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REVIEW

Chromosome instability and deregulated proliferation: an unavoidable duo

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Abstract The concept that aneuploidy is a characteristic of malignant cells has long been known; however, the idea that aneuploidy is an active contributor to tumorigenesis, as opposed to being an associated phenotype, is more recent in its evolution. At the same time, we are seeing the emergence of novel roles for tumor suppressor genes and oncogenes in genome stability. These include the adenomatous polyposis coli gene (*APC*), p53, the retinoblastoma susceptibility gene (*RB1*), and Ras. Originally, many of these genes were thought to be tumor suppressive or oncogenic solely because of their role in proliferative control. Because of the frequency with which they are disrupted in cancer, chromosome instability caused by their dysfunction may be more central to tumorigenesis than previously thought. Therefore, this review will highlight how the proper function of cell cycle regulatory genes contributes to the maintenance of genome stability, and how their mutation in cancer obligatorily connects proliferation and chromosome instability.

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Abbreviations

APC	Adenomatous polyposis coli
CDK	Cyclin dependent kinase
CIN	Chromosome instability
DDR	DNA damage response
LXCXE	Leucine-any amino acid-cysteine-any amino acid-glutamate
MMTV	Mouse mammary tumor virus promoter
OIS	Oncogene induced senescence
PARP	Poly ADP ribose polymerase
RB	Retinoblastoma
S-CIN	Segmental chromosome instability
W-CIN	Whole chromosome instability

Introduction

Genome instability is a broad term used to describe the failure of a cell to accurately pass on a copy of its genome to its daughter cells. There are several mechanisms by which this can occur, and these have been grouped into three broad categories. Microsatellite instability is caused by defective mismatch repair that leaves DNA replication errors uncorrected [1–3]. Nucleotide excision repair-related instability arises from defects that prevent removal and replacement of UV-damaged nucleotides [4–6]. The third type of instability, which will be the focus of this review, is chromosome instability (CIN), which can be further dissected into two types, whole chromosome instability (W-CIN) and segmental chromosome instability (S-CIN) (delineated by Geigl et al. [7]). W-CIN arises through the

gain and/or loss of whole chromosomes, which, if sustained through successive cell divisions, results in aneuploidy. Additionally, smaller regions of gain or loss, or changes in chromosome structure that do not result in copy number alterations, such as translocations or inversions, are broadly termed S-CIN [7]. A more in-depth discussion of the mechanisms by which CIN arises can be found in recent reviews by Aguilera et al., Holland et al., Tanaka et al., and Schvartzman et al. [8–11].

The concept that aneuploidy is a characteristic of malignant cells was first suggested by the work of von Hansemann and Boveri [12–14]. However, this theory was not verified until the early 1950s, when Sajiro Makino, and Levan and Hauschka among others [15–19] demonstrated that malignant cells have a unique chromosome compliment compared to their normal counterparts. Since then, chromosome instability has been observed to be tolerated, and even selected for, in many malignant cell types [20, 21]. Originally, such chromosome instability was thought to be a by-product, or a passenger that accompanied tumorigenesis. In other words, it was a cancer-associated phenotype, not a cancer-causing mechanism (Fig. 1a). However, in recent years, it has become evident that chromosome instability may exhibit a more causative role in the transformation of a normal cell into one that becomes cancerous (Fig. 1b). This shift in thought has been supported by several mouse models in which alterations of the spindle assembly checkpoint lead to higher than normal chromosome segregation errors, and offer proof of principle that chromosome instability alone can be the root cause of spontaneous tumors in mammals (reviewed in [11, 22]). In addition, the combination of these spindle assembly defects with other genetic lesions can enhance tumorigenesis, further demonstrating that CIN can stimulate progression of the disease [22]. Moreover, chromosome instability phenotypes are caused by mutations in tumor suppressor genes whose primary function resides in maintaining genome stability through repair and damage checkpoints, and/or the spindle assembly checkpoint, such as *BRCA1*, BubR1, and others [23–28]. These tumor suppressors, along with recently reported massive chromosome rearrangements (chromothripsis) that are evident in initial disease, and even in relapse [29], further argue that defects in chromosome stability can be central to cancer pathogenesis.

In a manner similar to our shift towards viewing chromosome instability as an active contributor to cancer as opposed to being an associated phenotype, our understanding of many well-known oncogenes and tumor

