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pRB, a Tumor Suppressor with a Stabilizing Presence

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Abstract

The product of the retinoblastoma tumor susceptibility gene (*RB1*) is a key regulator of cell proliferation and this function is thought to be central to its tumor suppressive activity. Several studies have demonstrated that inactivation of pRB not only allows inappropriate proliferation, but also undermines mitotic fidelity, leading to genome instability and ploidy changes. Such properties promote tumor evolution and correlate with increased resistance to therapeutics and tumor relapse. These observations suggest that inactivation of pRB may contribute to both tumor initiation and progression. Further characterization of pRB's role in chromosome segregation will provide insight into processes that are misregulated in human tumors and may reveal new therapeutic targets to kill or stall these chromosomally unstable lesions. Here, we review the evidence that pRB promotes genome stability and discuss the mechanisms that likely contribute to this effect.

Aneuploidy and CIN drive tumorigenesis

Genetic instability and the development of aneuploidy have long been linked to the acquisition of invasive and metastatic characteristics [1, 2], with the percent of aneuploid tumors increasing with both histological differentiation and tumor size to near 100% in advanced tumors [3–6]. Such genomic changes may be beneficial to cancer development. For example, genome instability, which includes chromosomal and subchromosomal alterations such as inversions, deletions, mutations, duplications and translocations of large chromosomal segments, has the potential to drive mutations in oncogenes and tumor suppressor genes. Aneuploidy, a state in which a cell exhibits an abnormal number of chromosomes, is a hallmark of many cancer cells. Several different defects can cause aneuploidy, including defects in mitosis that promote chromosome segregation errors. Underlying defects in mitotic chromosome segregation can result in a consistent elevated rate of gains and losses of whole chromosomes, a process that is known as chromosomal instability (CIN) [7]. Although cells can become aneuploid without displaying CIN, CIN necessarily results in aneuploidy. While aneuploidy has been shown to be detrimental to growth in otherwise normal cells and organisms [8–10], CIN can promote loss of heterozygosity (LOH), uncovering heterozygous mutations, thereby providing selective growth advantage in tumor cells [11].

The majority of solid tumors exhibit structural and numerical chromosome aberrations, with evidence of aneuploidy in even very early, benign lesions [12, 13]. This is illustrated by

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high-throughput genomic profiling studies showing that most human tumors display abnormalities in the number of whole chromosomes or chromosome arms [14–16]. Additionally, many tumors have been shown to be chromosomally unstable [7]. CIN and aneuploidy have been proposed to promote the evolution of tumor cells such that these genomic changes appear to promote metastasis and chemotherapeutic resistance, and correlate with poor patient prognosis [17–24]. Importantly, recent studies show that aneuploidy and CIN can have a causal role in tumorigenesis and relapse [10, 11, 25].

CIN is the result of an underlying defect in mitotic fidelity and several mechanisms that result in whole chromosome missegregation have been described. These include defects in bipolar spindle formation, errors in chromosome–spindle association, failed chromosome cohesion, and defects in the spindle assembly checkpoint [9, 11, 25–32]. In addition, studies of CIN cell lines suggest that merotely, an erroneous kinetochore attachment where a single kinetochore associates with microtubules from both spindle poles (box 1), is a dominant mechanism for chromosome missegregation in tumor cells [9, 28, 30, 33]. Consistent with this link between merotely, CIN and tumorigenesis, tetraploidy has been shown to be a sufficient precursor to chromosomal instability and tumorigenesis and this is thought to be due in large part to the presence of extra centrosomes that promote merotelic attachments [28, 30, 34, 35]. However, the majority of solid tumors exhibit a near diploid karyotype, making it unlikely that tetraploidy is an initiating factor in CIN or tumorigenesis in these cells [36].

