Relevance of pRB Loss in Human Malignancies

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ABSTRACT

The retinoblastoma tumor suppressor protein (pRB) is a known regulator of cell-cycle control; however, recent studies identified critical functions for pRB in regulating cancer-associated gene networks that influence the DNA damage response, apoptosis, and cell metabolism. Understanding the impact of these pRB functions on cancer development and progression in the clinical setting will be essential, given the prevalence of pRB loss of function across disease types. Moreover, the current state of evidence supports the concept that pRB loss results in pleiotropic effects distinct from tumor proliferation. Here, the implications of pRB loss (and resultant pathway deregulation) on disease progression and therapeutic response will be reviewed, based on clinical observation. Developing a better understanding of the pRB-regulated pathways that underpin the aggressive features of pRB-deficient tumors will be essential for further developing pRB as a biomarker of disease progression and for stratifying pRB-deficient tumors into more effective treatment regimens.

Introduction

The retinoblastoma protein (pRB) was the first protein identified as a tumor suppressor. pRB function is lost in 98% of the rare childhood malignancy retinoblastoma, in which the *RB1* gene was traced to a locus on chromosome 13 and subsequently sequenced (1–4). Outside of retinoblastoma, pRB loss is common across cancer types and is strongly associated with poor progression-free survival (PFS), disease-specific survival (DSS), and overall survival (OS; ref. 5). Despite this prevalence, the mechanisms by which pRB loss promotes cancer progression are not completely understood. Here, the clinical significance of pRB alterations across tumor types will be discussed, as well as the potential utility of pRB loss as a biomarker for prognosis and therapy resistance.

Mechanisms Driving Altered pRB Function in Human Malignancies

pRB exerts a well-understood function in regulating cell-cycle progression by inhibiting the activity of E2F transcription factors. In brief, pRB function is directly regulated by cyclin-dependent kinase 4 and 6 (CDK4/6) and cyclin D complexes. In response to mitogenic stimuli, CDK4/6 proteins are released from upstream CDK inhibitors (CDKI), such as $p16^{INK4a}$, and form complexes with cyclin D. These complexes then phosphorylate pRB, leading to a conformational change and release from E2F transcription factors, allowing for the activation of transcription and cell-cycle progression (6). As such, altered pRB function can be derived from multiple mechanisms including changes in the *RB1* gene itself, and altered function of upstream pathway regulators. Interestingly, these mechanisms tend to be disease type specific (**Table 1**) and are further discussed below.

RB1 gene modifications

Loss of pRB function in human cancer occurs predominately via deletion (one copy if heterozygous or two copies if homozygous) of the *RB1* gene, *RB1* promoter methylation, or mutations resulting in a nonfunctional protein. Deletion of the *RB1* gene occurs in 91%–100% of small cell lung cancer (SCLC), 72.2% of basal-like and 61.5% of luminal B breast cancers, 63% of osteosarcomas, 30% of non–small cell lung cancer (NSCLC), and 17%–33% of castration-resistant prostate cancer (CRPC; refs. 7–17). In retinoblastoma, loss of pRB is attributed to hypermethylation of the *RB1* promoter (16%) or *RB1* gene mutation (95%; refs. 18–20), indicating that loss of pRB can occur via multiple mechanisms within the same tumor type. Beyond retinoblastoma, mutations in the *RB1* gene that result in nonfunctional protein are infrequent (21), observed in less than 5% of osteosarcomas and locally advanced breast cancers (22, 23). Overall, alterations of the *RB1* gene are frequent across cancers and the type of alteration tends to be disease type specific. In addition to the *RB1* gene itself, pRB pathway alterations also occur in human malignancies and must be considered when examining pRB function in disease.

