (Mehine et al. 2013). In addition the genomic region involving *COL4A5* and *COL4A6* was recurrently rearranged leading to increased expression of the nearby gene *IRS4*, a downstream target of insulin-like growth factor I, which is known to be involved in leiomyoma development (Mehine et al. 2013).

Finally, the cataclysmic nature of chromothripsis allows simultaneous targeting of multiple cancer genes (Fig. 6). Analysis of chromothripsis in medulloblastoma revealed amplification of both *MYCN* and *GLI2* on a single double minute

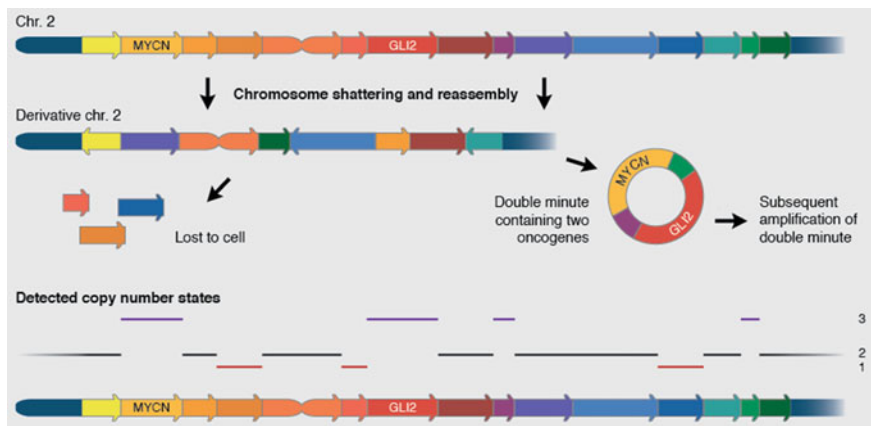


Fig. 6 Illustrative example of a double-hit by chromothripsis, based on the findings of Rausch et al. (2012). Chromothripsis leads to reshuffling of chromosome 2. During this event, a double minute chromosome is formed containing two known oncogenes, *MYCN* and *GLI2*. Subsequent amplification of the double minute chromosome -due to the presence of the oncogenes, which are beneficial to the cancer cell if present in higher numbers- leads to the presence of a third, highly amplified copy number state

chromosome resulting from chromothripsis (Rausch et al. 2012). Furthermore, several instances of double hits of cancer genes were caused by chromoplexy in prostate cancer, including formation of a *TMPRSS2-ERG* fusion gene together with disruption of the tumor suppressor *SMAD4* by a single chain of rearrangements. In exceptional cases three lesions were described that affected cancer genes, such as simultaneous disruption of *CDKN2A*, *WRN* and *FBXW7* in chordoma (Stephens et al. 2011).

Altogether, chromothripsis and other types of complex genome rearrangements are an important pan-cancer mechanism causing oncogenic lesions. Large-scale cancer genome analysis demonstrated that complex rearrangements are among the first rearrangements appearing in cancer genomes and may thus contribute to tumor initiation (Malhotra et al. 2013). Nevertheless, effects on cancer genes are not identified in all instances. Three neuroblastoma tumors were reported with chromothripsis on chromosome 5, without causing a notable tumor-driving lesion (Molenaar et al. 2012). In these cases chromothripsis may merely be a coincidental passenger event, apparently without a negative effect on cell survival. Such a scenario is underscored by the recent finding of temporal chromothripsis during the course of chronic lymphocytic leukemia development (Bassaganyas et al. 2013). This study indicated that a cancer subclone containing chromothripsis occurred as a secondary event years after initiation of disease. Subsequent rapid expansion of this subclone contributed to the aggressiveness, but the chromothripsis clone did not survive chemotherapeutic treatment and was not found in the relapse. Alternative explanations for the lack of oncogenic lesions resulting from chromothripsis could be the coupling of chromothripsis to a separate oncogenic mutation on the same

chromosome or yet unknown cellular consequences, such as global effects on chromosome structure and gene regulation.

10 Prognostic and Therapeutic Implications of Chromothripsis

With the overwhelming evidence that chromothripsis can promote formation of cancer-driving changes, the question is quickly raised as to whether chromothripsis represents a biomarker allowing clinical categorization of cancer patients. Several studies have now provided evidence for the association of chromothripsis with poor survival in different cancer types, including neuroblastoma, medulloblastoma, acute lymphoblastic leukemia and multiple myeloma (Rausch et al. 2012; Cai et al. 2014; Molenaar et al. 2012; Moorman et al. 2007; Magrangeas et al. 2011). It is unclear if chromothripsis in these tumors is only a biomarker associated with poor survival or whether the chromothripsis rearrangements themselves lead to more aggressive tumor growth. In the first case, chromothripsis may be a marker of severe genomic instability and a concomitant capacity of tumors to swiftly adapt as a response to changing environmental conditions. Consistent with this, association between chromosomal instability and poor clinical outcome has been observed in several human cancers (Carter et al. 2006). In the latter scenario, the actual genes affected by chromothripsis could result in a more aggressive disease course and lower survival. This option poses important opportunities for targeted therapy. For example, the *RELA* fusions observed in supratentorial ependymoma, which drive NF- κ B signaling, may be an interesting drug target specifically associated to chromothripsis (Parker et al. 2014). Both scenarios sketched above may be true, but likely depend on the tumor type and environmental conditions. This is underscored by the observation of chromothripsis in some cases of benign uterine leiomyomas (Mehine et al. 2013). Also, a transient subclone of chronic lymphocytic leukemia containing chromothripsis has been described (Bassaganyas et al. 2013). In these instances, chromothripsis likely represents a passenger event with little impact on disease course and is therefore not a valuable biomarker. However, the routine detection of chromothripsis breakpoints provides new opportunities for monitoring disease status and progression. Cancer-specific breakpoints can be used for the design of PCR amplicons to track the presence of cancer rearrangement in blood plasma, enabling personal diagnostic strategies (Leary et al. 2010; McBride et al. 2010). Circulating tumor DNA in blood plasma can even be used for direct sequencing and interrogation of cancer-specific chromosome aberrations, thus allowing non-invasive detection of tumors and their genetic changes (Berger et al. 2012). The same methodology can be applied to oncogenic chromothripsis rearrangements.

