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Review

When Genome Maintenance Goes Badly Awry

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Genetic abnormalities are present in all tumor types, although the frequency and type can vary. Chromosome abnormalities include highly aberrant structures, particularly chromothriptic chromosomes. The generation of massive sequencing data has illuminated the scope of the mutational burden in cancer genomes, identifying patterns of mutations (mutation signatures), which have the potential to shed light on the relatedness and etiologies of cancers and impact therapy response. Some mutation patterns are clearly attributable to disruptions in pathways that maintain genomic integrity. Here we review recent advances in our understanding of genetic changes occurring in cancers and the roles of genome maintenance pathways.

Introduction

Cancer builds on a foundation of germline variation and constant or even explosive damage to somatic genomes. Throughout the life of an individual, DNA is damaged as a result of ongoing endogenous processes and from environmental mutagens. The sum of genomic alterations is dependent on the repair processes our cells enact to manage perturbations, the landscape of which is beginning to be illuminated by whole-genome and exome sequencing. Whole-genome sequencing also allows a more complete assessment of chromosome structural variation and a wide-angle view of the genomic landscape. Sequencing predictions have often been confirmed by molecular and cell biological approaches, which are also uncovering novel cellular mechanisms. The pace of discovery in the last few years has been remarkable and provides promise that cancer will yield to our collective advances and novel biological insights.

DNA Damage: A Range of Sources

Sources and types of DNA damage are numerous (see Ciccia and Elledge, 2010 and references therein). Sun exposure to skin is one of the best-known sources of exogenous damage, often leading to pyrimidine dimers, as is cigarette smoke, which commonly results in DNA adducts. Radiation incurred from medical scans, radiotherapy, and other sources, or, in the extreme case, radioactive fallout, can result in double-strand breaks (DSBs). DNA damage arising through endogenous processes is as or more common as that from exogenous agents, including cytosine deamination, depurination, and base oxidation and methylation. Reactive oxygen species are well-known sources of DNA damage. More recently, endogenous aldehydes, including acetaldehyde and formaldehyde, which are also by-products of cellular metabolism, have been appreciated as an important source of endogenous DNA damage in animals, such that their inadequate repair is associated with cancer predisposition and other disease states (Langevin et al., 2011; Pontel et al., 2015).

DNA replication can lead to DNA breaks, especially at structures that are difficult to replicate, and base mismatches. Base mismatches are typically kept in check by the proofreading activities of DNA polymerases; however, missense mutations in the proofreading domains of the leading (*POLE*) and lagging (*POLD1*) strand polymerases are found in some cancers with ultramutated genomes (Cancer Genome Atlas Research Network et al., 2013; Palles et al., 2013; Seshagiri, 2013). Repair of the various lesions encountered in DNA involves specialized, often well-characterized pathways, including nucleotide and base excision repair, mismatch repair (MMR), nonhomologous end-joining (NHEJ), and homologous recombination (HR).

Because both strands of the DNA helix are disrupted, DSBs are considered particularly dangerous lesions that can jeopardize the stability of the genome. However, DSBs are intermediates in certain developmental programs, in particular during antigen receptor rearrangement and class switch recombination (Alt et al., 2013; Casellas et al., 2016). Astoundingly, during meiosis, which is key to the transmission of the genome, a couple hundred DSBs are introduced genome-wide (Cole et al., 2010), indicating that cells are able to repair high DNA damage loads with accuracy at least under some circumstances. Accurate repair may rely on particular aspects of the pathways involved in the repair of programmed DSBs, such as tight binding of the RAG recombinase to DNA ends during antigen receptor rearrangement; however, errors in the repair processes can give rise to oncogenic lesions (Alt et al., 2013; Casellas et al., 2016).

More recently, DSBs have been suggested to occur during another physiological program, i.e., during the rapid expression of immediate-early genes in response to neuronal activity (Madabhushi et al., 2015). DSBs in the promoters of these activity-induced genes, which are likely generated by topoisomerase IIβ, have been hypothesized to relieve torsional stress within topological domains to promote a rapid transcriptional response. Topoisomerase IIβ-generated DSBs in another context—nuclear receptor-induced genes—have been implicated in oncogenic rearrangements in prostate cells (Haffner et al., 2010; Lin et al., 2009). Within neuronal cells, recurrent DSBs have also been observed in genes rearranged in some cancers (Lyu et al., 2006; Wei et al., 2016).

A surprising source of exogenously induced DSBs is from microbial invaders (e.g., Nougayrède et al., 2006), such as from

Helicobacter pylori (Toller et al., 2011), which is associated with gastric cancer. In this case, DSBs appear to arise through nucleotide excision repair pathway components (Hartung et al., 2015). Microbial invaders can also lead to other types of DNA damage. Members of the APOBEC family of cytidine deaminases, which is involved in the defense against retroelements, can be induced by viral infection (Chan and Gordenin, 2015), for example, by HPV infection, which is associated with head and neck and cervical cancers. These tumors exhibit an overall high rate of mutations expected by APOBEC induction as well as specific mutations in genes linked to tumorigenesis, including *PIK3CA* (Henderson et al., 2014; Vieira et al., 2014).