pRB pathway alterations

Beyond pRB itself, alterations within the pRB pathway have also been defined as mechanisms of cancer development and/or malignant progression. Inactivation of negative regulators of the pathway, such as CDKIs, and increased activation of positive regulators of the pathway, including CDKs and cyclins, have been reported in almost all human cancers (24). Alterations in $p16^{INK4}$ (encoded by *CDKN2A*), a *bona fide* tumor suppressor, include genomic loss (2%–85%; refs. 25–35), somatic mutations (<15%; refs. 36–39), and altered expression driven by promoter methylation (8.5%–70%; refs. 40–45) across cancer types. However, CDK4 protein expression is often increased in cancer (20%–54%; refs. 46–49), and point mutations preventing binding to $p16^{INK4}$ and subsequent inactivation are observed in 16% of breast cancers (50, 51). Further, elevated protein expression of cyclin D1 due to amplification of the *CCND1* gene (2.5%–39%; refs. 52–55) and chromosomal rearrangement (16%–90%; refs. 56, 57) have been

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Table 1. Frequency of pRB pathway alterations in cancer.

Tumor	*RB1*	CDKN2A ($p16^{INK4}$)	CDK4	*CCND1* (cyclin D1)	Citations
Glioma		33%–85%—Genomic loss			(27, 29, 31)
Retinoblastoma	95%—Mutation 16%—Promoter methylation				(18, 20)
Esophageal		19%–70%—Promoter methylation		∼85%—Elevated protein	(36–38)
Head and neck		17%–27%—Promoter methylation 25%–66%—Genomic loss		26%–39%—Gene amplification	(33)
Mantle cell lymphoma				90%—Chromosomal rearrangement	(54)
NSCLC	30%—Genomic loss	31%—Promoter methylation <15%—Mutation		5%–30%—Gene amplification	(13, 36, 43)
SCLC	91%–100%—Genomic loss				(16)
Breast	72.2% Basal-like breast—Genomic loss 61.5% Luminal B breast—Genomic loss 2.7% Breast—Mutation	31%—Promoter methylation	16%—Mutation	15%–30%—Gene amplification	(19, 53, 54)
Colorectal		10%–40%—Promoter methylation		2.5%—Gene amplification	(42, 44)
Ovarian		7%–14%—Genomic loss			(26, 28, 55)
Bladder		10%–45%—Genomic loss			(30)
Endometrial		2%—Genomic loss 8.5%—Promoter methylation	20%–54%—Elevated protein	26%—Gene amplification	(25, 40, 47, 49)
Pancreatic		10%–37%—Genomic loss		25%—Gene amplification	(27, 34, 35)
Cervical		5%—Genomic loss			(25)
Prostate	17%–33%—Genomic loss				(7–12)
Multiple myeloma	50%—Genomic loss			16%—Chromosomal rearrangement	(57, 98)
Osteosarcoma	63%—Genomic loss <5%—Mutation	<15%—Mutation			(17, 22)
Melanoma				<25%—Gene amplification	(50, 51)

Note: The frequency and mechanisms of *RB1, CDKN1A* ($p16^{INK4}$), CDK4, and *CCDND1* (cyclin D1) alterations across disease types.

observed in specific cancers. Overall, alterations within the pRB pathway occur across pathway components, have proven to be context specific, and result in inactivation of pRB.

It has long been appreciated that mechanisms of pRB disruption are often tumor type selective, and emergent data strongly demonstrate that distinct mechanisms of pRB loss result in differential molecular and biological outcomes (16). To understand these differences and exploit them clinically, the molecular functions of pRB and the clinical implications of pRB loss must be examined.

