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Cancer Genomes Evolve by Pulverizing Single Chromosomes

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A report in this issue describes "chromothripsis," a new mechanism for genetic instability in cancer cells. Chromothripsis appears to be a cataclysmic event in which a single chromosome is fragmented and then reassembled. The phenomenon raises important questions of how chromosome rearrangements can be confined to defined genome segments.

We tend to think of tumor evolution as the gradual acquisition of mutations that can occur with a uniform chance across the whole genome: a series of genetic changes that stimulate growth, attenuate cell death, destroy checkpoint controls, promote further genetic instability, and enable metastasis (Stratton et al., 2009; Nowell, 1976). For many tumors, this idea of gradual alteration of the genome matches the appearance of tumors under the microscope, where malignant lesions can develop from benign lesions. However, this is not always the case. Cancer genomes can also evolve by "punctuated equilibrium"-like mechanisms in which one-off cataclysmic events generate the potential for multiple concurrent mutations. For example, critical shortening of telomeres triggers breakage-fusion-bridge cycles that result in gene amplification and other chromosome rearrangements (McClintock, 1941; Sahin and Depinho, 2010). Developing tumor cells can also make a single large evolutionary step by failing cytokinesis, whereby the doubling of the centrosome number produces a storm of aneuploidy (Fujiwara et al., 2005). In this issue of *Cell*, Stephens et al. (2011) describe a new type of cataclysmic event that they call chromothripsis (Greek; chromos for chromosome, thripsis for shattered into pieces) in which chromosomes are broken into many pieces and then stitched back together (Figure 1).

These findings come amidst a flood of information from the large-scale resequencing of cancer genomes, which is providing important insights into the evolutionary paths available to developing cancers (Stratton et al., 2009). Such efforts help to identify changes that contribute to tumorigenesis, but also may reveal "passenger" alterations that create potential burdens on tumor cells that could be exploited for therapeutics. Thus, understanding the ways that cancer genomes can evolve is important; the underlying evolutionary mechanisms should constrain the composition of the chromosomes in the mature tumor cell.

In their current work, Stephens et al. use paired-end next-generation sequencing across multiple cancer samples to determine chromosomal structure and, in particular, the breakpoints of copy number alterations. With this approach, they have identified a new type of chromosomal disruption in cancer whereby there are repeated switches in copy number state along the length of a chromosome or other genomic segment, often with hundreds of breakpoints within a chromosome arm. The chromosomal segments vary in copy number primarily by single segment changes: for example, a region with two copies would be followed by a single copy, followed by two, followed by three (Figure 1). Strikingly, these alterations are primarily limited to a single chromosome or, in some cases, a few chromosomes that appear to be co-coordinately altered. As the chromosomes appear to be shattered and then stitched back together, they have coined the term chromothripsis. A combination of genome resequencing and analysis of single-nucleotide polymorphism arrays in cell lines and primary tumors suggests that chromothripsis occurs in 2%–3% of cancers, spanning a wide variety of tumor types. In certain tumors, such as osteosarcomas and chordomas, chromothripsis is observed in up to 25% of samples.

Chromothripsis may lead to the generation of amplifications of one or more oncogenes or to the deletion of one or more tumor suppressor genes. For example, one small cell lung cancer cell line contains a normal copy of chromosome 8 and a massively rearranged derivative chromosome 8 with all the hallmarks of chromothripsis. This cell line also contains large numbers of double minute chromosomes comprised of 15 distinct segments of chromosome 8, all rearranged to one another and leading to amplification of the *MYC* oncogene. Most strikingly, fluorescence in situ hybridization (FISH) experiments demonstrate that the amplified sequences on the double minute chromosome are absent from the derivative chromosome 8. This strongly suggests that a single copy of chromosome 8 shattered and that most fragments were stitched together to generate the derivative chromosome, but other

fragments, including the *MYC* gene, were stitched together into a circular double minute chromosome whose amplification conferred a growth advantage—all occurring at the same time. In another example, a chordoma DNA sample exhibits a complex rearrangement that simultaneously disrupts the *CDKN2A*, *WRN*, and *FBXW7* tumor suppressor genes, each present at different locations in the genome. In principle, chromothripsis may also promote cancer by generation of new fusion genes as well. Given the complexity of chromothriptic alterations, it will be a challenge to find a statistical approach to determine the functional targets of these alterations.