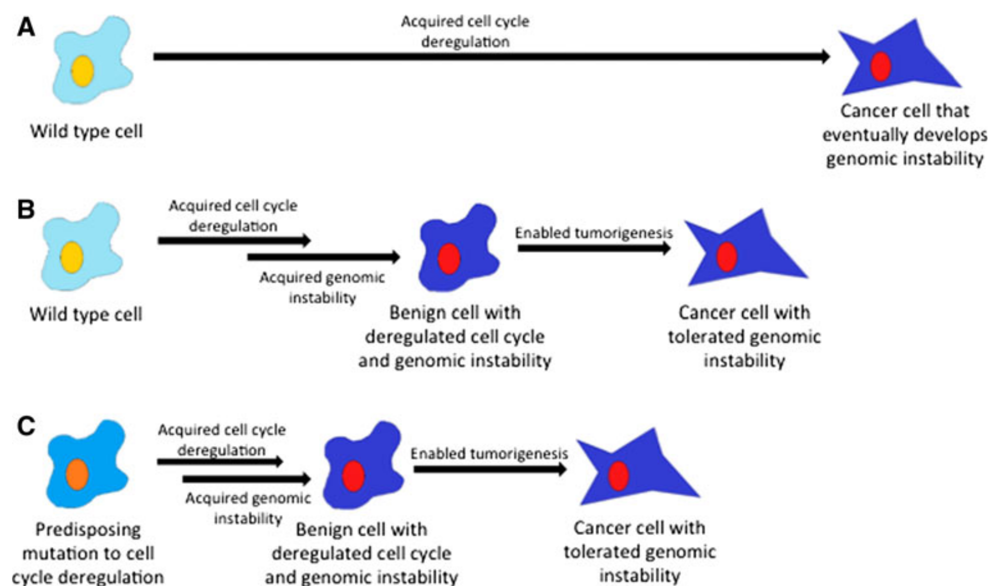

Fig. 1 Schematic diagram of cell cycle deregulation, genomic instability, and tumorigenesis. **a** Originally, genomic instability was thought of as a cancer associated phenotype—over time, a cell would randomly acquire cell cycle deregulation, leading to cancer formation. Then, as a result of uncontrolled proliferation, genomic instability would inherently arise in these cancer cells. **b** As discussed in this review, it is becoming apparent that deregulated cell cycle control can compromise genome stability. If the instability is tolerated, in combination with uncontrolled proliferation, a cell may acquire mutations more readily that enhance its tumorigenic potential (e.g., acquire mutations that help the cell evade apoptosis, initiate angiogenesis, and acquire other hallmarks of cancer cells). This in turn facilitates tumorigenesis in a shorter time frame, and, in theory, more quickly than if genome instability was acquired after a cell has become cancerous as shown in (**a**). **c** This may be even more true of those individuals born with a predisposing mutation that causes cell cycle deregulation, and, as a consequence, genomic instability. Because these individuals acquire cell cycle deregulation early on, they also have genomic instability, and are predisposed to tumorigenesis at a much earlier age in life. Examples of predisposing mutations are those in APC that lead to familial adenomatous polyposis (FAP), those in p53 that cause Li-Fraumeni syndrome, and mutations in *RB1* that cause retinoblastoma

suppressors have followed a similar path. The adenomatous polyposis coli gene (*APC*), p53, and the retinoblastoma susceptibility gene (*RB1*) were all initially discovered to function in growth control [30–36]. While this remains true, our expanding knowledge of these genes has revealed roles for them in the maintenance of genome stability and, in many cases, specifically in chromosome stability. As a result, chromosome instability caused by common genetic lesions in cancer may be more central to the process of tumorigenesis than is currently estimated (Fig. 1c).

In this review, we will present several examples whereby common tumor suppressor genes or oncogenes, with well-characterized roles in cell cycle control and cancer pathogenesis, also function to maintain a stable genome (Fig. 2). We will highlight how these genes are best known for regulating proliferation, and how they facilitate chromosome stability. In addition, we will explore how chromosome instability enhances the tumorigenic potential beyond deregulated proliferation that is caused by loss of these tumor suppressors, or gain of these oncogenes (Fig. 1c). Lastly, we will conclude by suggesting circumstances during which chromosome instability caused by these mutations may be exploited in the ongoing search for new cancer therapeutic strategies.

Adenomatous polyposis coli

Cytoplasmic growth regulator and mitotic spindle component

APC was identified through linkage analysis as the gene responsible for familial adenomatous polyposis, a type of colon cancer [37, 38]. In addition, *APC* is mutated in most cases of sporadic colon cancer [39]. Investigation into the functions of the APC protein product revealed that it is a cytoplasmic, β-catenin interacting protein [30, 31]. In the absence of stimulation by Wingless family ligands, APC

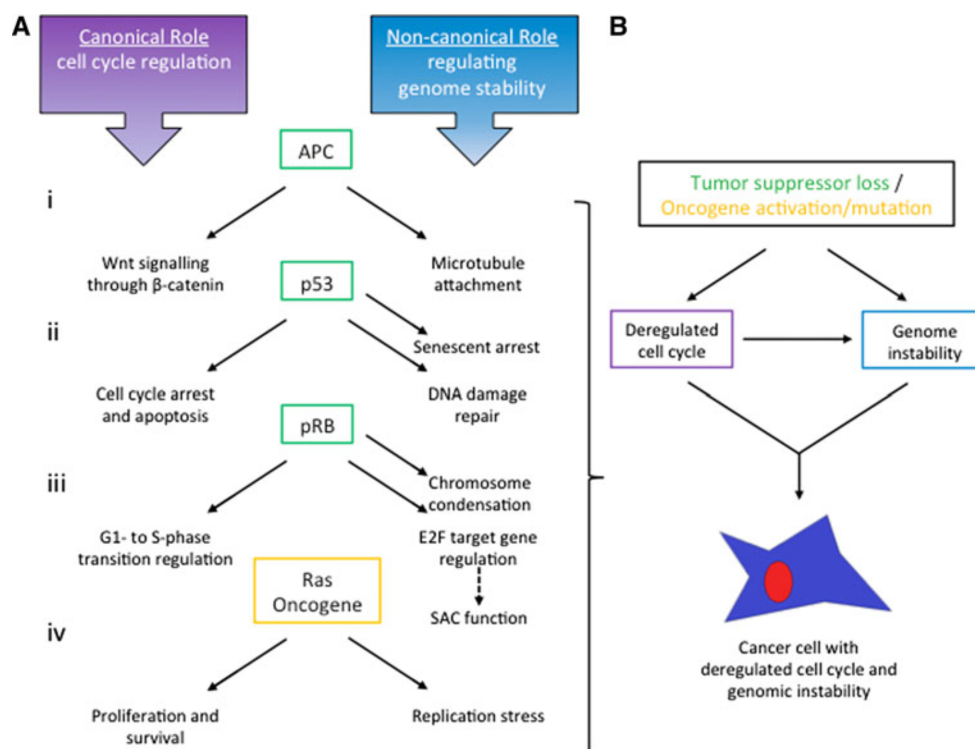

Fig. 2 Genomic instability goes hand-in-hand with cell cycle deregulation. **a** In a normal cell, tumor suppressors function to ensure proper regulation of the cell division cycle, and this is the canonical way in which we understand them to prevent cancer. However, many of these tumor suppressors (*i* APC, *ii* p53, *iii* pRB) have been found to also be involved in the maintenance of genome stability— a role that is also important to mediate their anti-tumorigenic effects. Similarly, proper regulation of proto-oncogenes ensures cell proliferation and survival (*iv* Ras). Upon loss of a tumor suppressor, or activation of a proto-oncogene, cell cycle regulation becomes compromised, and this is accompanied by a loss in genome stability. For tumor suppressors, this is due to a loss in their role in the maintenance of genome stability, and for proto-oncogenes, this results in a potential to promote genome instability as a by-product of uncontrolled proliferation. **b** Whether there is the loss of a tumor suppressor, or activation of an oncogene, deregulated cell cycle is subsequently followed by an acquisition of genome instability and ultimately cancer. This sequence of events may be true for other tumor suppressors and proto-oncogenes that are, as yet, unidentified, roles in the regulation of genome stability. *Green* tumor suppressor, *yellow* oncogene, *SAC* spindle assembly checkpoint

