MINIREVIEW

Retinoblastoma Tumor Suppressor: Where Cancer Meets the Cell Cycle

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The retinoblastoma tumor suppressor gene, *Rb*, was the first tumor suppressor identified and plays a fundamental role in regulation of progression through the cell cycle. This review details facets of RB protein function in cell cycle control and focuses on specific questions that remain intensive areas of investigation. Exp Biol Med 231:1271–1281, 2006

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Overview

Cell cycle control is a fundamental cellular process that governs cellular proliferation, and the retinoblastoma tumor suppressor protein (RB) is a critical component of the cell cycle control machinery. RB loss or inactivation is a major mechanism by which cancer cells attain a growth advantage during tumorigenesis. It is now apparent that RB impinges on a multitude of physiological processes not only in humans, but in plants and other organisms as well. These lines of investigation have demonstrated pivotal roles for RB in stem cell maintenance, tissue regeneration, differentiation, and developmental programs (1–5). Thus, delineating RB function in cell cycle control and tumor suppression may provide insight into the regulation of diverse biological processes.

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Background

Identification of the Retinoblastoma Tumor Suppressor. The retinoblastoma tumor suppressor gene (*Rb*) was initially identified as the causative agent whose loss results in the development of retinoblastoma, a pediatric tumor of the eye. Two clinically distinct forms of retinoblastoma occur, and were identified as the heritable and sporadic forms. The heritable form of the disease can be transmitted to successive generations in an autosomal dominant fashion and is characterized by early onset and increased multiplicity of tumors. By contrast, the sporadic form of the disease typically involves a single tumor. In the early 1970s, Dr. Alfred Knudson carried out statistical analyses of both disease forms and proposed that retinoblastoma arises after development of two genetic events (6). It was proposed that individuals with the heritable form of the disease carried one germline mutation and thus were at heightened risk for development of the disease. Following publication of this groundbreaking "two-hit hypothesis," multiple laboratories sought to elucidate the genetic basis for retinoblastoma. A key finding came from the observation that a specific chromosomal locus at 13q14 exhibited loss of heterozygosity in retinoblastoma lesions (7, 8). From these data it was hypothesized that retinoblastoma occurs after bialleic loss of a tumor suppressor, and through positional cloning strategies the *Rb* tumor suppressor gene was identified in the late 1980s by multiple laboratories (9–11). It was subsequently verified that *Rb* loss is indeed the causative factor for retinoblastoma, thus validating the concept of tumor suppressor action. Since these early studies it has been revealed that the RB protein is lost or inactivated in the majority of human tumors (>70%), encompassing a wide range of tumor types (12–16). As

such, there has been a concerted effort to delineate the mechanisms by which RB exerts its potent tumor suppressor activity.

Characterization of RB Protein Function. Cloning of the *Rb* tumor suppressor gene afforded the opportunity to discern its mechanisms of action. Early studies revealed that the RB protein is a 928 amino acid, nuclear phospho-protein that harbors no catalytic activity, and possesses weak, nonspecific DNA binding activity. In 1988 it was found that RB is sequestered by viral oncoproteins of DNA tumor viruses, including SV40 large T antigen, adenovirus E1A, and human papilloma virus E7 (17–20). It was correctly hypothesized that sequestration by viral oncoproteins disrupts the ability of RB to exert its tumor suppressor function, thus revealing one mechanism by which viral oncoproteins initiate tumor formation. Subsequent efforts to identify cellular proteins that associate with RB demonstrated that the cohort of RB interacting proteins belong to a wide array of disparate cellular processes (16, 21, 22), thus providing the indication that RB impinges on multiple biological pathways. However, it is now clear that many of the most critical RB binding partners participate in transcriptional control (16, 21). Paramount among these are the E2F family of transcription factors (23, 24). E2F and its heterodimer partner, DP, are central regulators of cell cycle gene expression, and directly modulate the expression of genes involved in DNA replication, DNA repair, and G_2/M progression (25–29). RB attenuates E2F action by recruiting transcriptional corepressors to these E2F-regulated promoters, thus mediating transcriptional repression of E2F-regulated genes (30–32). Currently, it is hypothesized that this function of RB is essential for regulating cellular proliferation (33–35). This model is supported by microarray studies, wherein the cohort of genes induced by E2F are summarily repressed by activated RB (36, 37). Thus, through its ability to recruit transcriptional repressor molecules and modulate E2F activity, RB is a potent regulator of genes required for cell cycle progression.