pRB Loss and Ki67 in Clinical Samples: Unexpected Findings

As a regulator of cell-cycle control, the effect of pRB loss on proliferation has been carefully examined across tumor types and found to be dependent on the mechanism by which pRB is lost, either loss of protein or inactivation of function. When examined across CRPC and lung adenocarcinoma cohorts, loss of pRB IHC staining did not negatively correlate with Ki67 (a marker of proliferation) staining (16, 58). These studies reveal that the impact of pRB loss in these contexts is beyond cell-cycle control, driving aggressive disease without altering proliferation. pRB inactivation via hyperphosphorylation, however, does correlate with a hyperproliferative phenotype. A study examining loss of $p16^{INK4}$ and phosphorylated pRB in triple-negative breast cancer (TNBC), which has the highest rate of $p16^{INK4}$ loss, found a strong positive correlation between phosphorylated pRB protein and Ki67 (59). These observations, along with previous functional studies indicating that pRB protein loss is not equivalent to pRB hyperphosphorylation (16), suggest that upstream alterations in the pRB pathway can drive a proproliferative advantage. In conclusion, the biological effect of pRB loss is reliant on the mechanism by which pRB function is lost and is thus crucial to understand the subsequent consequences driving disease progression and to appropriately target these diseases clinically.

Molecular Understanding of pRB Function and Loss

The molecular and cellular implications of pRB dysfunction are only recently being uncovered, in part as a result of next-generation sequencing technologies, advances in modeling, and the capacity to assess impact in clinical specimens. Notably, pRB has been found to interact with >200 proteins and regulate a large number of processes controlling cancer development and progression (**Fig. 1**).