binds to β-catenin leading to its phosphorylation by the GSK3 kinase, and subsequent targeting for degradation [40]. In simple terms, the presence of Wingless signaling inhibits β-catenin phosphorylation, releasing it from APC regulation, thereby allowing it to translocate to the nucleus and activate the transcription of genes that stimulate cell proliferation [40]. Wingless target genes that are stimulated by β-catenin include c-myc [41], n-myc [42], cyclin D [43, 44], survivin [45], and Id2 [46, 47]. Together, these target genes promote cell cycle progression and inhibit apoptosis. In familial colon cancer, germline mutations in *APC* lead to constitutive activation of these target genes, providing a distinct survival and proliferative advantage to polyps in these patients.

In addition to its role in proliferative control, APC has also been shown to function in the attachment of spindle microtubules to mitotic chromosomes [48]. During mitosis, APC localizes to the kinetochore through its interaction with the microtubule end binding protein, EB1, thereby facilitating the interaction of spindle microtubules with mitotic chromosomes [48–50]. *APC* mutant cells exhibit numerous unattached microtubules in metaphase and, not surprisingly, display chromosomal instability [51–54]. More recently, it has been proposed that APC also plays a role in regulating the amount of time microtubules persist at the kinetochore, which, when compromised by loss of APC, leads to lagging chromosomes [55].

Separating APC functions and their contribution to the maintenance of genome stability

A key question surrounding APC function is how the regulation of both β-catenin and microtubule attachment relates to chromosome instability, and how each contributes to APC's tumor suppressor function. A number of mutant forms of APC that can separate function in mitosis from proliferative control have helped to investigate this question. One frequently studied allele of APC, derived from a mutagenesis screen in mice, is designated Min, and encodes a protein containing the first 850 amino acids [56]. This mutation deletes the interaction domains for β-catenin, tubulin, and EB-1 [57]. In addition, a targeted allele that truncates Apc at amino acid 1638 (1638T), and by comparison only eliminates the tubulin and EB-1 interactions, acts as a valuable comparison [58]. Studies using embryonic stem cells homozygous for either of these mutations demonstrate a clear defect in mitotic spindle formation, characterized by improper connections of the mitotic spindle to the kinetochore [59]. This leads to gross aneuploidy (W-CIN) and polyploidy [59]. Moreover, because embryonic stem cells homozygous for the 1638T mutation can still regulate β-catenin, but exhibit chromosomal instability, this illustrates that the role for APC in maintaining chromosome stability is separate from deregulated proliferation caused by the Wingless signaling pathway [59]. In support of these findings, it has been reported that upregulation of β-catenin alone is not sufficient to cause mitotic defects and chromosome instability in 293 cells [60]. Further correlative data demonstrates that cells from the human colon cancer cell line SW480, containing mutations in APC, exhibit multinucleation (as can be caused by cytokinetic failure due to APC-related microtubule defects) [60]. In stark contrast, cells from the HCT116 human colon cancer cell line that has a stabilizing mutation in β-catenin and wild-type APC, do not [60]. Together, these studies strongly suggest that chromosome instability is a distinct effect of *APC* mutations.

Instability in mouse models of cancer

Given that the proliferative control and mitotic functions of APC are separable, mutant alleles in mice have also provided insight into their relative roles in cancer progression. From these analyses, mouse models generated to investigate the role for *APC* in colon cancer support a role for W-CIN in the progression of this cancer type. This is best exemplified by the phenotype of the heterozygous mouse strain ($Apc^{\Delta 716/+}$), which shows loss of heterozygosity eliminating wild-type *Apc* in adenomas of the small intestine in a manner similar to patients [61]. Additionally, the $Apc^{Min/+}$ mouse exhibits similar mitotic defects, including misoriented spindles and misaligned chromosomes in normal crypts with wild-type levels of β-catenin; these cells also exhibited a tetraploid genotype [59, 60]. Moreover, loss of heterozygosity of the wild-type *Apc* allele has been demonstrated in dysplastic intestinal crypts, revealing that genome instability caused by mutant *Apc* is evident early in colon cancer development [60]. Because these phenotypes are observed in normal cells, before adenomas form, and before there is upregulation of β-catenin, it argues that CIN contributes to colorectal cancer from the earliest stages.

Interestingly, $Apc^{1638T/+}$ mice that exhibit only CIN phenotypes due to defective interactions with microtubules and EB-1, but maintain normal regulation of β-catenin, are not cancer prone [58]. This suggests that, at least in this context, CIN caused by APC mutations is not sufficient to cause cancer and is most likely a contributor to progression. A key experiment, that is needed to fully understand the interplay between proliferative control by APC through β-catenin and maintenance of chromosome stability, is the generation of a mutant mouse model in which regulation of β-catenin is lost, but interactions with microtubules and EB-1 are preserved. Analysis of these mice would offer definitive insight into the degree with which CIN contributes to cancer progression stimulated by β-catenin in *Apc* mutant mice.

A role for APC in the spindle assembly checkpoint (SAC)

Finally, oncogenic APC mutants have been shown to deregulate the spindle assembly checkpoint; truncated mutants of APC (N-APC) associated with colon cancer have been shown to sequester soluble Mad2, and thereby reduce the potency of Mad2 activation of the SAC. Furthermore, this also reduces the interaction of Cdc20, Mad2, and BubR1, the "wait anaphase" complex [62]. As a result, there is increased CIN in cells expressing the oncogenic N-APC fragment, due to reduced activation of the SAC [63]. Interestingly, this does not occur in cells with complete loss of APC, as full length APC does not appear to bind Mad2, indicating that this is a gain of function of the N-APC truncated mutant [63]. However, it has also been suggested that full length APC plays a role in the regulation of the SAC by binding to Bub1 and Bub3 (SAC proteins), and being a substrate of the Bub1/BubR1 kinases in in vitro experiments [48]. In accordance with this data is the fact that $BubR1^{+/-}$;$APC^{min/+}$ mice develop ten times more colonic tumors than $APC^{min/+}$ mice alone [64]. While MEFs from the compound mutant mice exhibited higher levels of β-catenin and increased proliferation compared to wild-type and $BubR1^{+/-}$ MEFs, they were also able to proceed through mitosis when challenged with nocodazole. Compound mutant MEFs and exhibited increased genomic instability (as demonstrated by aneuploidy) in comparison with wild-type, $BubR1^{+/-}$, and $APC^{min/+}$ MEFs [59, 64–66].