Cell Cycle–Dependent Regulation of RB. Because cells must progress through the cell cycle to proliferate, there must be a means to regulate RB and antagonize its transcriptional repressor function. Such a mechanism is achieved through a series of phosphorylation events that regulate RB function in relation to cell cycle position and in response to mitogenic stimulation. In quiescence (G_0) and early G_1, RB is hypophosphorylated, and in this state RB is considered to be maximally active (e.g., able to bind and repress E2F activity with highest efficacy) (16, 38–41). In response to mitogenic stimulation, RB becomes progressively phosphorylated on discrete residues, rendering the protein increasingly less efficient at interacting with associated proteins (including E2F and the corepressor molecules), and thereby weakened in its ability to effect transcriptional repression (40, 42–44). Thus, phosphorylation of RB serves as a switch to progressively attenuate RB activity as a function of cell cycle progression. Importantly, RB phosphorylation is catalyzed by the activity of discrete cyclin-dependent kinase (CDK)/cyclin complexes.

The CDK/cyclin complexes that function in G_1 progression (CDK4/cyclin D and CDK2/cyclin E) are regulated in response to the cellular environment (12, 45–48). Universally, mitogenic signaling (e.g., growth factors) leads to activation of CDK/cyclin complexes, whereas antimitogenic signaling (e.g., as initiated by confluence or nutrient depletion) pathways inhibit activation of the G_1 CDK/cyclins. Each complex phosphorylates distinct residues of RB, and it has been postulated that this specificity provides a mechanism to temporally control RB during G_1 (39, 42, 43). Together, the G_1 CDK/cyclins catalyze sufficient RB phosphorylation to completely inactivate its transcriptional repressor function, thus allowing expression of E2F target genes, whose activity is essential for entry into S phase. It is imperative to note that RB is held inactive (e.g., hyperphosphorylated) throughout the remainder of the cell cycle, thus indicating that continued progression through S, G_2, and M phases require suppression of RB activity (44). Several kinases participate in this process, including CDK2/cyclin A and CDK1/cyclin B (44). Finally, in mitosis the activity of RB is reset through the action of a phosphatase activity (49–53), which restores RB to its hypophosphorylated state. Given the powerful role of the G_1 CDK/cyclins in initiating RB phosphorylation, their activities are tightly controlled through mechanisms including cyclin expression, cyclin stability, nuclear localization, CDK phosphorylation, or the influence of CDK-inhibitory molecules (CKIs) (12, 45, 47).

Together, these findings support an overall model (Fig. 1) wherein (i) RB in G_0 imparts cell cycle inhibition by repressing E2F activity; (ii) mitogenic signaling cascades overcome antagonistic signals in G_1 through activation of CDK4/cyclin D and CDK2/cyclin E complexes; these complexes phosphorylate RB and sequentially render it inactive; (iii) E2F-mediated gene expression is then elicited to promote gene expression programs that facilitate progression through S phase and G_2/M; and (iv) during the transition through mitosis RB is dephosphorylated through the action of a phosphatase to reactivate RB function. From these observations it is apparent that RB integrates both mitogenic and antimitogenic signals, and as such serves as a critical focal point to control cellular proliferation.

The RB Pathway and Cancer. As predicted based on the model of RB function, mutations, or alterations that disrupt the ability of RB to form transcriptional repressor complexes significantly impair its tumor suppressor activity (12–16). Several mechanisms have been identified in human tumors that ablate RB function. First, excessive expression of CDK4 or cyclin D (as achieved by amplification, mutation, chromosomal translocation, or other means) is known to occur with relatively high frequency in selected