11 Concluding Remarks

The discovery of chromothripsis has triggered a new field of research focused on understanding and discovery of complex genomic rearrangements in cancer. This has considerably changed our view on cancer evolution, which as we now think, can involve catastrophic events, in addition to a gradual pattern of mutation and selection. The field has now converged on three types of rearrangements, namely chromothripsis, chromoplexy and chromoanasythesis. Although chromoanasythesis displays distinct, replication-dependent genomic features, the difference between chromothripsis and chromoplexy is far less clear. Both phenomena appear to involve double-stranded DNA breaks in confined genomic regions and both lead to a typical pattern including deletions. Whether chromoplexy is a phenomenon, which is as widespread as chromothripsis is currently unclear. Chromoplexy may be a milder form of chromothripsis, generally involving fewer rearrangements. Due to stringent criteria for chromothripsis for the number of focal copy number changes (>10) (Table 1) chromoplexy events may have gone unnoticed in several studies. Furthermore, complex genomic changes in cancer genomes can be masked by simple variants, which have preceded or followed them. Whether chromothripsis and chromoplexy are truly two distinct phenomena or rather a more severe and milder display of the same event will likely be uncertain until the mechanism behind these phenomena is better understood, providing a more precise description of characteristics per event type. Until then, we may just speculate and come up with new models, which fit the experimental data, such as the recently proposed translocation-induced chromothripsis (Zhang et al. 2013). This model describes the occurrence of chromoplexy creating a platform for a chromothripsis event, possibly explaining the mechanism for chromothripsis involving multiple chromosomes. Whatever the mechanism, we do know that cancers may combine different complex changes, including chromothripsis, over consecutive cell divisions, altogether culminating into a chromosome configuration, which allows faster division, better survival and reduced death of the cancer cell.

Box 1: Detection of genomic rearrangements

Array CGH

Array CGH is a molecular cytogenetic method that compares a test sample to a reference sample by labeling DNA from both samples with different fluorophores. Labeled DNA is denatured and hybridized in a 1:1 ratio to a microarray containing specific cloned DNA fragments 100–200 kb in size. The ratio of test sample versus reference is determined for every fragment.

Deviations from the 1:1 ratio indicate the presence of a CNA in that specific region (Solinas-Toldo et al. 1997). Array CGH provides a quick, relatively affordable and genome-wide scan for CNAs, but is unable to detect copy neutral events.

SNP array

Unlike array CGH, SNP arrays are not performed by competitive hybridization; rather a pool of normal samples is used to estimate the expected intensity of a probe as a reference. A large number of SNPs spread out across the genome is tested. For each SNP, the probe intensity of the sample is compared to the reference. Like array CGH, SNP arrays are affordable and relatively fast and easy to analyze. An advantage of SNP arrays over array CGH is the determination of the allele frequency, which provides a percentage for the cells carrying the reference allele for every SNP (Conlin et al. 2010). In cancer research, the allele frequency is often used to determine the percentage of mosaicism of a sample, using bioinformatics tools such as Absolute (Carter et al. 2012).

Next generation sequencing (NGS)

Paired-end sequencing, is widely used for SV detection. Genomic DNA is fragmented to a specific size, varying from a few hundred bp up to 2–3 kb or even 10 kb in extreme cases. Paired reads from both ends of these fragments are generated and subsequently sequenced in a massively parallel fashion (Mardis 2009). In a typical sequencing run, 100 s of millions of short reads of 25–150 nucleotides are generated. De novo assembly, in which a genome is reconstructed from overlapping reads and subsequently compared to a reference genome to detect genomic rearrangements, is possible but highly complex due to the short read length that leads to fragmented assembled genomes. Instead, resequencing is normally applied, where paired sequencing reads are directly aligned to a reference genome. The vast majority of reads will map concordant, in which case the distance between the aligned reads is equal to the length of the original DNA fragment. Discordant reads indicate the presence of an SV, characterized by a difference in distance between the aligned reads and the length of the original DNA fragment (Fig. 2). Besides alignment distance another essential variable for SV detection is the orientation of the reads within the read pairs. The orientation of breakpoint junctions is indicated by using head (H), which indicates the 5' end of a fragment, and tail (T), indicating the 3' end of a fragment. Different rearrangement types lead to different orientation conformations (Fig. 2). Paired end sequencing also enables the detection of copy number events by analyzing read density. A deletion will lead to a decreased coverage for the deleted area when compared to the rest of the genome, a duplication to increased coverage. In order to reliably call an SV, multiple reads have to overlap a breakpoint, forming a cluster of discordant reads. The larger the number of reads overlapping the breakpoint, the more precise the breakpoint

location can be determined, from a relatively large breakpoint region up with low coverage to the determination of the breakpoint at the nucleotide level at high coverage. PCR and Sanger sequencing is generally used to validate breakpoints at high resolution.

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