APC summary

Regardless of the precise contributions of CIN to *APC* mutant cancers, the *APC* gene provides an ideal example for how oncogenesis due to deregulation of wingless signaling is enhanced by a chromosome instability phenotype. Because the vast majority of APC mutations in cancer truncate the protein eliminating both β-catenin and mitotic regulatory domains, the consequences of these deletions obligatorily affect both functions. For this reason, deregulation of proliferative control and CIN are intimately linked in this common type of cancer (Fig. 2ai, b).

Tp53

Tumor suppressor gene, global cell fate regulator

The p53 protein was first discovered because it is bound by a large T antigen from simian virus 40 [67–69]. In addition to its role in the viral transformation of cells, p53 was later discovered to be a potent tumor suppressor that is mutated in most families with Li-Fraumeni syndrome [70–73]. Furthermore, sporadic mutation of the *Tp53* gene also takes place in the majority of human cancers [74, 75]. Together, this background has established p53 as a critical target for mutation during tumorigenesis, whose function is compromised in virtually all forms of cancer.

Mechanisms of p53 regulation and function

Under conditions of homeostasis, the p53 protein is continuously translated and targeted for degradation via the 26S proteasome by the E3 ligase MDM2 (HDM2 in humans) [76–78]. Upon genotoxic stresses such as DNA damage or oncogene activation, the p53 protein becomes stabilized through phosphorylation by DNA damage responsive kinases such as Chk1, Chk2, and ATM, that block its interaction with MDM2 [79]. Furthermore, acetylation and methylation facilitate p53-dependent transcriptional activation of target genes to induce a cell cycle arrest or activate apoptotic signaling, among other responses [79]. Additionally, p53 can be stabilized and activated in response to aberrant proliferative signals. For example, E2F1 activates expression of $p14^{ARF}$ (also referred to by its murine nomenclature, $p19^{ARF}$) [80], which stabilizes p53 by sequestering MDM2 and preventing MDM2-mediated ubiquitination of p53 [81–83].

To mediate either a transient cell cycle arrest or a more permanent senescent arrest, p53 activates the transcription of $p21^{Cip1}$ [84], which in turn inhibits cyclin-dependent kinases, leading to an arrest of proliferation in any phase of the cell cycle [84–88]. To mediate apoptosis, p53 activates the transcription of genes encoding Bax, Noxa, and Puma, that stimulate apoptosis through the mitochondrial pathway [89–91]. Based on this summary of p53 function, it is clear that there is a direct connection between the maintenance of genome integrity through response to DNA damage and proliferative control, and through regulation of the cell division cycle. The many functions of p53 were conceptualized into a unified purpose when it was proposed to be the 'guardian of the genome' [35]. The ensuing paragraphs on p53 function will explain how its loss leads to an intimate association between chromosome instability and deregulated proliferation.

Guardian of the genome

Compromising the role of p53 in cell cycle and apoptotic regulation allows genetic change to accumulate over many cell generations. For example, fibroblasts from patients with Li-Fraumeni syndrome that exhibit LOH eliminating the wild-type *Tp53* allele, consequently display a significant increase in aneuploidy and structural chromosomal aberrations [92–94]. Furthermore, the ability to amplify drug resistance gene loci is dramatically higher in cells

harboring mutations in *Tp53* [95], further indicating that S-CIN is a consequence of defective p53 function. Finally, several studies of human cancers and human cancer-derived cell lines have demonstrated that there is a strong correlation between aneuploidy in tumors and *Tp53* mutations [96–98]. Together, these studies argue that defective p53 function leads to chromosome instability.

The ability of p53 to maintain a stable genome was originally thought to be a result of its inhibition of cell cycle progression, or induction of apoptosis in response to cellular stresses such as DNA damage. Failure to repair DNA damage before division, or survival of a cell following high levels of damage, both provide opportunities for chromosomal changes to arise [35]. Moreover, several studies have demonstrated that loss of p53 makes cells tolerant of both tetraploidy and aneuploidy [98–100]. However, other studies have suggested that p53 may play a more active role in the maintenance of genome stability (reviewed in [101]). For example, p53 has been reported to recognize DNA damage-related structures, and bind to ssDNA and dsDNA to promote strand exchange and mediate repair [102–105]; subsequently, a role for p53 in regulating homologous recombination and non-homologous end joining was uncovered [106–116]. p53 is also capable of binding proteins involved in homologous recombination repair and non-homologous end joining [117–124]. Presumably p53's activities in these different repair processes work to repair damaged chromosomes and prevent their accumulation. Whether p53 functions through active repair versus activating checkpoints and apoptosis is more important for suppressing CIN is not within the purview of this review. Simply, in all cases, cellular studies indicate that a lack of p53 function deregulates proliferation and leads to alterations at the chromosomal level and deficiency in *Tp53* is correlated with aneuploidy in tumors.