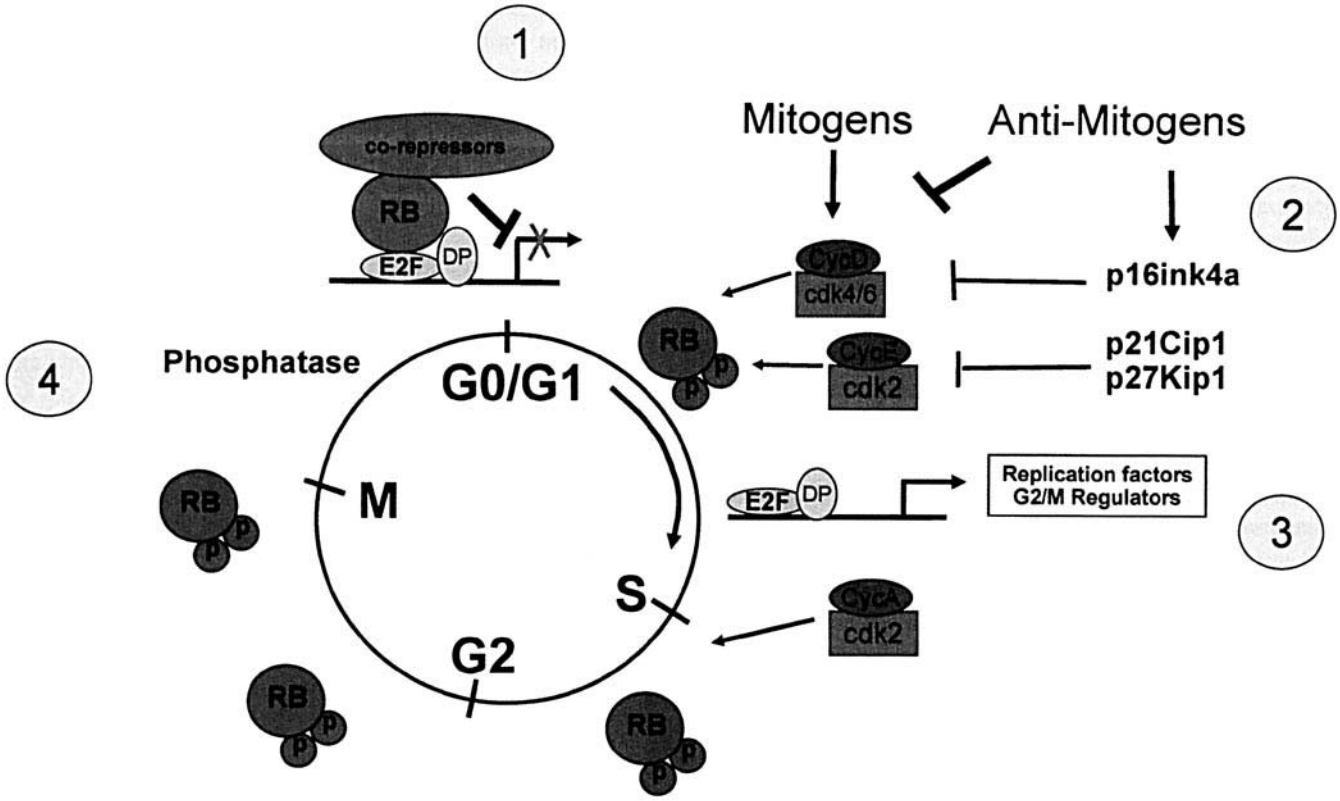

Figure 1. Schema of RB function. 1. RB in G_0 or early G_1 imparts cell cycle inhibition by repressing E2F-activity; 2. Mitogenic signaling cascades overcome antagonistic signals in G_1 to elicit activation of CDK4/cyclin D and CDK2/cyclin E complexes. These complexes phosphorylate RB and sequentially render it inactive; 3. E2F-mediated gene expression then promotes gene expression programs that facilitate progression through S phase and G_2/M. 4. During the transition through mitosis RB is dephosphorylated through the action of a phosphatase to reactivate RB function.

tumor types, and results in enhanced RB phosphorylation. Second, the p16ink4a CKI can be lost or mutated; this event also serves to induce excessive CDK4/cyclin D activity and RB phosphorylation, because p16ink4a is a critical inhibitor of CDK4/cyclin D function (12, 44, 47). Third, oncoproteins such as the human papilloma virus E7 protein can sequester RB and preclude association with corepressor molecules or E2F, thus compromising its ability to modulate gene transcription (18, 19). This mode of RB inactivation is frequently observed in cervical carcinoma and contributes to the development of disease. Lastly, the *Rb* locus itself can be mutated or lost (such as occurs in retinoblastoma and other tumor types) (16, 22), thus contributing to unchecked E2F activity. It has long been observed that while almost all human tumors inactivate RB function, the mechanisms by which this is achieved is tissue specific. For example, *Rb* mutation is observed at high frequency in small cell lung cancer, while it is a relatively rare event in non–small cell lung cancer, wherein loss of p16ink4a function is a predominant event (54, 55). The genesis and consequence of these tumor-specific alterations have not been addressed.