Box 1

Merotelic kinetochore attachments

- Form when microtubules from two spindle poles interact with a single kinetochore
- Formation is promoted by aberrant spindle geometry (multipolar spindle, supernumerary centrosomes) and/or centromere deformation (decreased cohesion or condensation, mis-localization/reduction of kinetochore proteins, etc), and/or hyper-stable kinetochore-microtubules attachments
- Do not signal the spindle assembly checkpoint and therefore allow anaphase progression prior to correction of erroneous attachments
- Often result in lagging chromosomes which may missegregate and/or be resolved into micronuclei during the subsequent G1
- Correction is influenced by a number of factors that regulate kinetochore-microtubule stability and mitotic timing
- Proposed to be a major mechanism of chromosome segregation errors in CIN cells

For cells to maintain CIN, they must additionally acquire tolerance of the resulting aneuploidy. In response to ploidy changes, non-transformed cells exhibit a strong proliferative defect [8, 9, 37], and in order to propagate their aneuploid genotype tumor cells must overcome or bypass such an effect. Although numerous pathways have been described that can contribute to chromosome segregation errors and tolerance of subsequent ploidy changes, the underlying molecular mechanism(s) behind such defects remains unclear. Given the broad array of factors that apparently contribute to mitotic fidelity and proliferation of aneuploid cells, it is possible that different subsets of defects exist in different tumors. Alternatively, a common defect may contribute to the susceptibility of tumor cells to both mis-segregate chromosomes and become tolerant of the subsequent

aberrant ploidy. Indeed recent data has implicated the pRB protein pathway in both the generation of CIN and the tolerance of aneuploidy, raising the possibility that pRB inactivation may contribute to many of the changes seen in tumor cells. Here, we discuss some of the evidence linking pRB to the maintenance of genome stability.

Loss of pRB promotes aneuploidy and chromosome instability

The retinoblastoma tumor susceptibility gene (*RB1*) was one of the first tumor suppressor genes to be discovered and most, if not all, tumors acquire lesions in the pRB pathway. While the loss of pRB function has been implicated in the development of numerous cancers [38], the significance of *RB1* inactivation is perhaps best illustrated by the pediatric cancer, retinoblastoma, for which the gene was named. In these tumors, the inactivation of both copies of *RB1* is rate-limiting for tumorigenesis and is thought to be an initiating event [39–41].

Inactivation of pRB impacts many cellular processes. For example, pRB-deficient cells have altered regulation of G1 checkpoints, changes in the control of cell cycle exit (differentiation, senescence, quiescence), and altered levels of autophagy, apoptosis, angiogenesis, and metastatic potential (reviewed in [38]). Many of these effects are thought to be a consequence of altered gene expression. The most intensively studied function of pRB is its ability to repress transcription of E2F-regulated genes, a role that enables it to regulate the expression of many genes that are needed in cell cycle progression and cell proliferation.

More recently, a series of studies have highlighted an additional consequence of *RB* inactivation that seems likely to impact tumorigenesis. In numerous *in vivo* and *in vitro* model systems, loss of pRB activity enhances genomic instability. These studies have linked the functional inactivation of pRB to various types of genomic change, including endoreduplication, increases in ploidy on both the chromosomal and subchromosomal (local amplifications, chromosome arm gains and losses) levels, and consistently high rates of chromosome segregation errors resulting in whole chromosome missegregation (CIN), as well as tolerance of such genomic variations [42–49] (Table 1). One potential reason for these changes is that the inactivation of pRB leads to defects during mitosis. Indeed, the mitotic defects of pRB-deficient cells have been characterized in detail and although less dramatic than those in G1 regulation that are evident earlier in the cell cycle, these subtle changes undermine the fidelity of chromosome segregation. The loss of pRB results in supernumerary centrosomes, centromeric defects, and formation of micronuclei. Remarkably, many of these changes are consistent with the formation of merotelic kinetochore attachments during mitosis (Box 1). It is well established that merotelic attachments promote whole chromosome missegregation and that frequent occurrence of such erroneous attachments, as is found in chromosomally unstable tumor cell lines, can result in aneuploidy (reviewed in [31]). Together this suggests that pRB loss of function leads to CIN in tumors by promoting merotelic kinetochore attachment (Table 2). Currently, there is scant evidence that pRB acts directly in mitosis. Instead, it seems probable that the loss of pRB function causes changes during earlier stages of the cell cycle that subsequently influence chromosome segregation. As described below, there is not one connection between pRB and mitosis; instead the mitotic defects seem likely to be the cumulative effect of several types of change resulting from the inactivation of pRB.