Explosive Events that Alter the Genome: Chromothripsis and Kataegis

A long-standing tenet in cancer etiology has been that mutations accumulate gradually over an extended period of time (see e.g., Jones et al., 2008). However, the advent of more advanced genome sequencing technologies has provided evidence that the relatively constant mutation rate may be interrupted by squalls of instability. Chromothripsis is a recently identified mutational process in which specific chromosomal regions undergo catastrophic shattering characterized by extensive genomic rearrangements (Stephens et al., 2011). Chromothriptic chromosomes can have dozens or hundreds of chromosome segments from one or a few chromosomes stitched together in random order and orientation with oscillating copy numbers (Korbel and Campbell, 2013). They have been observed in multiple tumor types and, surprisingly, even constitutionally in rare individuals (Kloosterman et al., 2012; Weckselblatt et al., 2015). Estimates are that up to 5% of tumors show evidence of chromothripsis, although some tumor types have higher frequencies (Kloosterman et al., 2014; Malhotra et al., 2013; Stephens et al., 2011). Chromothripsis can lead to disruption of tumor suppressor genes, oncogenic gene fusions, and oncogene amplification (Kloosterman et al., 2014; Leibowitz et al., 2015). Massive amplification associated with chromothripsis may involve double minute formation from excised fragments and subsequent reintegration as homogeneously staining regions (e.g., including the *MYC* locus) (Rausch et al., 2012; Stephens et al., 2011).

Two recent studies have provided possible mechanisms that could give rise to chromothripsis. Pellman and colleagues hypothesized that one route may involve DNA micronucleus formation, when the nuclear envelope reforms around chromosomes or chromosome fragments that become separated from the main chromosome complement during mitotic exit (Crasta et al., 2012). An attractive feature of this model is the physical isolation of DNA in micronuclei from bulk genomic DNA. Further, DNA in micronuclei undergoes breakage and extensive fragmentation (“pulverization”) likely due to asynchronous replication and collapse of the nuclear envelope (Crasta et al., 2012; Hatch et al., 2013). However, eventually micronuclear DNA can return to the nucleus for subsequent transmission to daughter cells. More recently, the complex genomic rearrangements consistent with chromothripsis have been confirmed by single-cell sequencing, providing evidence that chromothripsis occurs within a single-cell cycle and so is an episodic mutational phenomenon (Zhang et al., 2015). Of note, evidence for double minute formation during chromothriptic events was also provided.

Another new study from de Lange and colleagues has suggested that chromothripsis occurs as a consequence of telomere crisis resolution in the early stages of tumorigenesis (Maciejowski et al., 2015). Dicentric chromosomes that arise due to dysfunctional telomeres can form long-lived chromatin bridges that lead to nuclear envelope rupture during interphase (“NERDI”). Dicentrics have traditionally been thought to break due to forces pulling the chromosomes to opposite poles; however, Maciejowski et al. demonstrate that the cytoplasmic nuclease TREX1 localizes to chromatin bridges and gives rise to RPA-coated single-stranded DNA. Following telomere crisis resolution, clusters of genomic rearrangements were observed consistent with chromothripsis.

Disease causality or disease progression attributed to chromothripsis is difficult to determine with certainty. Attribution has been inferred in glioblastoma multiforme based on short latency, aggressive tumor biology, and high prevalence of chromothripsis (Malhotra et al., 2013). In childhood retinoblastoma, chromothripsis was identified as the mechanism of *RB1* loss, due to complex structural variation on chromosome 13 missed by conventional analysis (McEvoy et al., 2014), suggesting causality. In prostate cancer, the incidence of chromothripsis has been reported to be high, but it was of similar prevalence in both low-grade tumors that do not progress and aggressive high-grade tumors (Kovtun et al., 2015). This study identified no difference in clinical outcome that associated with the presence or absence of chromothripsis, and chromothriptic events did not involve the most common genetically altered drivers associated with prostate cancer. Thus, the data in prostate cancer suggest that chromothripsis is not related to cancer progression but may be related to cancer initiation given its relatively common occurrence in low-grade tumors. Chromothripsis is associated with poor prognosis in some reports, but evidence that its presence is an independent prognosticator is limited (Molenaar et al., 2012; Rausch et al., 2012).

A second catastrophic process is clustered mutagenesis, sometimes termed kataegis, which is typically associated with chromosomal rearrangements (Nik-Zainal et al., 2012; Roberts et al., 2012). Up to 50% of some tumor types show evidence of kataegis (Chen et al., 2014; Nik-Zainal et al., 2012). The mutations typically involve C to T transitions in TpC dinucleotides that arise from APOBEC3A/B acting on single-stranded DNA; in B cell lymphomas, a related APOBEC family member, AID, is also implicated in mutagenesis but at a distinct motif (Casellas et al., 2016; Chan and Gordenin, 2015) (Figure 1A). Single-stranded DNA can arise in cells through several cellular processes, including DNA replication, especially lagging strand synthesis, and end resection during DSB repair, to become a target for APOBECs (Chan and Gordenin, 2015; Haradhvala et al., 2016; Hoopes et al., 2016; Kazanov et al., 2015; Seplyarskiy et al., 2016). Further, the chromothriptic chromosomes described above that arose from telomere fusions show the characteristic hypermutation pattern of kataegis, suggesting that the single-stranded DNA in chromatin bridges is processed by APOBEC-mediated cytosine deamination (Maciejowski et al., 2015).