Combined, these findings provide a working model for elucidating the action of RB in cell cycle control and delineating the influence of RB loss in cancer. They also elicit critical questions that are still being addressed and for which a clear picture has yet to emerge: (i) How does RB govern cell cycle control?; (ii) What is the influence of RB loss on cell cycle control?; (iii) How does RB loss promote tumorigenesis?; and (iv) Does RB status modulate the treatment of cancer?

How Does RB Govern Cell Cycle Control? As summarized below, the mechanism through which RB acts to elicit cell cycle inhibition has remained a key question in the field for many years.

Role of RB in G1 control. Initially, it was speculated that RB functions in G_1 to limit cell cycle progression. This model was based on the observation that RB phosphorylation is completed by mid-G_1, suggesting that RB exerts its antimitogenic function prior to this point in the cell cycle. Attempts to uncover the mechanisms of RB activity were hindered by the fact that ectopically expressed RB is rapidly phosphorylated and inactivated in the majority of cell lines, thus precluding any effect on cell cycle position (40, 56). To overcome this challenge, several groups developed phosphorylation-site mutants of RB that rendered the protein refractory to CDK-mediated phosphorylation and inactivation (40, 56–60). As predicted, these constitutively active mutants were potent inhibitors of cell cycle progression and facilitated study of RB-mediated cell cycle control. Surprisingly, these analyses failed to identify G_1 targets as

critical for cell cycle inhibition. For example, cyclin E acts relatively late in G_1 and is a well-established E2F target gene. However, neither cyclin E–associated kinase activity nor events that result from cyclin E activity are altered by activated RB (57, 58, 61). Subsequently, microarray analyses confirmed that the vast majority of RB regulated genes are involved in S phase and G_2/M control (36, 37). While these data suggest that RB tumor suppressor activity lies beyond G_1 phase, it is possible that RB may have influence on G_1 progression that has not yet been revealed or is cell type specific.

Role of RB in S phase control. The initial indications that RB may function in S phase control came from three independent laboratories that demonstrated that cells arrested by active RB alleles accumulate with S phase DNA content (57, 58, 60). Subsequent studies using S phase synchronized cells showed that RB activation (as achieved via microinjection or through inducible expression) halts ongoing DNA replication (57, 60). Thus, evidence supports the current model of RB activity, wherein a principle execution point occurs in S phase. This function of RB is employed following DNA damage, cAMP signaling, and action of viral replication factors that limit cellular DNA replication (62–64). Given the importance of this activity for inducing cell cycle arrest, significant effort is devoted to determining the mechanisms by which RB governs DNA replication.

It has been postulated that RB may directly influence the DNA replication machinery. For example, it has been indicated that RB colocalizes with elements of the DNA replication apparatus, although there has been some controversy associated with this phenomenon (65–67). Additionally, RB has been reported to directly bind DNA replication factors (21, 68). Such findings would suggest that RB functions in *cis* to directly inhibit DNA replication. However, the negative action of RB on replication control can be overcome by providing cyclins and other factors in *trans,* suggesting that the function of RB is not through direct actions on critical replication factors (56, 59, 69, 70). Rather, these findings suggest that RB functions through a specific pathway, wherein the targeted constituent can be complemented in *trans.*