E2F-dependent mechanisms promoting CIN

The loss of pRB deregulates E2F. Comparison of gene expression data shows a significant overlap between the changes associated with CIN and the changes that occur in pRB-deficient cells, raising the possibility that the CIN signature may be, at least in part, a

consequence of pRB misregulation [50–52]. Well-characterized targets of E2F include multiple genes whose products are required for accurate chromosome segregation during mitosis, supporting the idea that one of the ways that pRB contributes to the maintenance of genome stability is through its regulation of E2F. Consistent with this idea, recent work has shown that upregulation of Mad2, one such E2F target that is deregulated by the inactivation of pRB, is sufficient to induce chromosome missegregation [25, 44]. In addition, the expression level and/or localization of several structural components of the kinetochore are also misregulated following pRB depletion [53–55]. Importantly, upregulation of at least one of these proteins, Hec1, has been linked to chromosome segregation errors [56].

A majority of solid tumors possess extra centrosomes, the presence of which can induce chromosome missegregation [28]. Centrosome amplification has been shown to result from E2F-dependent misregulation of several genes following RB loss [45, 57] and this may contribute to the chromosomal instability seen in pRB-depleted cells. However, it is not clear that all cells lacking pRB generate extra centrosomes, and in at least some cells that do, extra centrosomes are soon lost while chromosome missegregation continues [28, 45, 58]. While CIN is typically believed to be a cause of aneuploidy, recent data has raised the possibility that aneuploidy itself may be a cause of CIN, such that CIN becomes a self-propagating phenomenon [59]. It is possible, therefore, that transient defects, such as the presence of extra centrosomes, which can occur following pRB-depletion may be sufficient to initiate perpetual chromosomal instability.

E2F-independent functions of pRB that suppress CIN

Our recent work shows that pRB loss leads to structural defects at the centromeric region of chromosomes, changes that appear to result from a failure to properly recruit cohesin and condensin II components to chromatin [47]. The resulting centromeric defects, which manifest during mitosis as hyperstretched kinetochores, promote erroneous merotelic kinetochore attachments that lead to chromosome missegregation. In a related study, it was found that an pRB mutant ($Rb^{\Delta L}$) that retains its ability to interact with E2F likewise has defects in chromosomal stability, and reported that regulation of chromatin compaction via an interaction between pRB and the condensin II complex has a role in tumor suppression [43].

Interestingly, there are several lines of evidence indicating that these mitotic defects and chromosome segregation errors are separable from the regulation of E2F-dependent transcription. Overexpression of E2F1 is insufficient to produce centromeric defects seen following pRB depletion [47]. In addition, because the $pRb^{\Delta L}$ protein retains its ability to interact with E2F, it is competent to repress E2F targets like Mad2 [43, 46]. Moreover the mitotic defects of $Rb^{\Delta L/\Delta L}$ mice were evident in ES cells that lack normal programs of E2F regulation [43]. Studies in *Drosophila* have revealed an extensive co-localization between RBF1 and the Condensin II protein dCAP-D3 on polytene chromosomes that is independent of E2F/DP regulation [60]. This, together with evidence of physical interactions between pRB/RBF and Condensin II proteins, suggests that pRB has an E2F–independent function that promotes the normal recruitment of Condensin II components to chromatin. Together these studies suggest that changes in the recruitment of condensin II and cohesin complexes, resulting from either pRB loss or mutation, has functional consequences on both chromosome structure and genome stability.

pRB regulates programs of gene expression and many of the proteins that have been discovered to associate with pRB are transcription factors or chromatin associated proteins that impact transcription [38, 61–63]. Fitting with this trend, although best known for their roles in regulation of mitotic chromosome structure, recent studies have suggested that both

the cohesin and condensin complexes have additional roles in regulation of gene expression. Effects of cohesin on gene expression have been observed in numerous organisms (reviewed in [64, 65]) and, unlike the role of cohesin in mitosis, these effects are independent of cell cycle progression [64, 66, 67]. In addition, changes in gene expression have been noted in both mouse models and in human diseases associated with cohesin mutations, where surprisingly, effects on mitotic chromosome cohesion are minimal [68, 69]. Recent studies have found that cohesin physically and functionally interacts with the mediator complex, an important transcriptional regulator, at active genes [70]. Cohesin has also been shown to be recruited by, and to influence the activity of, the transcriptional regulator CTCF [71–73]. Likewise, Condensin II complex's effects on chromatin compaction have been suggested to alter the accessibility of chromatin to the transcriptional machinery, and potentially to regulate recruitment of various transcription regulators [74]. Indeed, both cohesin and condensin have been shown to influence enhancers, silencers and insulators of gene expression [75]. These observations raise the intriguing possibility that the connections between pRB proteins and cohesin and condensin II complexes may reflect a shared role in transcriptional control. Currently however, the loci that are targets of this co-regulation, or the context in which this occurs, are not known.

