

Chapter 2

The Generation, Detection, and Prevention of Genomic Instability During Cancer Progression and Metastasis

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Abstract Genome stability is tightly regulated through the cell cycle. Aberrations in genome structure and sequence are a hallmark of malignancy and these changes can allow abnormal cells to escape the regulatory mechanisms that would otherwise direct these cells into apoptosis or senescence. When genome instability occurs, it can happen as large or small structural changes in the genome, changes in gene expression, or even changes at the epigenetic level. There are many environmental factors that can induce DNA damage and strain the machinery that is responsible for maintaining genome stability. In some cases, such as UV light or chemical carcinogens, it is possible to avoid these factors and thus reduce the risk of cancer. But, in other instances, hereditary mutations impair the function of genes and their products, which normally protect the stability of the genome. While genomic instability offers selective advantages to the tumor, the tumor-specific loss of these pathways may provide therapeutic opportunities, which could be personalized through knowledge of the specific types of genomic instability that characterize an individual's tumor.

Keywords Genomic instability · Epigenome stability · DNA damage

Abbreviations

BER	Base excision repair
BFB	Break fusion break
CDK	Cyclin dependent kinase
CGH	Comparative genomic hybridization
CIN	Chromosome instability
CpG	C-phosphate-G
CRC	Colorectal cancer

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DDR	DNA damage response
DNA	Deoxynucleic acid
DSB	Double strand break
EMT	Epithelial to mesenchymal transition
HDR	Homology directed repair
LOH	Loss of heterozygosity
MET	Mesenchymal to epithelial transition
MIN or MSI,	Microsatellite instability
MMR	Mismatch repair
mtDNA	Mitochondrial DNA
NER	Nucleotide excision repair
NHEJ	Non-homologous end joining
NIN	Nucleotide instability
PCR	Polymerase chain reaction
SAC	Spindle assembly checkpoint
UV	Ultraviolet

Introduction

Cancer is a genetic disease. Tumor cells contain multiple mutations, ranging from single nucleotide changes to large-scale structural and numerical alterations of chromosomes. Collectively, these mutations are referred to as genome instability, which may be predisposed through inherited, germ-line mutations, as in the case of *p53*, *BRCA1* and *BRCA2*, or acquired as somatic mutations throughout an individual's lifetime. Among the currently known cancer causing genes, 82 are associated with germ-line mutations, 474 are associated with somatic mutations and 513 are associated with chromosomal alterations [1]. These mutations promote cell growth, inhibit cell death and are propagated through accelerated cell division. Moreover, specific genetic alterations among cancer cells can give rise to sub-populations of cells with growth advantages, as in the case of a metastatic cancer cell or a cancer stem cell. These aggressive cells often arise during later stages of tumorigenesis, and their genome may significantly differ from the initial tumor population. However, regardless of how or when these genetic mutations are acquired, alteration of critical genes can change a once-normal cell into a cancerous cell that divides uncontrollably, and additional genomic changes may allow them to gain further growth advantages and eventually spread throughout the body. Thus, the molecular process behind tumorigenesis can be viewed as the accumulation and evolution of genomic alterations.

During the life cycle of a normal cell, genome maintenance is tightly regulated to prevent neoplastic transformation or tumorigenesis. There are various caretaker processes throughout the cell cycle that strategically minimize genome instability, including high-fidelity DNA replication, accurate chromosome segregation and cell

cycle checkpoints. In addition, other mechanisms, such as DNA damage response (DDR) pathways, telomere stability and epigenome maintenance, prevent non-mutational genetic changes. Defects in these gene products and processes will compromise their ability to monitor genomic alterations and execute the appropriate damage responses, which include repair, induction of senescence or apoptosis. In most cases, alteration of a particular gene product is not sufficient for transformation but may fast-track a subset of pre-cancer cells to acquire additional genomic changes that allow them to gain further growth advantages. Accumulation rates and the types of genomic alterations may vary in distinct subsets of cancer cells, contributing to the heterogeneity observed in cancer.