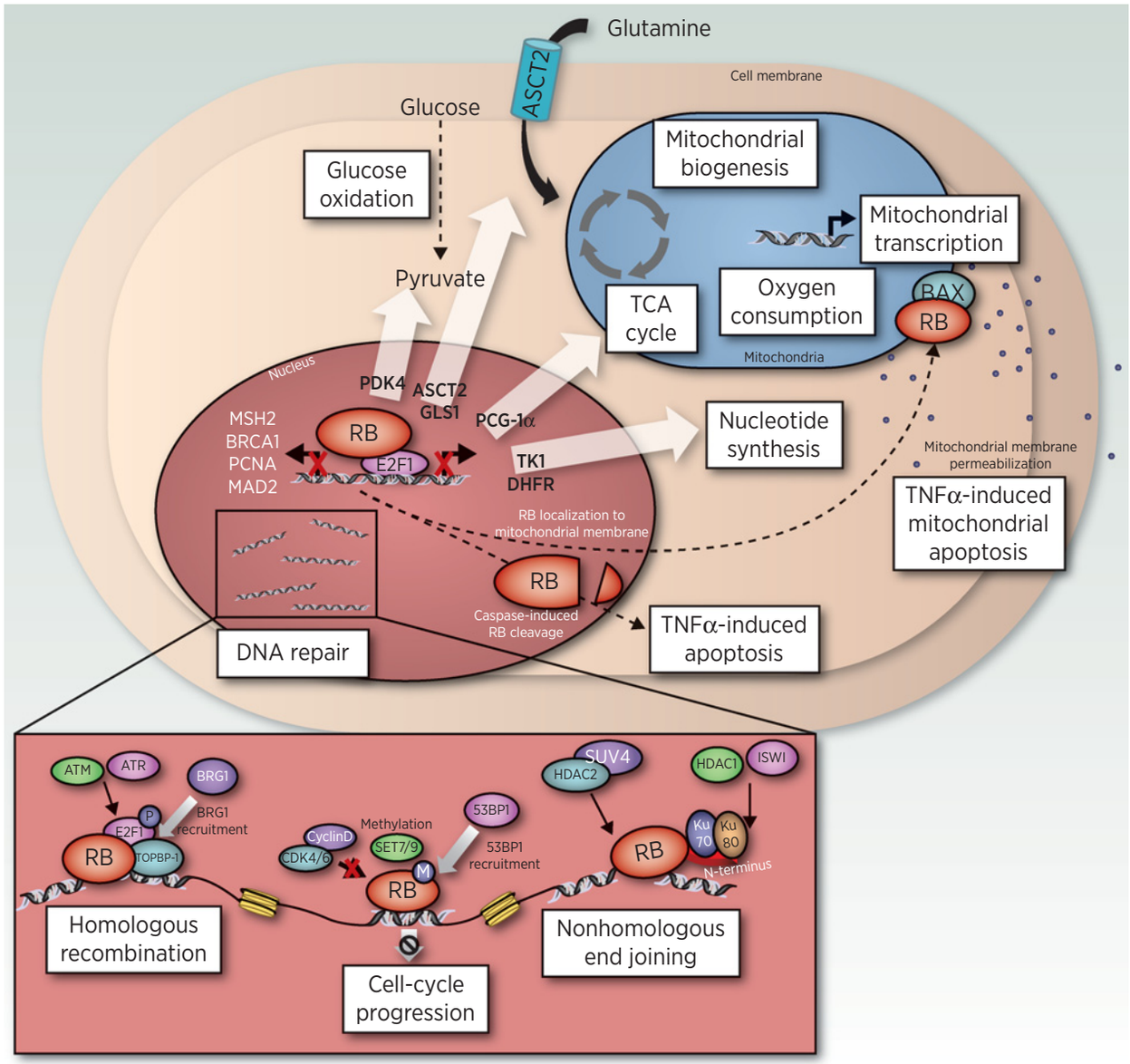

Figure 1.

Novel pRB functions beyond cell-cycle control. Through direct regulation of the E2F transcription factors, pRB has been identified to regulate glucose oxidation (PDK4), nucleotide synthesis (TK1, DHFR), glutamine metabolism (ASCT2, GLS1), TCA cycle, mitochondrial biogenesis, oxygen consumption, and mitochondrial transcription (PCG-1α), along with numerous DNA repair pathways (MSH2, BRCA1, PCNA, and MAD2). Through caspase cleavage or translocation to the mitochondria, pRB has been shown to directly regulate TNFα-induced apoptosis. Lastly, pRB has a critical role in directly regulating nonhomologous end joining (NHEJ), homologous recombination (HR), and cell-cycle progression.

DNA damage response

pRB plays an important role in the response to DNA damage both directly and indirectly, through regulation of E2F transcription. After pRB loss, E2Fs are deregulated, leading to the constitutive activation of target gene transcription. E2Fs regulate expression of genes encoding DNA repair factors including *MSH2, BRCA1*, and *PCNA* (60, 61), all of which play a critical role in DNA damage response. Beyond transcriptional control, pRB has been described as a regulator of nonhomologous end joining (NHEJ) and homologous recombination (HR) through direct interaction with DNA repair factors. pRB interacts with Ku70 and Ku80, resulting in the recruitment of chromatin modifiers known to mediate NHEJ (62). Consistently, pRB has been shown to interact with a breadth of additional chromatin modifiers including ISWI, HDAC1, HDAC2, and Suv4 (62–65). In the context of HR, pRB is recruited to sites of double-strand breaks in response to irradiation (IR) through ATM/ATR phosphorylation of E2F1, resulting in recruitment of BRG1 (66), an enzyme required for initiation of DNA end resection and subsequent repair (66). Further, pRB-deficient U2OS cells demonstrate increased sensitivity to IR compared with pRB-positive cells and are defective at resolving γ-H2AX (66), indicating

that pRB loss is associated with diminished repair capacity. Thus, studies discerning the impact of pRB status on DNA repair competency in the clinical setting may assist in the development of novel therapeutic interventions for pRB-deficient tumors.

Apoptosis

In addition to DNA damage response, pRB has been reported to significantly influence apoptosis. pRB has also been shown to localize directly to the mitochondria to positively regulate tumor necrosis factor (TNF)-induced mitochondrial apoptosis (67), and has been shown to be cleaved at a c-terminal consensus site that, when mutated, renders cells resistant to TNF-induced apoptosis (68–71). Further, genetically engineered mouse models of bladder cancer revealed that pRB loss results in reduced expression of p53, along with additional genes involved in apoptosis including *BAX, BAK, BID*, and *APAF1* (72); however, the mechanism is not fully understood. Thus, although pRB has been shown to influence apoptosis through multiple pathways, it remains crucial to fully understand these pRB functions and how they may affect therapy response.

