Leading Edge
Minireview

Cell

Chromothripsis and Human Disease: Piecing Together the Shattering Process

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DOI 10.1016/j.cell.2012.01.006

The unprecedented resolution of high-throughput genomics has enabled the recent discovery of a phenomenon by which specific regions of the genome are shattered and then stitched together via a single devastating event, referred to as chromothripsis. Potential mechanisms governing this process are now emerging, with implications for our understanding of the role of genomic rearrangements in development and disease.

Structural variation in the human genome has warranted considerable interest in the cancer community due to the potential functional consequences of these rearrangements in tumorigenesis. Through the acquisition of genomic rearrangements over time, a cell may tolerate the disruption of tumor suppressor genes, activation of oncogenes, or generation of fusion proteins that individually (or in combination) can promote tumor progression. Furthermore, the restricted expression of many resulting somatic gene fusions exemplifies the potential for discovering and developing novel targeted therapies. A recent discovery is the phenomenon whereby tens to hundreds of chromosomal rearrangements localized to a limited number of genomic regions can be acquired in a single catastrophic event termed chromothripsis (Greek, chromos for chromosome; thripsis, shattering into pieces) (Stephens et al., 2011). Cells that can survive such a catastrophic event emerge with a highly mutated genomic landscape that can confer a significant selective advantage to the clone, thereby promoting cancer progression. Initial screening indicates that chromothripsis is a widespread phenomenon occurring in ~2%–3% of different cancer types with some variability, as exemplified by the higher frequency observed in bone cancers. In addition to tumorigenesis, chromothripsis also appears to be playing a role during normal human development. Though the mechanisms behind chromothripsis are not yet fully understood, observations from recent work have provided some insights into the process.

Chromothripsis occurs through a single catastrophic shattering event followed by the stitching of genomic fragments into derivative chromosomes (Figure 1). Closer inspection of the phenomenon resulted in the formulation of six features that comprise a "signature of chromothripsis." These criteria are as follows: (1) multiple and complex rearrangements primarily alter a single chromosome, chromosomal arm, or region and, in some instances, concurrent rearrangements between chromosomes; (2) many regions show copy number changes alternating between two states, one copy (heterozygous deletion) or two copy (no loss or gain); (3) regions of single copy are not necessarily from simple deletions but are the byproduct of complex rearrangements spanning the region; (4) pronounced clustering of breakpoints; (5) the fragments residing in the clustered breakpoint regions do not reside in close proximity in the germline; and (6) breakpoints involving multiple chromosomes also show clustering. Subsequent cytogenetic confirmation suggests that genomic breakpoint clustering is not due to multiple, parallel rearrangements from various subclones (Stephens et al., 2011).

Implications of Chromothripsis in Cancer

Though the initial observation was made in a patient with chronic myeloid leukemia, additional screening revealed that ~2%–3% of patients across a broader range of human cancers show signs of chromothripsis. As a first pass, Stephens et al. screened single-nucleotide polymorphism (SNP) array data from 746 cancer cell lines, revealing 18 cell lines with genomic landscapes harboring the hallmarks of chromothripsis. In addition to hematological malignancies, this cell line subset included melanoma, lung, glioma, sarcoma, esophageal, colorectal, renal, and thyroid cancer. It is possible that this is an underrepresentation, as the heterogeneity in primary tumors may disguise the chromothripsis signature; closer examination of specific cancer types may show more variable rates. A recent study confirms the prevalence of chromothripsis in multiple myeloma (MM) and suggests that chromothripsis may be associated with a poor outcome in MM (Magrangeas et al., 2011). Recent work by Rausch et al. in this issue of *Cell* (Rausch et al., 2012) examined copy number data for 311 AML patients and found a significant association between chromothripsis and poor prognosis. Lastly, on an anecdotal level, the 62-year-old CLL patient from which chromothripsis was initially observed also showed rapid deterioration and relapsed quickly despite receiving alemtuzumab, a monoclonal antibody used in the treatment of CLL (Stephens et al., 2011).