The notion of genomic instability as a hallmark of malignancy has intrigued cancer biologists for over a century, from Theodore Boveri's hypothesis that highlighted chromosomal aberrations as the cause of cancer [2, 3], to the discovery of the "Philadelphia chromosome" that leads to the activation of the *Abl* gene [4] and then the identification of the first familial breast cancer susceptibility gene, *BRCA1* [5, 6]. High-resolution arrays, such as comparative genomic hybridization (CGH) and whole-genome sequencing, have identified recurrent alterations as well as genomic heterogeneity within clinically similar cancers. For example, genomic and epigenomic profiling allows for more precise classification of breast cancer subtypes, as well as the prediction of subtype-specific therapeutic targets [7].

However, despite our technological advances, we are still baffled by questions posited following Boveri's observation of genetic imbalances in sea-urchin eggs, such as: (1) how and when does genome instability occur?; (2) how many mutations does it take to cause cancer?; (3) is genome instability the driver for tumorigenesis, or simply a passenger of disease progression?; (4) what role does genome instability play in cancer evolution and metastasis?; and (5) how do we take advantage of this shared trait of all cancer cells to uncover new paradigms for prevention, diagnosis and responsive therapy?

Genomic Instability in Cancer

Genomic instability is a characteristic of all cancers and encompasses a variety of genetic alterations ranging from single nucleotide differences to large-scale changes at the chromosomal levels [8]. Genomic instability can be divided into three categories based on the degree and type of genetic alteration.

a. Nucleotide Instability (NIN)

Nucleotide instability includes base substitutions, deletions and insertions of one or a few nucleotides. These alterations result when errors occur during DNA replication or when the repair machinery malfunctions, such as nucleotide excision repair (NER) and base excision repair (BER) [8]. These alterations can cause dramatic

changes to gene structure and expression. For example, missense mutation in the *K-ras* gene occur in over 80 % of primary exocrine pancreatic tumors and their corresponding metastases [9]. NIN may also arise in mitochondrial DNA (mtDNA), and instability of mtDNA occurs in a variety of human cancers, including colorectal (CRC), gastric and lung [10].

b. Microsatellite Instability (MIN or MSI)

Microsatellites are short, two to six base-pair simple, or tandem, sequence repeats of DNA located throughout the genome. MIN occurs when the DNA mismatch repair (MMR) system is impaired, which results in the expansion, contraction, deletion and random insertion of microsatellites [11]. The MMR system identifies and binds to the mismatch, and excises the erroneous nucleotide and repairs the mismatch. MIN has been documented in a variety of cancers, including gastric, ovarian, lung, endometrial, and CRC [12–17]. To date, five MIN markers have been recommended by the National Cancer Institute for disease screening in patients susceptible to Lynch syndrome. MIN occurs in approximately 15 % of all CRC, which include both hereditary and sporadic forms of CRC, and are associated with a better prognosis than non-MSI tumors [18].

c. Chromosomal Instability (CIN)

CIN is the most prevalent form of genomic instability, observed in over 90 % of all malignancies, and is detected throughout the entire neoplastic transformation process, from premalignant lesions to metastatic lesions [19]. For example, chromosome 10 is often lost in glioblastomas, resulting in the inactivation of the tumor-suppressor gene, *PTEN* [20]. CIN refers to alterations of segments of chromosomes, or whole chromosomes, in terms of their structure or number, including amplifications, deletions, translocations, insertions, inversions, loss of heterozygosity (LOH) and homozygous deletions [8]. Change in chromosome numbers is a condition known as aneuploidy, while chromosome translocation involves the fusion of different chromosomes, or of two distant segments on the same chromosome, resulting in a chimeric chromosome [8]. Finally, the ploidy of the entire genome may deviate from the standard 2N complement of chromosomes and give rise to polyploid cells. CIN in tumor cells alters the expression of thousands of genes, which may help to explain why CIN tumors have a poorer prognosis than either MIN or NIN tumors [21].