Metabolism

Finally, emergent data have revealed critical roles for pRB in metabolic control. Repression of E2Fs by pRB has been identified as a major regulator of many metabolic pathways, including nucleotide biosynthesis, glucose oxidation, and mitochondrial function (73–78). Direct targets of E2F1 transcription include thymidine kinase (TK1) and dihydrofolate reductase (DHFR), enzymes required for nucleotide synthesis (73–75); pyruvate kinase dehydrogenase 4 (PDK4), an enzyme that contributes to the shift to a Warburg phenotype by preventing the entry of pyruvate into the citric acid cycle (76, 77); peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PCG-1alpha), which promotes mitochondrial transcription, biogenesis, and oxygen consumption; and genes involved in the electron transport chain and oxidative phosphorylation including the subunits for ATP synthase, cytochrome c oxidase, ubiquinol–cytochrome c reductase, and succinate dehydrogenase complex (76, 78). Further, pRB also plays a significant role in glutamine regulation, through the upregulated expression of the glutamine transporter ASCT2, and glutaminase (GLS1; ref. 79). pRB-deficient *Drosophila* models have shown an increased reliance on nucleotide metabolism and glutathione, both of which depend heavily on access to glutamine (80), suggesting an increase in dependence on glutamine upon pRB loss. Beyond E2F regulation, pRB is also implicated in regulating c-Myc, a known regulator of metabolic enzyme transcription (81–83). These studies highlight the critical impact pRB function has on metabolic transcriptional regulation, including but not limited to control of E2Fs.

As therapeutics targeting DNA repair, apoptosis, and metabolism are being introduced into the clinic, closing the gap between the biological and clinical impact of pRB loss on disease progression will be crucial to identify novel and effective treatments for these tumors.

The Impact of pRB Loss on Therapeutic Intervention

As discussed above, an abundance of observations in human tumors and defined model systems indicate that pRB loss promotes tumor development and progression through multiple biological mechanisms. Despite this knowledge, the relative impact of pRB status on therapeutic response remains loosely defined (**Fig. 2**).

pRB status and DNA repair targeted therapy

As discussed above, pRB is implicated as a critical factor in prompting DNA repair. Modeling pRB loss results in a switch from canonical NHEJ to a DNA-PK–independent mechanism of NHEJ, suggesting that clinically utilized DNA-PK inhibitors may not result in a favorable outcome against these pRB-deficient tumors (62). Further, as pRB loss results in defective HR repair and increased sensitivity to IR, pRB-deficient tumors may be sensitized to double-strand break-inducing agents in combination with targeted therapy such as PARP inhibition (66); however, this requires further investigation.

pRB status and chemotherapy

Previous studies have revealed that pRB-deficient tumors have a more favorable response to specific chemotherapeutics. A study of invasive breast cancer found that patients with pRB-deficient tumors had increased disease-free survival after chemotherapy including methotrexate and fluorouracil, compared with those that retained pRB positivity (84). As methotrexate and fluorouracil reduce purine and pyrimidine synthesis, this favorable response may be attributed to the heavy reliance pRB-deficient tumors have on nucleotide synthesis (80). Further, studies in breast, bladder, and pancreatic cancers have shown pRB-deficient tumors to be more sensitive to the platinum-based chemotherapy (85–87); however, the mechanisms driving this increased sensitivity remain to be defined.

pRB status and hormone therapy

pRB loss has been well defined as a mechanism driving resistance to hormone therapy in breast and prostate cancers. For estrogen receptor (ER)–positive breast cancer and androgen receptor (AR)–positive prostate cancer, the first line of treatment is hormone ablation therapy. Tumors eventually become resistant to therapy while retaining hormone receptor positivity. A study examining gene expression revealed that elevated expression of a 59 pRB-regulated gene signature correlates with failure of therapy in breast cancer patients treated with the antiestrogen tamoxifen (85). Further, studies examining primary breast tumors revealed that patients with a functional pRB pathway showed significantly increased recurrence-free survival when treated with tamoxifen, whereas those lacking pRB were significantly associated with impaired response (84, 88), suggesting loss of pRB as an indicator for poor response to hormone therapy. Moreover, in prostate cancer, *RB1* loss is found almost exclusively in CRPC (with little incidence in castration-sensitive disease), and is sufficient to promote therapy resistance through the deregulation of E2F1 and increased transcription of AR (13). These studies suggest that pRB loss can serve as a predictive marker of hormone therapy response in both breast and prostate cancers.