Because RB is a potent transcriptional regulator it is appealing to speculate that such *trans* acting factors are transcriptional targets of RB. Microarray analyses revealed a plethora of DNA replication factors that are repressed at the RNA level following the activation of RB (36, 37). However, during the analyses of an RB-mediated S phase inhibition, no attenuation of replication protein levels was observed (69). This observation is likely due to the stable nature of many of these proteins. In contrast, the cyclin A gene is not only efficiently repressed by RB, but its protein levels are dramatically attenuated with rapid kinetics (61, 69). This finding was significant, because cyclin A is a critical effector of S phase entry and DNA replication, and suggested that RB may act through cyclin A to regulate replication factor action. To delineate the ultimate influence of RB on replication machinery, the engagement of the replication apparatus with chromatin can be monitored. Analyses of multiple replication factors demonstrated that RB acts specifically to block the function of proliferating cell nuclear antigen (PCNA), the DNA polymerase processivity factor (69, 70). The ability of RB to attenuate PCNA was dependent on active transcriptional repression, and was mediated through down-regulation of cyclin A expression and attenuation of CDK2 activity (69, 70). Thus, a mechanism by which RB regulates DNA replication is manifested through depletion of cyclin A and resultant inhibition of PCNA activity. RB can also affect the dNTP synthetic machinery and with delayed kinetics down-regulates a myriad of additional replication proteins to further reinforce S phase arrest (69, 71).

Role of RB in G_2/M control. Because RB arrests cells earlier in the cell cycle there has been little investigation of the role of RB in G_2/M control. However, there are compelling reasons to believe that RB may in fact play an important role in coordinating progression through mitosis. First, many of the critical factors for G_2/M control (e.g., Cdk1, cyclin B, Plk1, Cdc20, Mad2) are regulated by the RB/E2F pathway (25, 36, 37). Second, several studies have linked RB action to mitotic control, although a detailed mechanism is lacking. For example, RB is proposed to assist in the maintenance of G_2/M checkpoints following DNA damage by mediating transcriptional repression of Cdk1 and cyclin B (72, 73). Future investigation into the role of RB in G_2/M control may reveal new facets of its tumor suppressor functions.

What Is the Influence of RB Loss on Cell Cycle Control?

Although much emphasis has been placed on delineating the mechanisms by which RB regulates the cell cycle, the consequence of RB loss has been less well described.

Models of RB loss. Dissecting the contribution of RB loss to specific facets of cell cycle control is dependent on the presence of reliable models. While many tumor cell lines lack RB, genetic variability between lines limits the utility of comparing nonisogenic cell systems. Alternatively, viral oncoproteins that inactivate RB have been utilized to reveal the consequence of RB loss. However, these oncoproteins often harbor additional activities that confound data interpretation, such as inactivation of the RB-related proteins p107 and p130 (74). Additionally, viral proteins can influence the activities of unrelated proteins such as p21, p53, or p300 (75–77). Although these models are frequently employed, much of what we know about RB loss has been determined by genetic deletion in mouse model systems.

Initial characterization of RB loss utilized murine embryonic fibroblasts derived from the mating of $Rb^{+/-}$ mice (78–80). More recently, several laboratories have taken advantage of mouse models wherein specific exons of *Rb* are flanked by LoxP sites (81, 82). In these models, the *Rb* gene can be functionally deleted through the action of

Cre-recombinase *in vitro*. These models are highly efficient and can be employed in the context of embryonic fibroblasts, adult fibroblasts, Schwann cells, or any other cell type that can be effectively maintained in culture (81, 82). In this model, the kinetics of RB protein loss can be followed, and corresponding molecular and biological effects assessed. The advent of RNA interference technologies also provides a new approach to delineate the outcome of RB loss (83, 84). In total, models to assess the impact of RB loss include use of RB null cells (as generated via controlled genetic disruption) and through indirect means (e.g., expression of viral oncoproteins).

RB loss and changes in gene expression. Based on the action of RB in transcriptional repression, loss of RB would be predicted to deregulate the expression of E2F-regulated genes. Analyses of chronic RB loss in mouse embryonic fibroblasts and subsequent microarray analyses demonstrated that as predicted, RB causes derepression of specific E2F-regulated genes (78, 85). Correspondingly, acute RB inactivation (as achieved via the Cre-Lox model) increases expression of E2F-regulated genes, including those encoding a large number of proteins associated with DNA replication proteins, G_2/M control, chromatin structure, cell death, and DNA repair (81)[1]. Thus, the hypothesis that RB plays a central role in E2F regulation has been validated by models of RB loss.