The genetics of p53 inactivation

In order to understand loss of p53 function in cancer and its impact on chromosome stability, it is also important to describe the mechanisms by which its function is eliminated. p53 is unique among tumor suppressors because it is organized into a homotetrameric transcription factor [125, 126]. This creates the opportunity for its function to be disrupted by dominantly acting mutations. For this reason, one allele of p53 can be eliminated by random mutation, which can then be followed by loss of heterozygosity to remove the remaining wild-type allele, much like other tumor suppressor genes are thought to be lost [70–73]. A number of means by which mutant p53 can gain function have been suggested. For example, mutant p53 can oligomerize with wild-type p53 as well as p53 family members p63 and p73, thereby inhibiting their ability to transactivate target genes [127–135]. Furthermore, p53 mutants have also been shown to activate the transcription of new target genes encoding growth stimulators including c-myc [136], cyclins and cdks [137], and hTert [138], all of which provide survival and proliferative advantages (reviewed by [139, 140]). Alternatively, regulators such as MDM2 can become oncogenically activated leading to loss of p53 function [141, 142]. Cancer patients with missense mutations in *Tp53* often have a poorer prognosis than those lacking *Tp53* entirely, as the lack of wild-type *Tp53* confers only loss of tumor suppressor function [143], while the presence of dominantly mutated p53 not only confers loss of tumor suppressor activity but also provides a gain of function that is selected for in malignancy [143]. This gain of function concept is supported by the fact that Li-Fraumeni patients with mutated *Tp53*, as opposed to no p53 expression, have a significantly higher incidence of cancer that also occurs at an earlier age of onset [144]. For these reasons, loss of wild-type p53 function is variable in its effects on malignant progression, and this can impact the degree of chromosome instability exhibited from one mutation type to the next.

Instability in *Trp53* mouse models of cancer

Numerous transgenic and gene-targeted mouse models that manipulate the murine *Tp53* gene (called *Trp53*) have been generated. In many regards, the analysis of these animals has revealed that mutations in *Trp53* predispose to cancer and cause chromosome instability in a manner similar to what is suggested from cell culture and clinical data. However, a small number of reports using gene-targeted *Trp53* mice offer a clear demonstration of the connection between defective cell cycle control and CIN, and they are outlined below.

Trp53 knock out mice develop primarily lymphomas in a relatively short period of time [145–147]. Flow cytometric and cytogenetic analyses of these tumors suggests the emergence of aneuploidy, but maintenance of a near diploid karyotype [148–150]. Spectral karyotype analysis of chromosome spreads from these tumors indicates that chromosomal translocations are rare [149]. Depending on perspective, this can be considered either validation that loss of *Trp53* results in CIN, or a suggestion that these phenotypes are remarkably mild considering p53's role in maintaining genome stability. However, as noted above, null alleles are less severe than dominantly acting point mutations. Data on aneuploidy in primary cell culture and tumors from mice harboring the R172H and R270H mutants are scarce. These mutations represent two of the most common dominant negative point mutations found in human p53, and likely offer a better model of how p53 function is most often altered in human cancer [151, 152].

These mouse strains exhibit highly aggressive forms of cancer reminiscent of Li-Fraumeni syndrome, that are distinctly more metastatic than lymphomas found in *Trp53*$^{-/-}$ animals [151, 152]. Taken together, this implies that these point mutations in murine p53 recapitulate the human syndrome well, and suggests that they likely accommodate genome instability. Another mouse line that harbors a point mutation in *Trp53* and mimics a human cancer-derived allele with dominant properties very elegantly demonstrates the connection between CIN and defective proliferative control by p53. The substitution of R172P (R175P in humans) creates an allele of *Trp53* that eliminates p53 dependent apoptosis but retains some transcriptional activation function for the cyclin-dependent kinase inhibitor p21 [153]. Mice homozygous for this mutation are considerably more resistant to spontaneous tumor formation than *Trp53*$^{-/-}$ alone [153]. These mice also develop lymphomas, but, surprisingly, their ploidy remains strictly diploid. This suggests that defective apoptotic regulation is not linked to CIN and implicates a role for p53 in cell cycle control as its means to inhibit CIN. Further experimentation with these mice revealed that crossing them to p21-deficient strains advanced the onset of tumor formation and led to widespread aneuploidy in the resulting tumors [154]. Thus, analysis of the R172P mouse offers a very compelling case that defective cell cycle control by p53 is tightly linked to chromosomal aberrations.

Tp53 summary

In sum, discussion of the function of p53 in cell cycle control, the genetics of mutations that inactivate its function, and gene-targeted mouse models, demonstrates linkage between defective cell cycle control and CIN. In Li-Fraumeni patients, *Tp53* mutations are the initiating event and this ensures that, in these cancers and likely in many others that suffer sporadic loss of p53, CIN is a reliable companion that can facilitate genetic change on the pathway to tumorigenesis. Considering that point mutations which dominantly inactivate p53 are found in most human cancers, and that these mutations accompany aneuploidy, it suggests that CIN is a frequent consequence of mutations that drive cancer cell proliferation (Fig. 2aii, b).

Retinoblastoma (RB)

The original tumor suppressor

The retinoblastoma susceptibility gene is the prototypical tumor suppressor gene. It was first cloned through positional mapping and identification of regions on chromosome 13q that were deleted in retinoblastomas and osteosarcomas [155]. Initially, it was expected that *RB1* would function in a relatively specialized role in the few tissues where its loss of function contributed to hereditary cancer development. However, studies of the transforming activity of oncogenic viral proteins such as adenovirus E1A, simian virus 40 TAg, and human papilloma virus E7 indicated that inactivation of the *RB1* protein (pRB) was a requirement for transformation [156]; this work suggested that pRB may function more broadly in an anti-oncogenic manner. Subsequent studies later revealed that pRB regulates the transition from the G1 to S-phase of the cell division cycle and that this universal role in proliferation transcends all cell types [157]. Since deregulated proliferation is key to cancer initiation and progression, genetic alterations that eliminate pRB function are a hallmark of nearly all cancers [158, 159].

The mechanism of pRB cell cycle control

The means by which pRB regulates G1 to S-phase progression in the cell cycle is through the control of E2F transcription factors [157]. In G1, pRB binds to transcriptional activation domains of E2Fs and inhibits the expression of genes that are required for S-phase progression [160, 161]. Concomitantly, pRB interacts with a number of cellular proteins that exhibit enzymatic activity capable of remodeling chromatin including histone deacetylases [162–164], histone methyltransferases [165, 166], DNA methyltransferases [167], and helicases such as BRG1/Brm [168]. This leads to heterochromatinization of E2F-target gene promoters and further inhibition of their expression. Under growth arrest conditions such as quiescence, pRB is hypophosphorylated and binds stably to E2Fs and chromatin regulators, preventing E2F-target gene transcription [157]. Upon mitogenic signaling, cyclinD/cdk4, followed by cyclinE/cdk2 complexes, hyperphosphorylate pRB, releasing both chromatin remodeling proteins and E2F transcription factors; E2Fs are thus free to activate the transcription of genes required for S-phase progression, and this irreversibly drives the cell cycle forward [159].