The association of chromothripsis with more aggressive tumors is quite logical given the potential impact that a single catastrophic event may have on a cell. A progressive model of tumor cell evolution suggests the gradual accumulation of

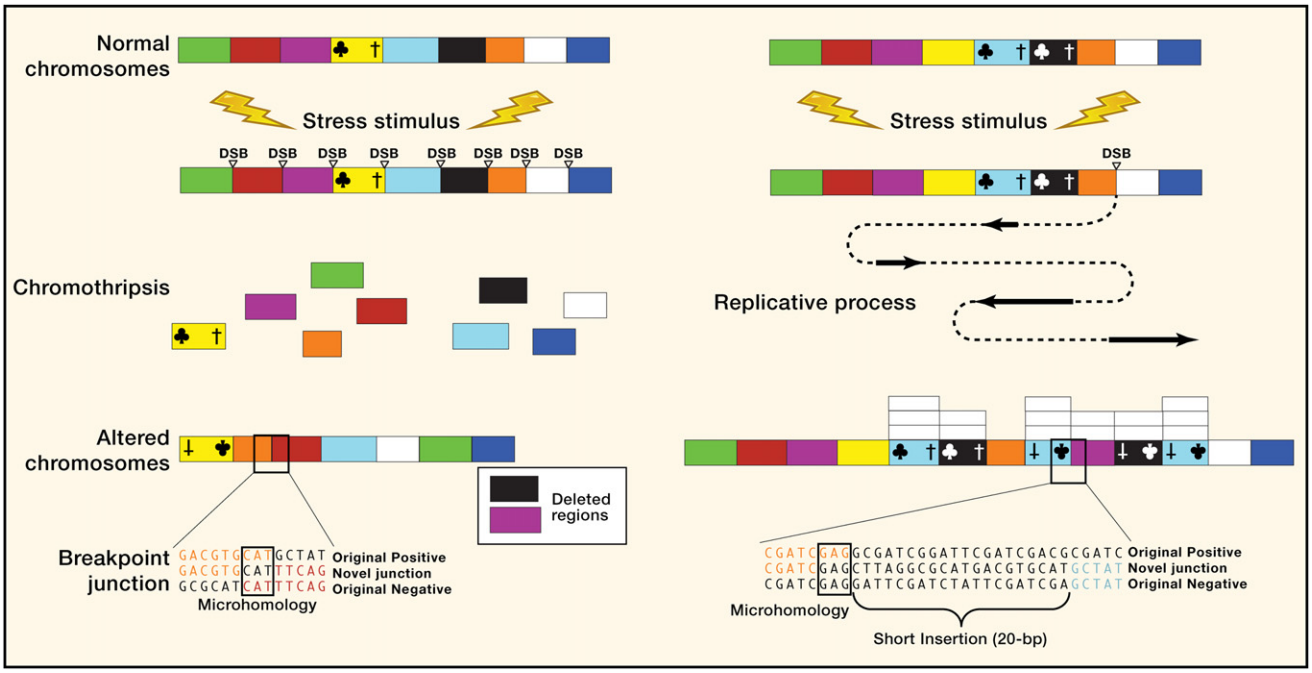

Figure 1. Chromothripsis Reshapes the Genomic Landscape in a Single Devastating Event
Overview of chromothripsis. Stress stimulus may help to trigger the shattering process in localized regions that are subsequently stitched back together. (Left) Stress simultaneously generates double-strand breaks (triangle) that are joined together to generate a derivative chromosome, potentially resulting in regions being deleted. The breakpoint junction reveals microhomology, without insertions, thereby supporting NHEJ. (Right) A replicative stress generates a nick in the chromosome, causing a replication fork to collapse. MMBIR results in the duplication and triplication represented by two or three rectangles above the altered chromosome, respectively. An example breakpoint junction reveals microhomology as well as a short insert.

mutations randomly throughout the genome over time. However, shattering a genome into tens to hundreds of fragments and then stitching them back together, in a seemingly random process, produces highly derivative chromosomes. This can result in the concurrent generation of numerous mutations that individually, or in combination, provide a selective advantage for a cell. As such, it is believed that chromothripsis can accelerate the evolutionary process of a tumor cell.