Elevated levels of DNA damage in pRB mutant cells

In addition to structural changes to chromatin, several studies have reported that loss of pRB renders cells more prone to DNA damage, suggesting that pRB family members may be generally required to maintain genome integrity (Table 1). Just as there are several ways in which the loss of pRB can influence progression through mitosis, there are several ways in which the inactivation of pRB may lead to the accumulation of DNA damage. For example, pRB is important for the maintenance of DNA damage-induced cell cycle arrest during G1 [76, 77]. In addition, although cells lacking pRB family members are able to initiate G2 arrest in response to DNA damage, they are not always competent to maintain the arrest and instead enter mitosis with broken chromosomes [78]. Other links stems from the importance of pRB in regulation of replication and in controlling gene expression. Recent work shows that misregulation of the pRB pathway leads to nucleotide deficiency, and consequent replication-induced DNA damage [79]. Also, since pRB and pRB-related proteins regulate the expression of DNA damage repair factors [77, 80] they may also indirectly affect the efficiency of repair processes. An additional connection is suggested by the recent evidence that pRB affects the distribution of cohesin complexes. Cohesin complexes have been shown to play a role in regulating efficient repair of double strand breaks (DSBs) [81], so this may represent an additional way in which pRB may influence the DNA damage response.

Finally, we note that pRB interacts with many chromatin regulators and, in addition to their roles in transcriptional control (reviewed in [63]), several of these regulators are important for the formation of heterochromatin and, like cohesin and condensin II complexes, may have effects on both centromeric and telomeric structure [61, 62]. The loss or mutation of pRB leads to changes in chromatin structure, both in the local vicinity of promoter regions and in the organization of chromosomal structures. It is possible that the compound effect of all of these changes may make chromatin more vulnerable to damage. For example, general changes in chromosome architecture may predispose the cell to chromosome fragility and/or segregation errors. Such global effects would be difficult to attribute to any specific gene or binding partner.

pRB's regulation of genome stability: an additional tumor suppressive role?

pRB is a multifunctional protein. In addition to its role as a negative regulator of the G1 to S transition, pRB has also been shown to promote cellular differentiation, modulate cell fate decisions, be important for oncogene-induced senescence, and affect cellular sensitivity to apoptosis (reviewed in [38]). Currently it is unclear which of these activities are most important for tumor suppression and, depending on the context, their relative importance is likely to vary. There is increasing evidence that CIN and aneuploidy have causative roles in tumorigenesis and in the evolution of cancer cells. Given the extensive changes seen in pRB-deficient cells it seems likely that pRB's role in maintaining genome stability also contributes to its tumor suppressive activity [43].

Mutation of RB1 is a rate-limiting event in the development of most retinoblastomas. Recent studies suggest that homozygous mutation of RB1 leads to the appearance of benign retinomas that subsequently progress to retinoblastoma [40]. The role of pRB in E2F-regulated promotion of cell cycle progression, as well as promotion of differentiation and senescence explain why loss of pRB activity would be beneficial at the initial stages of tumor development. It is generally thought that there is a temporal aspect of tumor evolution in which cells gradually acquire numerous mutations [82]. The presence of chromosomal instability would promote such evolution and, indeed, the malignant progression from retinoma to retinoblastoma has been correlated with greatly increased levels of aneuploidy and genomic instability [40].