Inactivation of pRB function in cancer

The regulatory pathway that controls cell cycle advancement through G1 is often referred to as the RB-pathway. It includes cyclin D/cdk4, cyclin-dependent kinase inhibitors (CKIs) such as p16Ink4a, and finally pRB; in the vast majority of cancers, the retinoblastoma signaling pathway is compromised [158, 169]. The means by which it can be disrupted include (1) direct mutation of the retinoblastoma susceptibility gene, rendering it non-functional [170], (2) inactivation and degradation of pRB as caused by the

human papilloma virus E7 oncoprotein, with effects similar to those resulting from direct mutation [171], (3) constitutive hyperphosphorylation of pRB in cancer by overexpression of cyclin D/cdk4 complexes, which is most commonly observed [158], or (4) inactivation of CKIs [158]. Functionally, all of these result in the loss of regulation of E2F transcription and inappropriate entry into the cell division cycle.

Inactivation of the *RB1* gene in retinoblastoma has become a paradigm for the inactivation of tumor suppressor genes. The original 'two-hit' hypothesis predicted the need for both alleles of *RB1* to be eliminated in order for its function to be compromised. It posited that elimination of each allele was an independent event [172]. Thus, for *RB1* to be inactivated by direct mutation, and deregulate the RB pathway as described above, both copies of the gene need to be affected [173]. Elegant work by the White laboratory has revealed a number of chromosomal aberrations that can facilitate the loss of the remaining wild-type *RB1* allele in a heterozygous, premalignant cell that already has one mutated copy of *RB1* [174]. Thus, while rare, chromosomal aberrations are intimately linked to the elimination of pRB function in cancer initiation. For these reasons, it is important to distinguish the sporadic chromosomal segregation errors and abnormalities that can lead to loss of wild-type *RB1* in $RB1^{+/-}$ cells, from the CIN phenotype that arises as a consequence of complete deficiency for pRB.

Multiple mechanisms allow the retinoblastoma protein to prevent CIN

As described above, much attention has been focused on pRB's ability to regulate E2F transcription factors at the G1 to S-phase transition, as this regulates a cell's commitment to replicate its DNA and divide. There are two general divisions in which to categorize pRB's functions that maintain genome stability. The first is as a consequence of deregulated E2F transcription. As detailed above, the vast majority of cancers possess mutations that disrupt regulation of the RB pathway, leading to uncontrolled E2F transcription. For this reason, missexpression of genes early in the cell cycle can result in chromosome re-replication or missegregation later, as is the case with deregulation of the E2F-target genes, cyclin E and MAD2, respectively [175, 176]. In addition, E2F-independent regulation of the chromatin structure of mitotic chromosomes has also emerged as a means by which the retinoblastoma protein contributes to the maintenance of genome stability [177–179]; both mechanisms will be discussed below.

Among E2F transcriptional targets, a number stand out as known causes of chromosome instability when overexpressed. First, both cyclin E1 and E2 isoforms are E2F target genes; their stable overexpression leads to abnormally elevated cyclin-dependent kinase activity, ultimately leading to aneuploidy or polyploidy [175]. Furthermore, a number of components of the spindle assembly checkpoint are E2F target genes, including Mad2 and BubR1, whose overexpression leads to enhanced checkpoint activity [11]. This in turn delays progression through mitosis and manifests as chromosome segregation errors [176]. These examples of deregulated E2F target gene-induced expression reveal how elevated levels of these gene products drive CIN, and offer a simple connection between loss of proliferation control and aneuploidy.

The first reports to suggest a role for pRB in maintaining a stable genome independently of E2Fs, and thereby G1 to S-phase regulation, demonstrated defects in chromosome structure or maintenance. In a study by Zheng et al. [180], it was demonstrated that $Rb1^{+/-}$ and $Rb1^{-/-}$ mouse embryonic stem cells exhibit a high frequency of loss of a selectable chromosomal marker compared to wild-type. Furthermore, loss of drug resistance was due to complete absence of the selectable marker, implicating chromosomal loss or rearrangement as the explanation for genetic change [180]. Similarly, it was also observed that cells deficient for all pRB family proteins display lengthened telomeres and centromere fusions [165, 181]. Metaphase spreads from these cells are characterized by chromosome fusions and tetraploidy [165].

Interestingly, similar centromere, aneuploidy, polyploidy, W-CIN, and S-CIN phenotypes have been observed in cells with defective condensin I/II complex function [182–186]. The condensin II complex facilitates chromosome condensation during prometaphase, and is important for maintaining chromosome structure and architecture during mitosis, particularly at the centromere [185, 187, 188] (reviewed by [189]). The association of the condensin II complex with pRB is lacking in both *RB1* null cells, as well as those containing a targeted mutation in pRB that eliminates just LXCXE type interactions with the pRB pocket domain [177, 178, 190]. Defective condensin II function offers an explanation for the observed hypocondensation at centromeres, centromere fusions, and increase in whole chromosome gains and losses [177, 178, 190]. Presumably, defects in condensation lead to misshapen centromeres and merotelic attachments by spindle microtubules, which leads to missegregation of chromosomes without activating the spindle assembly checkpoint [191, 192]. Defects in S-CIN have also been reported in pRB-family-deficient fibroblasts, suggesting that chromosome structure defects may be more prevalent than at centromeres alone [179].

Defects in E2F regulation lead to elevated levels of aneuploidy because of improper regulation of DNA replication and activation of the spindle assembly checkpoint [175, 176]. The connection between chromosome instability caused by defective condensation in pRB mutants,

and deregulated cell cycle control is less clear. For example, wild-type embryonic stem cells lack a pRB-dependent G1 arrest mechanism [193], but display CIN phenotypes caused by pRB deficiency [180]. For this reason, it remains to be determined whether elevated cyclin-dependent kinase activity (the most common way of eliminating pRB function in cancer) compromises pRB's role in chromosome condensation, beyond causing aneuploidy through elevated E2F-mediated transcription.