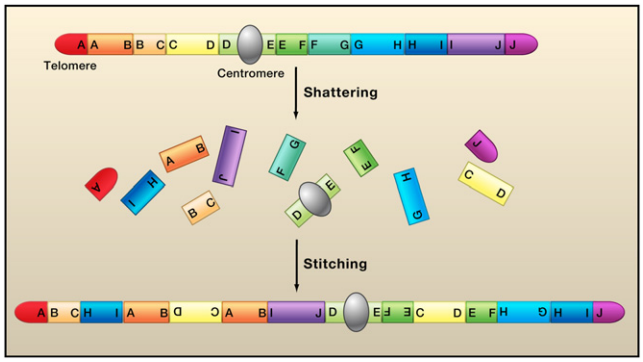

Figure 1. Stitching Together Shattered Chromosomes by Chromothripsis

Chromothripsis is proposed to involve the shattering of a single chromosome, a small group of chromosomes, or a single chromosome arm. The fragments, or a subset of the fragments, are then stitched together by nonhomologous end-joining. The mechanism by which these alterations are confined to a small segment of the genome is not defined.

The authors argue that the chromothriptic events are likely to occur in a single catastrophic event rather than a series of subsequent and random alterations. Three pieces of evidence suggest the possibility that chromothriptic changes have occurred in a single event. First, the number of copy number states found on the altered chromosome is restricted to two; under a model of progressive alterations, many copy number states would be expected. Second, in the higher copy number states, heterozygosity is preserved; if there were progressive alterations, any early occurring deletion would eliminate heterozygosity. Finally, the alterations cluster to a greater degree than would be expected from sequential alterations in the chromosome. A statistical analysis based on Monte Carlo simulations of the progressive model is also consistent with the view that the limited number of copy number states is very unlikely to have occurred by chance through sequential alteration, again arguing for a catastrophic or "punctuated equilibrium" model.

What mechanisms could produce such massive but highly localized changes in the genome? The first interesting question is how does the chromosome get shattered? One well-known mechanism by which chromosomes can be "pulverized" is premature chromosome compaction (PCC), a phenomenon that was first observed in cell fusion experiments (Rao and Johnson, 1970; Sperling and Rao, 1974). When chromosomes from an S phase nucleus are induced to undergo chromosome condensation by signals from chromosomes derived from a cell in mitosis, the incompletely replicated chromosomes from the S phase nucleus shatter. It is therefore tempting to speculate that chromothripsis could initiate during mitosis by a PCC-like mechanism.

The next question is how the fragments might be stitched together. In principal, some information about the initial shattering as well as the stitching together might be gleaned from the sequence of the junctions of the fragments on the derivative chromosome. For example, telomere fusions between sister chromatids are expected to produce a large number of head-to-head duplications (Murnane, 2006). However, chromothripsis produces highly complex derivative chromosomes that lack an identifiable signature—the segments on the derivative chromosome have been joined by a seemingly random mechanism. The sequence at the junction of each segment shows either a lack of homology or microhomology between the joined segments. Thus, the main conclusion we can draw from the sequence analysis is that the ends are likely joined by the nonhomologous end-joining DNA repair system.

Finally, we are left with the fascinating puzzle of how the pulverization is confined to one or two chromosomes or to a single chromosome arm. The underlying mechanism is unclear; however, the authors speculate that this could possibly be linked to critical telomere shortening (Pampalona et al., 2010). Short telomeres can cause chromatid fusion and the bridging of dicentric chromosomes across the cytokinetic furrow. The resolution of bridging chromosomes is known to produce nuclear protrusions and fragments that, in principle, could spatially isolate a chromosome.

Altogether, the discovery of chromothripsis by Stephens et al. reveals a new way that cancer genomes can evolve. In what appears to be a single step, numerous genes can be mutated, amplified, and rearranged. Because chromothripsis occurs in such a wide variety of tumors, the underlying mechanism is likely to reflect as yet undefined general properties of human cancer.

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