pRB status and CDKI

Pharmacologic inhibition of CDK4/6 is the most clinically mature means to target the pRB pathway, serving to induce tumor suppressor function by dampening the action of inhibitory kinases. The current FDA-approved CDK4/6 inhibitors include palbociclib, ribociclib, and abemaciclib, which are approved for hormone receptor–positive, HER2-negative advanced breast cancer in combination with the estrogen receptor antagonist fulvestrant (89, 90). Three individual clinical phase III studies investigating these CDK4/6 inhibitors found that palbociclib, ribociclib, and abemaciclib significantly improved PFS when treated in combination with fulvestrant compared with fulvestrant alone (90–92). However, although at the time of approval pRB positivity was not required for inclusion in each trial, there is little molecular basis to target the pathway upstream after pRB loss,

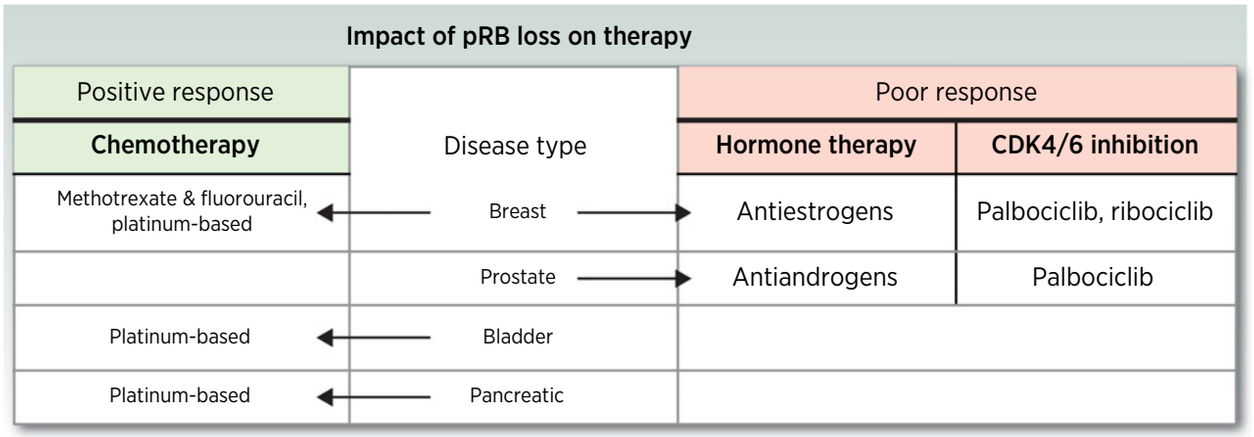

Figure 2.
The impact of pRB loss on therapeutic intervention. Studies have observed that pRB loss promotes a positive response to chemotherapy in breast, bladder, and pancreatic cancers. Conversely, pRB loss has been identified as a mechanism of resistance to hormone therapy and CDK4/6 inhibition in breast and prostate cancers.