RB loss and cell cycle deregulation. Because RB loss results in deregulation of many genes associated with cell cycle control, it was predicted that significant alterations in cell cycle distribution and cellular proliferation would ensue. However, low-passage RB-deficient cells exhibit a cell cycle profile comparable to that observed in RB-proficient cells (78, 81, 82). It has therefore been proposed that RB holds minimal influence on cell cycle control in the absence of cellular stress and in the presence of ample mitogen. This model predicts that conditions of genomic insult or cell stress may heighten the requirement for RB activity; indeed, such factors invoke more pronounced effects on cell cycle progression in RB-deficient cells. For example, the ability of transforming growth factor-β to retard cell cycle progression is diminished after RB loss (78). Particularly striking is the response to DNA damaging agents, wherein loss of RB leads to an uncoupling of the normal inhibition of cell cycle progression (62, 81, 86–88). As such, RB-deficient cells bypass cellular checkpoints and continue to progress through the cell cycle in the presence of DNA damage. Combined, these studies indicate that RB loss has a significant effect on the cellular response to antimitogenic signals.

RB loss and senescence and differentiation programs. The loss of RB and corresponding changes in cell cycle control are critically linked to two additional processes that are associated with terminal proliferative arrest: cellular senescence and differentiation (89–91).

The engagement of the senescent state is a highly controversial process that can be invoked by replicative telomere shortening or via a multitude of stresses (e.g., DNA damage or oncogene activation). RB loss can bypass the proliferative cessation that is associated with extended time in specific cell culture systems (82), but is not believed to facilitate cellular immortality in the face of telomere erosion (92). In the context of induced senescence programs, RB has been shown to play a pivotal role downstream of both oncogene activation and DNA damage stresses (16, 82, 83, 93). For example, oncogenic Ras induces a senescent phenotype in primary human fibroblasts, and this senescence phenotype is partially reversed via disruption of the RB pathway (83). Therefore, loss of RB is proposed to represent one means for such challenged cells to progress to tumor development.

Cellular differentiation is critical for tissue function and generally restricts subsequent cellular proliferation. As such, differentiation can function in specific cell types as a tumor suppressive mechanism. RB action has been analyzed in a host of differentiation paradigms in both cell culture and animal models. For example, in myogenic differentiation RB is required for the full spectrum of muscle markers to be expressed (94). Additionally, the RB-deficient muscle that forms has the capacity to re-enter into the cell cycle (94–96). Correspondingly, RB loss influences adipose and bone differentiation programs (97–99). In many of these processes not only does RB loss modify cell cycle withdrawal associated with differentiation, but interestingly, RB actually functions as a transcriptional activator for many differentiation associated transcription factors (e.g., MyoD in muscle differentiation or core binding factor A (CBFA) in osteogenic differentiation) (4, 97, 100, 101).

How Does RB Loss Promote Tumorigenesis?

One of the critical focus areas for RB research is to develop an understanding of the mechanisms through which loss of RB contributes to tumor development.

Models. The vast majority of functional studies on RB and tumor suppression have been carried out in either cell culture or mouse models.

Using cell culture models RB-deficient cells are often viewed as being predisposed to transformations by various oncogenes. Such a model was initially supported by studies demonstrating cooperation between viral oncoproteins that target RB (e.g., adenovirus E1A or HPV-E7) and other oncogenes (e.g., Myc) (93, 102). More recent studies suggest that specific RB loss is pivotal in transformation (103, 104). However, this effect of RB is dependent on the oncogene utilized and recent studies paradoxically suggest that loss of RB actually inhibits Ras-mediated transcription (105–107). Thus, the activity of RB in suppressing tumorigenesis is likely context dependent.

In the mouse, heterozygosity for *Rb* predisposes to the development of pituitary and thyroid tumors (108–112). The

[1] Markey *et al.*, submitted.

penetrance of these tumor types varies depending on the mouse strain, but all occur with loss of heterozygosity at the *Rb* locus. Homozygous *Rb* gene inactivation results in embryonic lethality, thus to investigate tissues outside of the pituitary and thyroid, investigators have utilized targeted expression of Cre or Flp recombinase to ablate RB function in a tissue-specific fashion (113–121). From these studies it is apparent that RB loss is insufficient to stimulate tumor development in most tissues. However, these models revealed at least two means through which RB loss and concomitant cell cycle deregulation can contribute to tumor development.