Telomere Maintenance in Cancer

Telomeres are unique G-rich repetitive sequences (TTAGGG) located at the ends of the eukaryotic chromosomes [22, 23]. Telomeres protect the ends of the chromosomes and preserve their integrity [24]. As telomeres will gradually shorten with

each round of cell division due to chromosome end-processing, telomere maintenance is necessary for continuous cell division and the addition of telomeric repeats must be catalyzed by telomerase reverse transcriptase (hTERT) [25]. In most somatic cells, insufficient telomerase activity will lead to telomere shortening and the induction of cellular senescence [26]. Thus, telomerase inhibition may be a promising target in cancer therapy [27–29].

Inhibition of telomerase activity in a variety of cancer cell lines resulted in accelerated telomere shortening, cell death and differentiation [27–29]. However, due to mutations in the tumor suppressor p53, cancer cells frequently bypass senescence, and continue to divide, which promotes genome instability due to chromosome fusions [24]. Telomere shortening will signal the DDR pathway [30, 31], which may promote CIN, tumor initiation and progression [32, 33]. As an example, telomerase knockout (*mTR*^{-/-}) mice significantly increase the incidence of spontaneous tumor formation (4–6 fold compared to the wild-type population) due to telomere shortening [34]. These *mTR*^{-/-} tumors have 3–18 fold more chromosome fusions and a two-fold increase in aneuploidy compared to *mTR*^{+/+} tumors [34]. Conversely, telomerase activity also promotes tumorigenesis [35]. Thus, telomere-associated tumorigenic processes are stage specific; telomere shortening is critical to the accumulation of genetic mutations needed for cancer initiation while, in later stages, telomerase activity promotes cell proliferation necessary for cancer expansion and metastasis.

Epigenome Instability in Cancer

Epigenetics is defined as all heritable changes that may modify gene expression without affecting the primary DNA sequence, such as DNA methylation and chromatin remodeling. DNA methylation and histone modification are the most well understood epigenetic processes, and preservation of these epigenetic markers during cell division is vital for gene regulation. In cancer, epimutations may result in dysregulation of critical genes either independently, or in conjunction with deleterious genetic mutations. Moreover, these epimutations are inherited through clonal expansion, which can promote cancer initiation and progression [36, 37]. Although it is unclear whether these epigenetic alterations are causative or a consequence of tumorigenesis, it is certain that epigenome instability is a prominent feature in cancer.

a. DNA Methylation in Cancer

DNA methylation is a covalent modification where a methyl group is added to the carbon-5 position of cytosine nucleotides followed by a guanine (CpG) via a group of DNA methyltransferase (DNMT) enzymes [38]. DNA methylation can result in gene silencing, and occurs primarily at CpG islands within heterochromatin [38]. An individual cell's DNA methylation pattern is important for its ability to establish

tissue-specific gene expression or maintain pluripotency. Global DNA hypomethylation and site-specific hypermethylation are two key epimutations that occur in cancer [36]. DNA hypomethylation can lead to aberrant overexpression of oncogenes, such as *R-Ras* in gastric cancer, *S-100* in colon cancer [39] and *IGF2* in Wilms' tumor [40]. Hypomethylation of retrotransposons and specific repeat sequences can also result in genomic instability by promoting chromosome rearrangements [41, 42]. Global hypomethylation in many cancers, such as in the breast, brain and cervix, is positively correlated with increased grades of malignancy [43]. On the other hand, hypermethylation contributes to tumorigenesis by silencing the transcription of tumor suppressor genes, such as *Rb*, *BRCA1* and *p16* [36], which may act as a second hit as described by Knudson's hypothesis [44].

b. Histone Modification in Cancer

At the chromatin level, modifications of the four core histones, H2A, H2B, H3 and H4, regulate gene expression. These histone modifications include acetylation, deacetylation, methylation, phosphorylation and ubiquitination [45]. For example, methylation of histone H3 lysine 9 (H3K9), H3K27, H4K20, and H3 arginine 2 (R2) are indicative of heterochromatin formation and transcriptional repression, while acetylation of H3 and H4 promote euchromatin formation and gene transcription [45].