In an alternative view, recent work by several groups highlights the idea that not all tumor progression is gradual and that occasional isolated events can occur that greatly advance tumor evolution in a single step (punctuated equilibrium). One example of this is cytokinesis failure and the generation of a tetraploid cell. In the context of p53 mutations, tetraploidy has been shown to be initiating for tumor formation, and the subsequent presence of extra centrosomes promotes CIN through the formation of merotelic attachments [28, 34]. A second example of such a disastrous event is the recently described chromothripsis, in which a single chromosome is shattered and then haphazardly pieced back together, resulting in massive rearrangements, deletions and amplifications along a single chromosome [83], potentially leading to oncogene amplifications or tumor suppressor deletions. That usually only one chromosome is involved suggests that the affected chromosome is spatially separated. This may occur by resolution of chromosome bridges following cytokinesis, as proposed by the authors, or alternatively by the formation of micronuclei, which occasionally result following merotelic attachment. Interestingly, work by David Pellman and colleagues shows that chromatin located in micronuclei accumulate damage (personal communication). Merotely can also give rise to lagging chromosomes during anaphase that are positioned under the cytokinetic furrow. Subsequent furrow ingression can cause chromosome breakage [84, 85]. In support of this idea, recent work shows that DNA double strand breaks are apparent following merotelic attachments [86] and exciting new work by Medema and colleagues show that following cytokinesis, cells predisposed to forming merotelic attachments exhibit increased levels of DNA damage and subsequent chromosomal abnormalities (personal communication). This suggests that the increase in merotelic attachments that occur when pRB is lost may lead not only to the missegregation of whole chromosomes, but may also predispose afflicted chromosomes to catastrophic damage, increasing the chance of tumorigenic mutations.

Although increased severity of chromosomal changes and aneuploidy correlate with tumor progression, the generation of aneuploidy by increasing chromosome missegregation rates alone is growth inhibitory in culture [8, 9, 37], and results in few tumors in only a subset of

tissues, and late in life, when examined in mouse models [10, 87, 88]. The fact that many other tumors are able to tolerate such ploidy changes and continue to propagate with an ever-changing genome indicates that they have acquired specific adaptive mechanisms and these can perhaps be targeted to halt tumor growth [89]. Recent work has linked growth arrest in newly aneuploid cells to metabolic abnormalities [90] and activation of the p53 pathway[59]. A series of studies have shown that pRB loss is sufficient for cells to acquire ploidy changes and to remain competent to proliferate (see Table 1). This suggests that corruption of pRB pathway activity is likely to be a significant factor contributing to tolerance of aneuploidy. Understanding how pRB activity contributes to genome stability, ensuring accurate chromosome segregation and the intolerance of ploidy changes, could prove useful in devising therapeutic approaches that target aneuploid cancers.

These observations illustrate the point that the inactivation of pRB has the potential to cause multiple types of changes (Figure 1). For example, the links to condensin and cohesin connect pRB to the organization of chromosome structure, gene regulation, and DNA damage responses and repair. Potentially, each of these roles may have a variety of consequences for tumor cells. The centromeric dysfunction and merotelic attachment seen when pRB is lost can result in aneuploidy, chromosome instability, and genomic instability, and each of these phenomena presents a different set of risks for additional copy gains or losses and/or mutation of oncogenes and tumor suppressor genes.

Taken together, the current information shows that pRB loss promotes defects in mechanisms of chromosome segregation, instigating changes in whole chromosome copy number as well as more complex subchromosomal changes. The loss of pRB causes a consistent, low level of chromosome missegregation (CIN) and this effect is likely to involve both E2F dependent and E2F-independent pathways. pRB pathway lesions also impair the DNA damage response pathway. Through these changes, disregulation of the pRB pathway promotes cell cycle progression and mitotic failure, resulting in genomic instability and aneuploidy (Figure 1). Data showing the prevalence of merotelic attachments in CIN tumor cells, together with new findings suggesting that such erroneous attachments can lead to both whole chromosome segregation defects and the accumulation of DNA damage, highlight the potential importance of pRB's influence on mitotic fidelity.

The details are important

A recurring theme in the RB literature is the observation that pRB's role is highly context dependent. pRB can interact with many different proteins, and both its biological function and binding partners vary greatly. Among the challenges for future studies of the link between pRB and genomic instability is the need to determine which type(s) of tumor cells are most vulnerable to genetic change following the inactivation of pRB, and to discover which of the molecular consequences of pRB inactivation drive genomic instability in these cells.