Multiple lines of evidence have been proposed to support a single catastrophic event versus a progressive model. First, the number of copy number states following chromothripsis is predominantly restricted to two states. Under a progressive model, the number of states would be expected to increase during the accumulation of rearrangements. To further support this, Stephens et al. performed a Monte Carlo simulation of the progressive model of acquiring rearrangements to demonstrate that a cell harboring the observed quantity of breakpoints in four samples having undergone chromothripsis (SNU-C1, PD3172, 8505C, and TK10) should have resulted in more copy number states. Second, regions with higher copy number retain heterozygosity following chromothripsis. However, under a progressive model, an early occurring deletion would eliminate heterozygosity. Third, under a progressive model, a random distribution of rearrangements would be expected. This is in sharp contrast to the high level of breakpoint clustering, suggesting a single catastrophic event. Overall, given the interrelatedness of the rearrangements and spatial localization, it is unlikely that the signature originated from independent, consecutive events.

Recent work by Rausch et al. using whole-genome sequencing (WGS) and array-based approaches has revealed a link between both germline and somatic *TP53* mutations and chromothripsis. WGS of a Sonic-Hedgehog subtype of medulloblastoma (SHH-MB) from a female patient with Li-Fraumeni syndrome (LFS), a disorder with germline *TP53* mutations that increase susceptibility to cancer, revealed a signature of chromothripsis. Furthermore, germline DNA, available for six of the ten SHH-MB patients showing signs of chromothripsis, was used to confirm five patients with germline *TP53* mutations. These five patients represent previously undiagnosed LFS cases. Overall, germline mutations of *TP53* in SSH-MB patients suggest that it occurred prior to chromothripsis and may be involved in the initiation and/or response to chromothripsis.

Chromothripsis and Human Development

Two recent studies have suggested that chromothripsis may also contribute to structural variation in the germline. In the first study, a family trio that includes a child with severe congenital abnormalities underwent mate-pair sequencing. This work provided evidence that chromothripsis may be generating structural variation in the germline that results in congenital defects (Kloosterman et al., 2011a). In the second study, 17 individuals showing developmental delay and cognitive anomalies were analyzed with array comparative genomic hybridization (aCGH) and breakpoint sequencing, resulting in the observation of a mechanism similar to chromothripsis. This suggests that catastrophic events may be occurring throughout the life cycle of an

organism (Liu et al., 2011). Furthermore, in addition to the inversions and translocations that had previously been associated with chromothripsis, the individuals showed extensive duplication and triplication. These differences in the observed altered chromosomes between Stephens et al. and Liu et al. are suggestive of differences in the cause and mechanism of repair. Overall, it is clear that chromothripsis plays a significant role in human development, and through the advancement of recent technologies, we can begin to dissect the potential mechanisms.

Mutational Mechanisms Governing the "Shattering" Process

Three areas that will garner significant interest in discovering the underlying causes of chromothripsis are the genomic localization, the mechanism driving the shattering process, and the stitching of the fragmented segments.

Existing data have shown that the shattering appears to involve only a subset of chromosomes, a single chromosome, a chromosomal arm, or even a few megabases of a chromosomal band. Though it seems puzzling, the localization of the shattered genomic fragments may offer a few possibilities as to the mechanism. For instance, the ability to accomplish such confined damage, exemplified by high-density breakpoint clusters, suggests that the chromosomes are likely condensed, and therefore the shattering event may occur during mitosis. Furthermore, instances involving multiple chromosomes suggest a spatial proximity during the shattering event, resulting in the random stitching confined to a subset of chromosomes. Though it is astounding that a cell can survive such a catastrophic event, it is plausible that localized shattering represents the upper limit of what a cell can tolerate and still survive. Therefore, a contrasting view is that chromothripsis events are not always restricted to specific regions; however, any event involving a greater number of chromosomes may have had lethal consequences and therefore is not observed.