The global loss of H4K16ac and H4K20me3 has been recognized as a hallmark of almost all human cancers [46], while other changes in histone modification are used as prognostic markers [47–51]. These epigenetic marks are catalyzed by various histone-modifying enzymes, such as lysine methyltransferases, arginine methyltransferases, serine-threonine kinases, histone deacetylases and acetyltransferases [45]. Aberrant expression of these enzymes results in changes in histone modification, which can dysregulate subsequent DNA repair, gene transcription and growth-promotion. For example, histone deacetylase 1 (HDAC1) is reduced in gastric cancer, while reduced HDAC5 and HDAC10 associate with poor prognosis in lung cancer [52]. Changes in histone modification patterns can also lead to an overall change in the chromatin structure, which increases the risk of translocation of random transposons and chromosome breakage during mitosis.

c. Nucleosome Remodeling in Cancer

Nucleosomes are the basic unit for DNA packaging, and the architecture of these nucleosomes determines chromatin structure and the accessibility of regulatory DNA sequences to transcription factors [53]. Nucleosome remodeling includes the repositioning of the nucleosome, as well as changes in the content of histone proteins within the nucleosome [53]. In a cancer epigenome, the sliding of pre-existing nucleosomes and incorporation of new nucleosomes, determines chromatin

accessibility and gene expression [53]. For example, in hereditary nonpolyposis colon cancer, three *de novo* nucleosomes are present within the promoter CpG island of the *MLH1* gene, which is a homolog of the *E. coli* DNA mismatch repair gene [54]. Moreover, substitution of the canonical histone proteins with non-canonical histone proteins within the nucleosome can also influence nucleosome occupancy and gene expression [53, 54].

Crosstalk Between Genomic and Epigenomic Instability

Despite the differences between DNA mutations and epimutations in cancer, their effects on gene regulation are ultimately the same. Indeed, these two processes work symbiotically and synergistically with genetic mutations in epigenetic regulators resulting in epimutations, and vice versa. For example, hypermethylation of key tumor suppressor genes, such as *Rb*, *PTEN*, *BRCA1* and *MLH1*, is a common phenomenon observed in cancers [54, 55]. On the other hand, genetic mutations of key epigenetic modifiers, such as different DNMTs, histone modification proteins and chromatin remodeling proteins, can also impair epigenome stability [56]. Both genomic instability and epigenomic instability are phenomena observed in almost all cancers at every stage of cancer evolution [56, 57]. The crosstalk between these two phenomena adds complexity to cancer biology but also offers potential novel therapeutic targets [58, 59].

Mechanisms for Genome and Epigenome Stability

For a normal cell, the end goal of cell division is to accurately duplicate its genome and distribute its genetic material evenly between the two daughter cells. To maintain genome integrity during proliferation, four major mechanisms are in place: (1) high-fidelity DNA replication during S-phase; (2) accurate chromosome segregation during mitosis; (3) sporadic DDR throughout the cell cycle; and (4) quality control checkpoints regulating cell cycle progression (Fig. 2.1).

a. Error-Free DNA Replication

The duplication of the genome during S-phase is under tight regulation to ensure copy number and temporal (once per cell cycle) control, known as replication licensing [60, 61]. Replication licensing is highly conserved throughout evolution and is regulated by cyclin dependent kinases (CDKs). Replication is initiated with the assembly of a pre-replication complex assembled at the replication origin. Untimely initiation can cause re-replication and aneuploidy, and low replication-initiation density can lead to unfinished replication of the whole genome [62]. During