While the loss of pRB function promotes genomic changes through misregulation of chromosome structure, the tolerance and effect of such structural changes may manifest differently in tumors of different origin. The effects of pRB loss are very likely to be strongly influenced by mutations in other tumor suppressors or oncogenes. For example, loss of pRB can result in p53-dependent cell death. In addition, p53 has roles in the DNA damage checkpoint and regulation of response to aneuploidy. Therefore, a tumor is more likely to benefit from genomic instability and chromosome mis-segregation imparted by loss of pRB when the p53-pathway has also been functionally inactivated.

A second complication is that pRB can be functionally compromised in tumor cells by several different types of changes. In addition to the loss or mutation of *RB1*, the mutation or

lowered expression of $p16^{INK4A}$ or the overexpression of Cyclin/Cdks alter the phosphorylation state of pRB. Currently it is unclear how the changes in genome stability resulting from *RB1* mutation compare with those that occur in cells where pRB is deregulated by cdk-phosphorylation. In addition to targeting pRB, G1 cdks phosphorylate two pRB related proteins, p107 and p130, that, in certain cell types, can compensate for some effects resulting from the loss of pRB. Differences in the type of lesion in the pRB pathway, as well as cell type-dependent differences in the degree of functional overlap/redundancy between pocket protein family members (p107 and p130) and potential mechanisms of suppression/compensation may contribute to the variation in type and degree of genomic instability seen in the vast array of tumors that have lost pRB function.

CIN and aneuploidy are thought to have causative roles in tumorigenesis and tumor evolution, and the gene expression signature following pRB loss is able to predict poor survival in some human cancer [48], suggesting that pRB's role in maintaining genome stability may be an additional important tumor suppressive function of this remarkable protein. Many questions remain to be answered (Box 2). It will be important to determine the impact of pRB's role in genome stability and how the genomic changes resulting from pRB inactivation of contribute to tumor growth, evolution, and response to treatment in various cancer models. Recent work has shown that enhancement of microtubule dynamics is one strategy to suppress CIN in tumor cells [26] and a better understanding of the mechanism(s) by which the loss of RB leads to chromosomal instability may uncover additional novel therapeutic targets. On the one hand these defects may represent an "Achilles heel" that can be specifically enhanced in tumor cells. Alternatively, it may be possible to suppress these changes, helping to stabilize the cancer genome and impairing tumor evolution, effects that that may enhance the potency of traditional therapeutics. For these ideas to become a reality, it will likely be important to better define the context in which pRB inactivation leads to genomic instability, and the situations in which this instability changes the biology of the tumor.

Box 2

Outstanding Questions

- Is genomic instability resulting from mitotic defects a consequence of pRB loss of function in transcription, replication, DNA damage repair, chromatin structural changes at the telomere and centromere, mitotic progression, or a combination of these changes?
- What aspect(s) of genomic instability is influenced by pRB's role in transcription (E2F-dependent and otherwise)?
- Does pRB affect mitotic fidelity directly at the time of cell division, or are pRB defects generated earlier in the cell cycle and then manifest during mitosis?
- Is pRB regulation of cohesin and condensin II the result of a direct physical interaction, or mediated through pRB's regulation of histone modification?
- Under what context does pRB loss of function promote genome instability? (i.e. cooperation with other mutations or LOH, type of pRB pathway lesion, etc)
- To what extent does tumor type/tissue of origin influence pRB's role in genome stability?
- How does pRB's role in maintenance of genome stability influence tumor growth and evolution?

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Glossary

Cohesin	A protein complex that associates with chromatin and is best known for its role in holding sister chromatids together. Cohesin is loaded on chromatin concurrent with replication and is maintained there until mitosis when it is removed sequentially from chromosome arms in response to phosporylation, and subsequently from the centromeric region by cleavage upon anaphase onset
Condensin	A protein complex structurally similar to cohesin whose chromatin association is cell cycle regulated. Mammalian cells have 2 described condensin complexes Condensin I and II, which differ in a subset of their components. Chromatin association of condensin complexes drives coiling of interphase chromatin and compaction prior to mitosis known as condensation
Centromere/ Kinetochore	The primary constriction during mitosis where sister chromatids are held together. The centromere is a specialized condensed region of each chromosome upon which the proteinaceous kinetochore structure is built. The kinetochore mediates association between the chromosome and microtubule fibers of the mitotic spindle to allow for chromosome movement and ensure accurate segregation during cell division
Centrosome	The microtubule organizing center of the cell. The centrosome is duplicated in preparation for cell division, with each centrosome forming one of two mitotic spindle poles. Presence of extra centrosomes is implicated in multipolar spindle formation, merotelic attachment and chromosome segregation errors