The mechanisms driving chromosomal translocations have been a major area of interest in cancer biology, and therefore understanding the shattering process poses a new challenge. It is unclear what caused the double-stranded DNA breaks, but an environmental stimulus, such as free radicals or ionizing radiation, may serve as the trigger (Lieber, 2010; Tsai and Lieber, 2010). For instance, exposure to ionizing radiation while the chromosomes are condensed during mitosis would offer an opportunity to intensely shatter a localized region from a chromosome, or multiple chromosomes, in close spatial proximity.

DNA replication stress may serve as a stimulus to chromothripsis. This can occur through an increase in the number of stalled DNA replication forks by inhibitory agents of DNA replication or decreased stability of stalled forks by altered DNA replication checkpoint proteins. Interestingly, it has been shown that precancerous cells with activated oncogenes have prematurely terminated DNA replication forks and DNA DSBs that form specifically in S phase. Regardless of the specific DNA replication stress, a DNA replication fork collapse at a specific chromosomal loci, potentially a common fragile site, can generate the genomic configurations associated with chromothripsis (Halazonetis et al., 2008). Further, recent work by Crasta et al. shows that micronuclei, generated from mitotic chromosome segregation errors, have persistent DNA replication (Crasta et al., 2012). The authors go on to show that aberrant DNA replication can produce DNA damage and mutagenesis or chromosome pulverization within the micronuclei. As observed during chromothripsis, the partitioning of a chromosome into micronuclei also offers an explanation for extensive DNA damage being restricted to a single chromosome.

As the shattering typically involves the telomeric region, there may be a link to telomere shortening, suggesting a breakage-fusion-bridge cycle (Pampalona et al., 2010). Following telomere loss, end-to-end chromosome fusions form and are subsequently pulled to opposite daughter cells via their centromeres, thereby forming the anaphase bridge (McClintock, 1941; Sahin and Depinho, 2010). Though this model offers a mechanism for potential localization, it is typically associated with amplicons, or regions of high copy number, and results in head-to-head duplications, whereas chromothripsis produces highly complex derivative chromosomes with two or three copy states (Murnane, 2006; O'Hagan et al., 2002).

Interestingly, the shattering observed during chromothripsis shows similarities to the dramatically altered chromosomes involved in the previously characterized process of premature chromosome compaction (PCC) (Rao and Johnson, 1970; Sperling and Rao, 1974). The process of PCC has been shown to occur when chromosomes from an S phase nucleus are induced to undergo chromosome condensation by signals from chromosomes derived from mitosis. This, in turn, results in the "shattering" of the incompletely replicated chromosomes. Given the similarities in the intense genomic disruption, it is plausible that chromothripsis could be due to a similar process.

A different perspective is that chromothripsis might be caused by an apoptotic mechanism (Tubio and Estivill, 2011). Here, a cell would undergo apoptosis due to the stress of an external stimulus, such as ionizing radiation. Though the majority of cells would ultimately die, a subset of the population may survive and subsequently undergo DNA repair that can introduce rearrangements (Stanulla et al., 2001). Overall, several models have been proposed, additional experimental work is necessary to fully elucidate the specific mechanism(s).

Given the importance of *TP53* for maintaining genomic stability, the recent work by Rausch et al. suggests that *TP53* mutations may predispose cells to chromothripsis. This can be exemplified by its potential role in a number of the proposed mechanisms. For instance, if the chromothripsis is driven by the generation of DSBs following exposure to ionizing radiation, a cell harboring *TP53* mutations may show a preference toward low-fidelity repair mechanisms such as nonhomologous end-joining (NHEJ). In the context of telomere shortening, cells with mutant *TP53* are likely to show an increased delay at the G2/M transition and shorter telomeres and are more prone to end-to-end fusions. Lastly, mutant *TP53* may contribute to altered control of the G2/M transition checkpoint, thereby contributing to premature chromosome compaction. In addition to contributing to the mechanism driving chromothripsis, impaired *TP53* may also facilitate cell survival following a catastrophic event.