providing the rationale for using pRB positivity as a biomarker for CDK4/6 inhibitor sensitivity. Further, resistance to CDK4/6 inhibitors is common, and a key mechanism by which tumors gain this resistance is through pRB loss (85). Specifically, studies in breast cancer models showed that either pRB protein loss or increased cyclin E1 expression leads to resistance to CDK4/6 inhibition (93, 94). Concordantly, genomic characterization of ribociclib-resistant patient-derived xenograft models identified an acquired *RB1* frameshift mutation leading to reduced pRB expression (93). Further studies indicated that 50% of models with aberrant pRB gene signature expression showed reduced copy number of *RB1* by the time of palbociclib resistance, further supporting a role for pRB loss in acquired resistance to CDK4/6 inhibition (94). In addition to pRB loss, pRB inactivation has also been identified as a resistance mechanism of CDK4/6 inhibition through elevated expression of cyclin D1, leading to restored pRB hyperphosphorylation (95). A molecular understanding of these resistance mechanisms to CDK4/6 inhibitors will play a critical role in explaining tumor response in the clinic and further provide a rationale to clinically examine pRB loss in patient tumors. As such, although pRB loss portends CDK4/6 inhibitor resistance, the presence of pRB alone is not sufficient to predict responsiveness.

Current Prognostic Value of pRB in Human Malignancies

In addition to identification as a biomarker for predicted response to therapy, pRB loss is also implicated as a prognostic marker for clinical outcome and disease stage in tumors with relatively high frequency of pRB deficiency including multiple myeloma (MM); NSCLC; breast, prostate, and bladder cancers; and osteosarcomas. While altered patterns in pRB function and expression have proven to be useful indicators of disease development and progression, their use as prognostic clinical markers remains controversial.

Multiple myeloma

Loss of pRB is observed in up to 50% of MM tumors (96, 97), and deletion of the *RB1* gene significantly correlates with shorter OS, supporting additional studies suggesting that deletion of pRB has independent prognostic value in MM (98–100). Furthermore, a significant number of patients with tumors lacking pRB were found to have stage III disease at diagnosis, suggesting that pRB may also serve as a marker of disease stage in this tumor type as well (98); however, this requires further clinical investigation.

NSCLC

The use of pRB as a prognostic marker has been investigated in NSCLC; however, results remain inconsistent. When examined exclusively at early stage of disease, pRB-deficient cases exhibited a tendency for shorter OS (101–103). More specifically, when examining adenocarcinoma alone, pRB-deficient tumors were associated with shorter OS compared with pRB-positive tumors (101). However, additional smaller studies have not found pRB to provide significant prognostic value in NSCLC (104, 105); despite cohort size limitations, pRB-deficient tumors did display a trend toward poorer OS, suggesting larger cohorts are needed to fully assess the effect of pRB loss (105). Together, these studies suggest that pRB loss may portend poor outcome for patients and may be useful as a prognostic marker in specific subtypes of NSCLC; however, more targeted studies are needed to test these hypotheses.

Breast cancer

In breast cancer, the use of pRB as a marker of disease stage or clinical outcome has been shown to be subtype specific. A large, retrospective study of 1,806 patients with breast cancer revealed that loss of pRB protein was associated with TNBC and basal core phenotype subtypes (106–108), suggesting pRB loss may be a useful marker of disease subtype. However, there was no significant association with disease-free survival or OS within these subtypes, indicating the prognostic value of pRB on patient outcome may be lacking (88, 106, 109). Conversely, in ductal carcinoma *in situ* tumors, pRB protein loss was strongly associated with recurrence of invasive breast cancer, suggesting that the value of pRB status as a prognostic marker in this stage of disease may have utility (110). In total, although pRB status has not been confirmed as a prognostic marker for OS, it has prognostic value in specific breast cancer subtypes and needs to be further explored.

Prostate cancer

Loss of *RB1* gene expression is found almost exclusively in CRPC, suggesting that genomic *RB1* loss can be utilized as a marker

for stage of disease (13). Moreover, the emergence of pRB loss signatures has become an increasingly utilized tool. pRB loss gene signatures are strongly represented in advanced disease and associated with reduced recurrence-free survival (13, 16). Furthermore, an independent pRB loss gene signature for dual copy *RB1* loss developed using The Cancer Genome Atlas Pan-Cancer data set across cancer types found that high expression of this signature significantly correlated with shorter PFS, DSS, and OS when examined across prostate cancer cohorts (5). These data implicate pRB loss and pRB loss gene signatures as a surrogate for pRB loss as prognostic markers for disease. Understanding the biological consequences in these tumors is required for complete understanding of disease progression and development of therapies to target disease.