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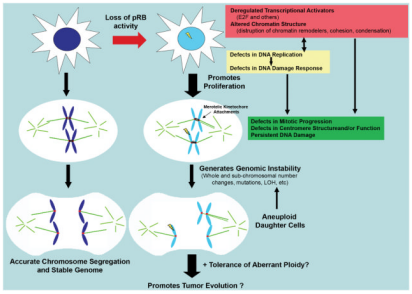

Figure 1. Complex role of pRB in maintenance of genomic stability

Functional inactivation of pRB has been shown to result in numerous changes, including transcriptional changes (local effects: regulation of specific transcriptional activators: E2F, etc; broad effects: regulation of heterochromatin formation, including telomeric and centromeric regions; and global effects: chromosome condensation and cohesion levels [purple box, top right]). Each of these defects in turn influence DNA replication, mitotic fidelity and the DNA damage response machinery through the transcriptional regulation of important players (yellow and green Boxes; DNA damage represented by yellow lightning bolt). In addition, transcription-independent interactions of pRB with components of many of these pathways have been identified, raising the possibility that pRB's influence on these downstream processes may be independent of its role in transcription (yellow and green Boxes). Importantly, defects in each of these cellular processes have been shown to result in genomic instability. In addition, pRB loss has been implicated in tolerance of aneuploidy, thereby allowing the propagation of these newly aneuploid cells. The molecular mechanisms behind many of these effects remain unclear and the complex interplay between different effectors of pRB loss makes it difficult to tease apart functional relevance. The importance of individual effects of pRB inactivation under different cellular contexts may in part explain the varying degree of genomic instability seen in tumors possessing pRB pathway lesions.