Hyperplastic proliferation. Aberrations in cell cycle control can lead to unscheduled cellular proliferation and contribute to hyperplastic proliferation in selected tissues. Such hyperproliferative responses would be expected and have been documented in skin, lung, prostate, pituitary, and additional models of RB deletion (113–118, 120, 121). However, the hyperplastic phenotypes rarely progress to tumor formation. The lack of neoplastic disease could be attributed to the relatively limited life span of mice or potentially to other barriers to tumor progression that are lost at relatively low frequency in murine models. Two clearly defined barriers to tumorigenesis with loss of RB are the p53 tumor suppressor and the RB-related protein p107. The p107 protein has been shown to compensate for RB loss in multiple settings (82). In the mouse, coordinate loss of RB and p107 initiates tumor formation in many tissues wherein singular RB loss is insufficient for this process (120, 122–125). For example, retinoblastoma formation requires loss of both RB and p107 (123, 124). By contrast, concurrent loss of the p53 tumor suppressor cooperates with RB deletion in the genesis of small cell lung, medulloblastoma, and ovarian tumors (114, 116, 117). Thus, cooperative genetic events are required in most mouse models to stimulate tumor formation after RB loss.

Genomic instability. An alternative means through which cell cycle deregulation can influence cancer is via a breakdown of genome integrity. RB loss was initially suspected to compromise genome stability based on studies with viral oncoproteins (126). Subsequent studies have indicated that loss of RB compromises genome integrity, in many instances via inducing a polyploidy phenotype (67, 81, 84, 127–131). The mechanism through which RB loss leads to these changes in genome stability is postulated to arise from at least two discrete mechanisms. First, specific target genes of RB have the capacity to deregulate cell cycle transitions and in so doing compromise DNA ploidy control. Perhaps the strongest data supporting this model come from analyses of RB and MAD-2 (84). MAD-2 gene expression is regulated via E2F, and the MAD-2 protein negatively regulates mitosis. As such, RB-deficient cells exhibited elevation of MAD2 expression, and this event was causally associated with mitotic delay and corresponding failures in appropriate chromosomal segregation leading to aneuploidy (84). It is likely that other RB-regulated target genes could contribute to aneuploidy by relaxing the normally tight coordination between completion of mitosis and the initiation of DNA replication. Second, RB loss has been associated with modifications of chromatin (132). These changes in chromatin could compromise centromere function and similarly compromise the integrity of chromatin segregation. While genome instability fueled by RB loss could promote cancer, most evidence supporting this model has been obtained in cultured cells. Although few animal model studies have assessed the effects of RB loss on genome integrity, targeted RB deletion in the murine liver alters ploidy in hepatocytes (119). However, the consequence of RB-mediated ploidy control and genome stability on tumorigenesis has yet to be firmly determined.

Does RB Status Modulate Response to Cancer Therapies?

Given the critical action of RB in tumor suppression and stress responses, it is appealing to speculate that RB loss will have a significant influence on therapeutic strategies used in the treatment of cancer.

Action of RB loss on cytotoxic therapies. Because many DNA damaging agents are utilized in cancer therapy, multiple studies have evaluated the influence of RB loss on cytotoxic chemotherapeutic agents that function to induce DNA damage (62, 81, 86–88). These agents (e.g., cisplatin, ionizing radiation, camptothecin) inhibit cell cycle progression in cells containing a functional RB protein. However, DNA replication proceeds unabated in RB-deficient cells, thus resulting in aberrations of DNA ploidy (67, 81, 133). This effect of RB loss would be expected to exert two possible influences on cytotoxic therapies. First, it may be predicted that RB loss could render cells permissive for a high mutation burden, thereby facilitating tumorigenesis (128). Remarkably, such an effect has not been documented in published studies. Second, aberrant cell cycle progression in the presence of DNA damage could enhance the burden of damage and stimulate cell death. This latter effect has been observed in multiple studies, and in fact, RB loss results in a significant increase in secondary forms of DNA damage due to aberrant cell cycle control (86, 87, 134). Importantly, loss of RB is considered a "proapoptotic effect" and numerous studies have demonstrated that RB-deficient cells are particularly susceptible to cell death (134–138). The basis for this is complex and likely due to the deregulation of E2F activity, which in certain contexts, is a highly apoptotic signal (137, 139–144). Specifically, the proapoptotic protein E2F-1 has been shown to induce the expression of apoptotic genes (137, 138) as well as to influence DNA damage signaling pathways (140, 141, 145, 146) to mediate cell death. Depending on the agent, it is probable that both cell cycle deregulation and deregulated expression of specific proapoptotic genes coordinately contribute to the sensitivity of RB-deficient cells to cytotoxic therapeutics.