Stitching Chromosomes Back Together

Independent of the mechanism causing chromothripsis, examination of the resulting genomic features in conjunction with

nucleotide resolution of the breakpoint junctions, from high-throughput sequencing, offers some insights into how the fragments are joined together (Figure 1). The first mechanism is NHEJ, a template-independent DNA double-strand break repair mechanism that ligates two broken DNA ends with a concomitant loss or gain of nucleotides (Lieber, 2010). In addition to NHEJ, replicative processes have been associated with the generation of complex genomic rearrangements such as fork stalling template switching (FoSTeS) and microhomology-mediated break-induced repair (MMBIR). MMBIR is based on the notion that the replication fork collapses when it encounters a nick, or single-strand break, and has therefore been the more supported mechanism attributed to the observed chromothripsis events (Hastings et al., 2009). The breakpoint junctions derived from replicative processes are expected to have microhomologies, insertions, and relatively long templated insertions. Furthermore, in contrast to NHEJ repair of simultaneously generated DSBs, replicative processes offer an explanation for the duplications and triplications that have been reported (Liu et al., 2011).

To date, the breakpoints generated via chromothripsis in human cancer appeared to have limited sequence overlap, thereby suggesting that, following shattering, via double-stranded DNA breaks, genomic fragments were stitched together by an NHEJ mechanism, as opposed to homologous recombination, to form complex derivative chromosomes. For instance, the breakpoint junctions from LFS SHH-MB cases revealed short (less than four base pairs) microhomology tracts and instances of short insertions of nontemplate DNA sequence consistent with NHEJ. The reassembly of the derivative chromosomes may be guided by microhomology or is completely random and based on the spatial proximity of the genomic fragments (Kloosterman et al., 2011b; Stephens et al., 2011).

In contrast to somatic alterations that occur in differentiated cells, germline rearrangements discovered in genomic disorders were found to occur during gametogenesis or early postzygotic development. Kloosterman et al. observed patterns among the breakpoints, indicative of simultaneous double-stranded breaks in patients with congenital abnormalities. The breakpoint junctions revealed microhomology, a lack of homology, or small insertions and deletions, supporting a nonhomologous mechanism of break repair. In contrast, Liu et al. observed small template insertions and microhomology at the breakpoint junctions, suggestive of a replicative process such as FoSTeS (Lee et al., 2007) or MMBIR (Hastings et al., 2009). Overall, though initial studies suggest that NHEJ seems to be a predominant model in somatic structural variation, our understanding of the stitching process in human development remains an area of significant research.

Impact on Patient Care

Only through additional screening across larger patient cohorts will there be sufficient strength to establish clinical associations with patients having undergone chromothripsis. However, some headway into the contributing factors of chromothripsis has already been made, as exemplified by the high incidence of chromothripsis in SHH-MB patients with TP53 mutations, many of which are germline TP53 mutations. Furthermore, this association is not restricted to medulloblastoma, as preliminary evidence shows that, in LFS patients, excluding SSH-MB, chromothripsis occurs at a higher rate (36%) than overall incidence in cancers (2%–3%) (Rausch et al., 2012). This is clinically significant in that these patients may benefit from regular screening and may warrant careful treatment strategies involving DNA-damaging agents and radiotherapy in order to reduce the incidence of therapy resistance and ultimately increase survival rates. Chromothripsis demonstrates how the analysis of large-scale genomics data can have profound effects on human development and tumor cell progression.

ACKNOWLEDGMENTS

C.A.M. is funded by an NIH grant R00CA149182-03 and a Prostate Cancer Foundation Young Investigator Award.

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