Table 1

pRB Loss Undermines Genome Stability: an Overview

Described effect on Aneuploidy, CIN and/or DNA Damage response	Type of Lesion/model	Date	Ref(s)
Cell cycle analysis of Rb deficient fibroblasts shows they have an increased incidence of aneuploidy	Primary fibroblasts isolated from *Rb* mutant mouse embryos	1996	[91]
Rb loss or inactivation allows for re-replication following drug induced mitotic arrest	$Rb^{-/-}$ mouse embryonic fibroblasts (MEFs); E7 expressing fibroblasts, $p16^{-/-}$ and $p21^{-/-}$ MEFs	1997/1998	[92] [93]
Loss of Rb, but not p107 or p130 impairs G1/S checkpoint response to damaging inducing agents	Rb−/− MEFs	1998	[94]
Loss of the Rb family prevents appropriate G1 arrest in response to DNA damage	$Rb^{-/-}$; $p107^{-/-}$; $p130^{-/-}$ Triple Knockout (TKO) MEFs	2000	[95, 96]
pRB interacts with Hec1 and hsRB expression in a yeast temperature sensitive hec1 allele suppresses segregation errors	Heterologous yeast system expressing human pRB and human Hec1	2000	[55]
Deficiency of Rb causes increased loss of a marker gene	$Rb^{-/-}$ mouse embryonic stem (ES) cells with an inserted chromosomal marker	2002	[97]
Rb null cells exhibit increased levels of aneuploidy	$Rb^{-/-}$ MEFs	2002	[98]
pRB depletion leads to upreguation of the mitotic spindle assembly checkpoint protein Mad2 and near diploid aneuploidy	$Rb^{-/-}$ MEFs, mouse and human cell lines expressing pRB-targeting short hairpins or E1A expression constructs	2004	[44]
Loss of Rb leads to increased ploidy and failure of the DNA damage checkpoint response	Conditional *Rb* knockout in Mouse Adult Fibroblasts (MAFs)	2004	[99]
Cells lacking Rb function exhibit increased double strand breaks and compromised cell cycle arrest following genotoxic stress.	Conditional *Rb* knockout in (MAFs)	2004/2005	[76, 80]
Rb loss leads to increased ploidy following serum starvation	$Rb^{-/-}$ and $Rb^{-/-}p107^{-/-}$ MEFs	2005	[100]
Rb loss leads to increased levels of aneuploidy and tetraploidy, independent of p53 status	$Rb1^{-/-}$, $p107^{-/-}$; $p130^{-/-}$, and TKO MEFs	2005	[62]
Loss of pRB leads to E2F1-mediated accumulation of DNA double strand breaks	Acute depletion of pRB in human fibroblast and osteosarcoma cell lines	2005/2006	[101, 102]
Acute loss of Rb induces centrosome amplification and aneuploidy	Conditional *Rb* deficient MEFs	2006	[45]
Disruption of Rb LXCXE pocket reduces pericentromeric H4K20me3 and induces ploidy changes	$Rb1^{\Delta L/\Delta L}$, $Rb1^{-/-}$ mouse ES cells	2006	[46]
Acute and sustained liver specific Rb loss promotes ploidy changes	Liver-specific conditional *Rb* knockout mice	2005/2007	[48, 103]
Rb loss leads to deregulation of DNA synthesis and elevated ploidy	Conditional *Rb* knockout in MAFs	2007	[49]
pRB inactivation compromises the G2/M DNA damage checkpoint	Stable pRB depletion in U2OS cells	2007	[104]
Whole chromosome gains and losses are coincident with progression from retinoma to retinoblastoma	Genetic *RB1* mutation/human tumor samples	2008	[40]
Rbf1 loss leads to defects in condensin II chromatin association and chromatin condensation, and abnormal chromosome segregation	*Drosophila Rbf1* mutants	2008	[60]
Acute depletion of pRB leads to supernumerary centrosomes, formation of micronuclei and aneuploidy. Depletion of CENPA partially suppresses the number of aneuploid cells.	Acute siRNA pRB depletion in human fibroblasts and HCT116 tumor cells	2009	[42, 105]
Suggests a functional link between pRB and the kinetochore protein Hec1 influences chromosome segregation	Transient and stable depletion of pRB in HCT116 tumor cells	2010	[53]

Described effect on Aneuploidy, CIN and/or DNA Damage response	**Type of Lesion/model**	**Date**	**Ref(s)**
pRB loss causes defects in chromosome cohesion and condensation, centromere dysfunction, and promotes high rates of chromosome missegregation	Acute and stable depletion of pRB in human epithelial cells; *Drosophila* Rbf1 mutants	2010	[106]
An Rb1 mutant that retains it's ability to regulate E2F exhibits defects in chromatin condensation, promotes more aggressive tumors, and accelerates loss of heterozygosity in a tumor model	$Rb1^{\Delta L/\Delta L}$; $TRP53^{+/-}$ and $Rb1^{\Delta L/\Delta L}$; $TRP53^{-/-}$ knock in mouse tumor models and MEFs and mouse ES cells	2010	[107]
Loss of the Rb family of proteins compromises the G2 DNA damage checkpoint, allowing cells to enter mitosis with unrepaired damage, thereby promoting genome instability and aneuploidy	TKO MEFs	2010	[78]
Replication induced DNA damage due to pRB pathway defects is caused by nucleotide deficiency and leads to genome instability	Primary keratinocytes infected with E6/E7 expression vector; Cyclin E overexpression in BJ fibroblasts	2011	[79]

Table 2

Mitotic defects identified following pRB pathway lesions are consistent with the presence of merotelic kinetochore attachments

Mitotic Delay and Increased Mitotic Index	[42, 44, 47]
Supernumerary Centrosomes	[45, 57, 58]
Micronuclei Formation	[42, 43]
Changes in Heterochromatin Formation (i.e. Centromeric Regions)	[43, 46, 47, 60–62, 108, 109]
Changes in expression, recruitment and/or localization of kinetochore or centromere proteins	[44, 53–55, 105, 110]
Changes in Mitotic Spindle Assembly Checkpoint Control	[44, 53]
Lagging chromosomes during anaphase	[47, 105]