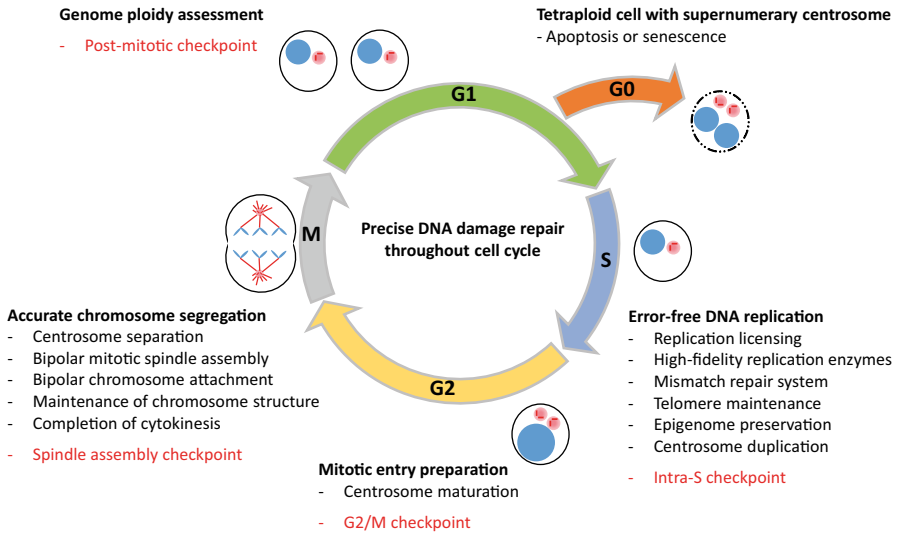


Fig. 2.1 Mechanisms to prevent genome instability throughout the cell cycle. The cell employs many mechanisms through the cell cycle to prevent genome instability. The important processes that occur during each phase of the cell cycle are highlighted in *black* while cell cycle checkpoints are highlighted in *red*

the extension phase of DNA replication, replication forks often pause and restart to ensure replication completion. Failure to restart these replication forks will result in double-strand breaks, single-strand DNA breaks and hyper-recombination, which all need to be resolved by the DDR pathway before mitotic entry to maintain genome stability [62]. Replication on the lagging strand occurs through the production of multiple short Okazaki fragments, these fragments need to be ligated to maintain the structure of the lagging strand [63]. Moreover, there is an RNA primer and a short DNA segment at the 5' end of each Okazaki fragment, which all needs to be removed prior to ligation of the Okazaki fragment [63]. Failure to remove these elements affects Okazaki fragment maturation, and can result in genomic alterations [63]. El-Khamisy and colleagues (Chapter 3) provide an in-depth discussion of the molecular control of DDR pathways.

b. Bipolar Spindle Assembly and Accurate Chromosome Segregation During Mitosis

After error-free DNA replication during S-phase, genome stability requires accurate chromosome segregation during mitosis. Chromosome segregation is a multi-step process, which requires faithful centrosome duplication, assembly of a bipolar mitotic spindle, proper attachment between the chromosomes and the mitotic spindle, and completion of cytokinesis. Dysregulation of any of these processes may result in aneuploidy.

The centrosome is the primary microtubule organizing center in the eukaryotic cell and is composed of a pair of centrioles surrounded by a cloud of proteins that promote microtubule nucleation and cilia formation [64, 65]. During mitosis, the centrosome directs mitotic spindle formation at the poles, which contributes to faithful chromosome segregation and cleavage furrow formation [65, 66]. Since the mitotic spindle is a bipolar structure, the centrosome must be duplicated only once during every cell cycle and in a semi-conservative manner. Thus, centrosome duplication occurs simultaneously with DNA replication during S-phase, and is tightly regulated.

Centrosome amplification, the presence of more than two centrosomes, is a common characteristic of almost all cancers, which frequently accompanies aberrant chromosome numbers [67]. The presence of supernumerary centrosomes during mitosis is problematic as it can cause multipolar mitoses, chromosome missegregation, cytokinesis failure and subsequent chromosome imbalances that promote tumorigenesis [67]. Due to dysregulation of various tumor suppressors and oncogenes, supernumerary centrosomes can arise from centrosome overduplication, *de novo* assembly, or previous mitotic failure [64, 67–70]. Centrosome duplication errors result from multiple daughter centrioles forming around a single mother centriole, or multiple rounds of centrosome duplication and paired centriole duplication [64, 67–70]. Extra centrosomes can also be made from *de novo* assembly, where an acentriolar centrosome is made without a pre-existing centriolar centrosome as the template [71–73]. Various mitotic catastrophes can also result in supernumerary centrosomes. For example, loss of mitotic spindle integrity due to inadequate cross-linking by microtubule-associated proteins can cause a centrosome to split with each individual centriole capable of functioning as a spindle pole, or the formation of an acentriolar spindle pole [74–79]; aborted cytokinesis, mitotic slippage and DNA damage are additional mechanisms for cancer cells to obtain extra centrosomes [80].