Chromosome instability in pRB mouse models of cancer

Heterozygous *Rb1*$^{+/-}$ mice are cancer prone and develop pituitary tumors by 1 year of age, the majority of which have lost the remaining wild-type *Rb1* allele [194]; in this regard, they recapitulate the steps of the two-hit hypothesis quite faithfully. We are unaware of attempts to evaluate CIN in these tumors, or any others created by conditional deletion of *Rb1* in a specific tissue. However, it would be difficult to discern the contribution of CIN to an *Rb1*-deficient cancer model in isolation from the effects of deregulated proliferation when using null alleles of *Rb1*.

Two mouse models offer a glimpse at the effects of deregulated E2F target gene expression. One is from transgenic overexpression of a non-degradable cyclin E in the mammary gland, and the other is overexpression of Mad2 in the same tissue; both cause mammary cancer and result in chromosomal abnormalities [195, 196]. While cyclin E clearly causes proliferation, analysis of *MMTV-cyclin E*;*Trp53*$^{+/-}$ mice reveals that elevated cyclin E hastens the loss of the remaining wild-type *Trp53* allele.

A recently generated mouse strain called *Rb1*$^{\Delta L}$ has demonstrated a connection between pRB-mediated chromosome condensation and tumor suppression [177]. This mutation in *Rb1* abrogates the ability of cellular and viral proteins with the LXCXE peptide motif to bind to the pocket domain [197]. Importantly, this mutant form of pRB retains the ability to bind to, and regulate, E2F transcription factors such that Mad2 and other E2F target genes are expressed normally in cells from these mice [197]. *Rb1*$^{\Delta L/\Delta L}$ mice do not develop spontaneous tumors; however, cells from these mice exhibit a significant increase in mitotic abnormalities [177, 197]. *Rb1*$^{\Delta L/\Delta L}$;*Trp53*$^{+/-}$ mice succumb to cancer significantly sooner than *Trp53*$^{+/-}$ mice, and because both genotypes lose their remaining wild-type *Trp53* allele in the process, it implies that CIN caused by the *Rb1*$^{\Delta L}$ mutation accelerates cancer pathogenesis [177].

RB1 summary

Based on the available data from these mouse models of cancer, loss of pRB function contributes to both deregulated proliferation and chromosome instability. As shown with p53, this section of the review offers another example of a frequent target for inactivation in cancer that facilitates both chromosome instability, and deregulated proliferation in a largely inseparable manner (Fig. 2aiii, b).

Are there roles for oncogenes in genome instability?

So far, the proteins discussed here have been tumor suppressors whose loss leads to increased proliferation. Furthermore, in addition to traditional tumor suppressive mechanisms, their involvement in the maintenance of genome stability is a contributor to their tumor suppressive mechanisms.

What about oncogenes? Cells are unlikely to have a protein whose role is to cause genome instability. As such, it follows that there would be no proto-oncogene whose mutation into an oncogene would be directly involved in generating genome instability. However, it is possible that, by somewhat indirect mechanisms, activation of oncogenes can cause chromosomal instability as a by-product of increased proliferation.

The Ras pathway in proliferation and instability

The prototypical oncogene for this example is Ras. Under normal conditions, mitogenic signaling promotes GTP binding by Ras, and this leads to elevated growth signals through a number of pathways (reviewed in [198]). In cancer, mutations preferentially affect H-Ras or K-Ras and prevent GTP hydrolysis, causing constitutive signaling [198]. As a result, Ras activation by mutation leads to transcriptional activation of many genes, and these downstream effectors drive cell proliferation and survival [199, 200]. The contribution of Ras to chromosome instability comes from two perspectives. First, it has been reported that both human and rodent tumor cells with an activating mutation in one of their Ras isoforms exhibit heteroploidy, as well as chromosome breaks and rearrangements [201–203]. A more mechanistic explanation comes from studies of Ras in oncogene-induced senescence (OIS) [204]. In this case, senescence is induced by a robust DNA damage response (DDR) that becomes activated from partially replicated DNA caused by the repeated firing of DNA replication origins as a result of Ras induction of abnormally high proliferative signals [205]. This partially replicated DNA is a source of S-CIN; accordingly, loss of heterozygosity (LOH) at common fragile sites in OIS has been demonstrated with Ras overexpression [205]. Furthermore, genomic instability resulting from oncogenic Ras can be detected following just one round of DNA replication [201].

The acquisition of mutations that accommodate activated Ras, such as loss of DDR mediators including ATM, p53, and

ATR, permit these cells to proliferate [205]. In this way, Ras-induced hyperproliferation can cause chromosome instability as a by-product of its proliferative signals (Fig. 2aiv, b). By extension, cells with oncogenic activation of receptor tyrosine kinases upstream of Ras, or activating mutations in downstream effectors such as Raf, can readily be envisioned to cause similar effects. In this regard, the phenotype of *MMTV-cyclin E* overexpressing mice, described above as causing over-replication and genome instability, may also exemplify over-active growth stimulating signals, as well as loss of growth inhibiting mechanisms [196].

Summation of proliferative control defects and chromosome instability in cancer

Each of the genes described above represent common mutational events in cancer. *Tp53* is the most frequently mutated gene in cancer with mutations present in more than 50% of cases, irrespective of disease site [74, 75]. Direct mutation of pRB is relatively rare, but other alterations to its regulators ensure that it is functionless for E2F regulation in the vast majority of cancers [158]. *APC* mutations are uncommon outside of colorectal cancer, but occur very frequently in this cancer type [37–39]. Since colorectal cancer is one of the most common cancer types in western nations such as Canada and the USA, this further emphasizes the abundance of tumors with a defect in APC-mediated proliferative control and chromosome stability [133, 206]. Lastly, Ras mutations are present in approximately 10–90% of cancers, depending on disease site [198]. All told, the summation of these common mutation types, and their effect on chromosome instability, indicates that CIN is likely to be as intimately associated with cancer as its most well-known characteristics, such as deregulated proliferative control.