RB loss and directed therapeutics. In contrast with cytotoxic agents, relatively little is known regarding the consequence of RB loss on noncytotoxic therapeutics.

Examples of this class of agents would include hormonal therapies (as used in the treatment of breast and prostate cancer) or other directed therapies (e.g., CDK inhibitors, epidermal growth factor receptor inhibitors) that may exert most of their influence through cell cycle inhibition. There is reason to suspect that RB loss will have significant effects on the response to these agents. For example, hormonal therapy of both breast and prostate cancer cells is associated with the activation/dephosphorylation of RB that is believed to be required for the cessation of proliferation (147, 148). Thus, RB loss could represent a means to bypass these commonly utilized therapeutic modalities. Additionally, the CDK4 inhibitor from PD-332991 fails to influence RB-deficient cells (149). As new drugs are developed, RB status is rarely examined as a determinant for therapeutic efficacy. However, studies that do investigate RB function could provide insight into the spectrum of tumor that may be expected to respond to a particular agent. With discovery efforts directed at molecular targets that ultimately interface with the cell cycle machinery, it will be critically important to understand the influence of RB loss on the efficacy of these agents to accurately monitor their utility.

Conclusions and Lingering Questions

With in excess of 10,000 articles published that relate to RB, many facets of its function have been elucidated. Importantly, these studies provide a clear framework in which to interrogate RB action in cell cycle control. However, many fundamental questions remain to be clearly addressed.

Tumor Suppression. Surprisingly, a universal understanding of the mechanisms through which RB functions to suppress tumorigenesis has eluded investigators for the last 20 years. Critical studies in mouse and cell culture models support the possibility that the action of RB in tumor suppression is likely context specific. Clearly, there are tissues that are significantly more susceptible to tumorigenesis arising with RB loss than others (e.g., retina in humans and pituitary and thyroid in mice). In these tissues, loss of RB compromises critical facets of proliferation such that tumors readily arise from a field of aberrant cellular proliferation. In some tissue types, loss of RB could abrogate differentiation programs, whereas in other tissues RB loss would compromise genomic stability in a manner promoting tumorigenesis. Therefore, it is likely that each tissue will have to be tackled separately to understand the action of RB in suppressing tumorigenesis.

Influence of the RB Pathway on Clinical Outcome. Numerous studies have evaluated the influence of RB loss on clinical parameters. The clearest example of this is retinoblastoma, in which the *Rb* gene is analyzed in individuals with retinoblastoma to delineate heritable versus somatic forms of the disease (150–152). In contrast, other tumor types have provided a murky picture for the role of RB on prognosis in any given tumor type. In many tumor types there are data that paradoxically implicate RB loss for both favorable and poor prognoses. The bases for these contradictory data are often elusive. However, in many tissues RB expression levels are relatively low or RB loss is mediated via nongenetic mechanisms, thus hindering assessment of RB status. As such, the use of surrogate markers of RB function, such as the gene expression signatures of RB loss, may provide an advantage in evaluating RB activity in tumor specimens. Therefore, advances in the detection of functionality status will likely be required to interrogate the RB pathway for prognostic significance.

Targeting RB Loss in Cancer Therapy. Activation of tumor suppressor pathways may represent ideal avenues for intervention, and have already proven successful in preclinical studies with p53 (153, 154). Efforts to exploit direct RB loss have largely emanated from the field of oncolytic viruses, wherein viruses have been engineered to preferentially replicate in RB-deficient cells (155, 156). The efficacy of such agents as therapeutics remains unclear. As discussed above, conventional assessment of cytotoxic agents has indicated that RB loss in specific cell-based assays enhances cytoxicity. However, the extent to which this phenomenon holds true in tumor types that have lost both RB and facets of the apoptotic machinery is less clear and needs to be evaluated in appropriate models.

In sum, while significant milestones have been reached in elucidating RB function in model systems, implementation of this knowledge to develop novel therapeutic intervention strategies has yet to achieve fruition. Future investigations into the biological consequence of RB loss are expected to delineate the full range of mechanisms involved in tumor suppression and provide tools to predict course and direct the most efficacious treatment for disease.

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