The presence of supernumerary centrosomes may not be good news even for cancer cells, as catastrophic aneuploidy can result in nonviable daughter cells [81]. However, cancer cells have developed mechanisms that overcome this fate by clustering multiple centrosomes [70, 81–84]. These mechanisms can dampen high level aneuploidy and extreme CIN to avoid programmed cell death [85, 86]. Given that centrosome clustering may be advantageous for the survival of cancer cells, this process may be an attractive and specific therapeutic target [83, 84, 87].

The mitotic spindle, a microtubule-based bipolar structure, is the cellular machinery responsible for the distribution of genetic material between the progeny cells. The mitotic spindle will capture the chromosomes at the kinetochores, align them along the cell's equator, and then pull them towards each spindle pole. Mitotic spindle integrity is a vital tumor suppressor pathway that requires microtubule crosslinking and motor protein movements to establish spindle length, position and orientation [88–92]. Bipolar chromosome attachment generates tension across the sister kinetochores, and this signals for chromatid separation and mitotic progression. Erroneous attachments engage the spindle assembly checkpoint (SAC), which prevents the onset of anaphase until the errors are corrected. However, if the SAC

fails to sense the misattached chromosome, the misattached/unattached chromatid will lag behind, which could mean the loss or gain of whole or part of a chromosome in the daughter cells [93, 94].

Cytokinesis partitions the cytoplasm of the mother cell between the two daughter cells concurrent with nuclear membrane formation. The process of cytokinesis includes the specification of the cleavage plane, ingression of the cleavage furrow, and abscission of the midbody, which are all heavily dependent on microtubule and actin networks. Cytokinesis failure may occur when mitotic spindle elongation and positioning are perturbed, thus disrupting delivery of activation signals to the cortex for cleavage plane formation [95]. Aborted cytokinesis will result in a cell with double the genetic material (tetraploid) and double the number of centrosomes. This tetraploid state is considered an intermediate for the aneuploid state frequently observed in cancer cells [96], and the fate of these tetraploid cells heavily depends on p53 [97–100].

Telomere-associated genome instability occurs when inappropriate DNA repair (i.e. non-homologous end joining (NHEJ) and homology directed repair (HDR)) takes place at dysfunctional telomeres. Inappropriate NHEJ produces dicentric chromosomes [30, 31], which are highly unstable with a tendency to break during mitosis [30, 31]. Repair of these new breaks can propagate new dicentric chromosome formation [30, 31]. This process is called the breakage-fusion-bridge (BFB) cycle, which can continue over multiple cell divisions and generate complex chromosomal rearrangements [101, 102]. This BFB cycle generally has three outcomes: (1) LOH due to breakage sites or asymmetric segregation of chromosomes after breakage; (2) gene amplification due to breakage sites; and (3) unbalanced translocations [30]. During HDR, inversions, deletions and translocations are generated when recombination occurs at a highly homologous stretch of telomeric DNA either on the same chromosome or between two different chromosomes [30]. Activation of the HDR pathway increases the formation of anaphase bridges, which must be resolved to prevent cytokinesis failure and aneuploid progenies [103]. El-Khamisy and colleagues (Chap. 3) provide an in-depth discussion of the molecular control of NHEJ and HDR pathways.

c. Cell Cycle Checkpoints

Cell cycle checkpoints are the cell's quality control mechanisms that coordinate the progression of the cell cycle and delay entry into the next stage in the presence of genome damage. Under circumstances where the detected genome damage is not fixed, these checkpoints will trigger senescence, mitotic catastrophe or apoptosis to eliminate high-risk cells. The G1/S (and post-mitotic) and G2/M checkpoints can recognize DNA lesions and abnormal chromosome structures, whereas the spindle assembly checkpoint is an intra-mitosis surveillance mechanism that monitors the interaction between chromosomes and the mitotic spindle.

The post-mitotic checkpoint monitors the state of the genome after the previous round of the cell cycle, and delays replication in the presence of damaged DNA.

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