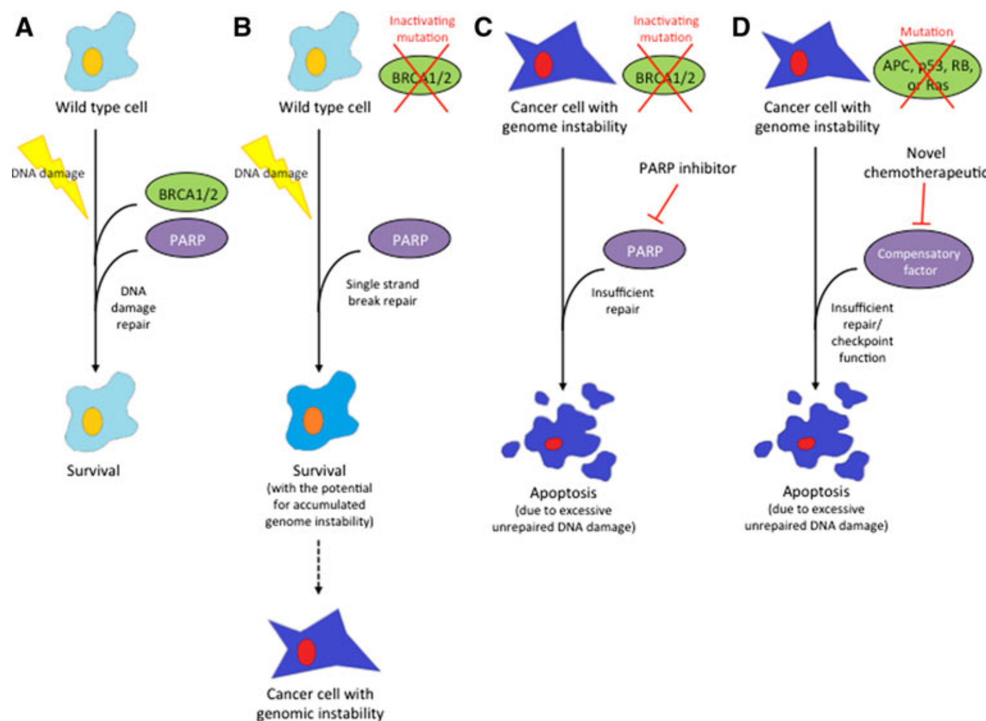

Fig. 3 Model for the exploitation of genome instability to induce apoptosis in cancer. **a** In wild-type cells, DNA damage causing double-strand breaks can be repaired by homologous recombination (HRR) mediated by BRCA1/2. **b** Inactivating mutations in either BRCA1 or BRCA2 compromise HRR, and DNA damage is more difficult to repair. In the case of BRCA-deficient cancers, repair of single-stranded lesions by PARP-mediated DNA damage repair is critical because subsequent replication fork stalling cannot be repaired by homologous recombination. **c** New chemotherapeutics for cancers with BRCA1 or BRCA2 mutations include the treatment of these tumors with PARP inhibitors. PARP inhibitors disable one of the remaining mechanisms by which these cancer cells repair DNA. This creates synthetic lethality in which BRCA mutant cells display greatly elevated sensitivity to PARP inhibitors compared to BRCA wild-type controls. **d** We suggest that this principle of synthetic lethality can be extended to more frequently affected tumor suppressors or oncogenes. There may be signaling pathways that cancer cells with common *APC*, *Tp53*, *RB1*, or Ras mutations rely on to compensate for the chromosome instability that these mutations create. Targeting these pathways with novel chemotherapeutics may lead to more anti-cancer agents akin to PARP inhibitors, except with a broader range of susceptible tumors because of the common nature of linked defects in proliferative control and genome instability described in this review

Prospects for harnessing the linkage between chromosome instability and deregulated proliferation

While it is obvious that chromosome instability facilitates the acquisition of potentially oncogenic mutations, it has also been proposed to act as a tumor suppressive mechanism in cell-specific contexts [22, 207]. In such circumstances, cancer-promoting alterations to chromosomes gained in one cell division cycle may rapidly be lost in successive rounds of cell division, resulting in a lack of persistence of a malignant clone. Additionally, as discussed previously, there is presumably a threshold level of DNA damage above which cell cycle arrest and subsequent senescence or apoptosis occur. Therefore, it follows that cells with high levels of chromosome instability may selectively undergo senescence or apoptosis. In this way, high levels of chromosome instability can act as a tumor suppressive mechanism. Taking advantage of chromosome instability for cancer treatment likely involves converting low levels of instability, that are tolerated and permissive to cancer pathogenesis, into higher levels that arrest proliferation or lead to cell death.

An example of how to capitalize on chromosome instability defects and exacerbate them to cause lethality in chromosomally unstable cancer cells has been demonstrated in tumors deficient for *BRCA1* or *BRCA2*. These tumor suppressors function in homologous recombination repair of stalled replication forks (Fig. 3a) [188]. Cancers deficient for BRCA function can be selectively killed by agents that increase fork stalling, such as inhibition of the poly ADP ribose polymerase (PARP) [208]. This predicts that disruption of both repair pathways will be synthetically lethal, and has given rise to the therapeutic approach of treating *BRCA1* or *BRCA2* mutant cancers with PARP inhibitors (Fig. 3b). The addition of PARP inhibitors to such mutant cancers leads to single strand break accumulation that cannot be fixed during replication by homologous recombination [142, 209]. As a result, these cells die from unrepaired double-stranded breaks; cells with functional homologous recombination are orders of magnitude less sensitive (Fig. 3c). BRCA mutant cancers are relatively rare, and moreover not all BRCA mutant cancers exhibit this synthetic lethality; therefore, this limits the applicability of this therapy. In comparison, the tumor suppressor and oncogene mutations described in this review likely cause CIN on a much larger scale. We suggest that there are likely synthetic lethal combinations between common tumor suppressor pathway mutations that cause CIN, and other as yet to be determined genome maintenance mechanisms (Fig. 3d). We propose that, in addition to further investigation of the tumor suppressor and oncogenes described here and how they cause CIN, future research should also embrace the pursuit of synthetic lethal combinations with these gene mutations. This approach promises to translate our basic knowledge of relatively difficult drug targets such as pRB, Ras, or p53 into future treatment options.

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