Other solid tumors

pRB status has also been studied in bladder cancer and osteosarcomas. Multiple studies have found a strong correlation between pRB loss, tumor grade and stage, and OS in bladder cancer, providing a rationale for the utilization of pRB as both a prognostic and staging marker in this disease type (111–113). In osteosarcoma, studies have reported a significant difference in event-free survival (between tumors with loss of heterozygosity (LOH) of pRB and those without LOH (22, 114, 115), suggesting that pRB status can be utilized as a marker for prognosis.

RB1 Germline Mutations and Cancer Predisposition

RB1 germline mutations, resulting in loss of pRB protein, have been reported as predictive markers of cancer initiation and development. Specifically, in retinoblastoma, about 40% of patients who develop the disease have a hereditary predisposition caused by a heterozygous *RB1* germline mutation. Unfortunately, patients with these germline mutations have an increased risk of second primary malignancies such as osteosarcoma, lipomas, soft-tissue sarcomas, melanoma, and cancers of the brain (116–119). Examination revealed that the mutations occur throughout the *RB1* gene and no correlation between the mutations and the type of secondary malignancy diagnosed have been observed (120). A better understanding of the impact *RB1* germline mutations have on the risk for secondary cancer development will have a critical influence on patient outcome. Identification of patients predisposed to secondary malignancies will allow for more comprehensive cancer screenings, earlier diagnosis, and more effective treatment.

Clinical Barriers of Defining pRB Loss as a Biomarker

Although supportive evidence exists for the use of pRB status as a prognostic or predictive biomarker, it is important to note that utilizing the loss of a tumor suppressor as a biomarker introduces numerous complicating factors. Although sequencing can readily detect deletion or mutations of the *RB1* gene, defining pRB status is often done through IHC staining of tumor samples. Histologic staining introduces a number of challenges as pRB protein expression is generally heterogeneous within tumors. Studies that have examined pRB status define pRB positivity by describing staining at null/weak, intermediate, or high, or compare staining to surrounding nonneoplastic cells that retained nuclear pRB positivity. As such, there remains to be a standardized method and threshold to define pRB positivity in clinical samples. Additionally, factors such as subclonal loss (121) and distinguishing between 1 and 2 copy loss are difficult to determine, especially in low tumor content samples (16). Interestingly, single-copy *RB1* loss has proven to be sufficient to induce aggressive phenotypes (13). Thus, although tumor heterogeneity remains to be an obstacle, *RB1* haploinsufficiency may be specifically beneficial in defining pRB status as a biomarker. Beyond the use of pRB status alone, the use of gene signatures has also proven to be difficult as measuring gene-expression changes requires a specific threshold of expression be defined. Although multiple pRB loss gene signatures have been defined and shown to accurately predict pRB loss across cancer types (5, 16), the addition of gene-expression threshold changes may improve the success in defining pRB status in tumors. Overall, although the use of pRB loss as a clinical biomarker introduces a number of challenges, advancement in the clinical methods of detecting pRB status will provide a critical avenue to target and treat tumors that lack defining oncogenic mutations.

In addition to pRB loss alone, it is critical to understand how pRB loss in combination with loss of additional tumor suppressors may affect clinical response. A study of 260 MM patients found no prognostic significance of pRB loss alone on OS or time to disease progression. However, for patients with combined pRB and p53 alterations, a decrease in OS was observed, suggesting that pRB status may have prognostic value when combined with other correlates (122). Interestingly, combined loss of *RB1* and *TP53* occurs in nearly 100% of SCLC and >53% of neuroendocrine prostate cancers (123, 124). However, it is important to note that alterations of these genes often occur through complicated rearrangements which may or may not be detectable through exome or panel-based next-generation sequencing (NGS; ref. 123). Thus, the method of tumor suppressor status detection

Table 2. Clinical trials using pRB status as a biomarker.

Tumor	Interventions	Significance of pRB status	Study phase	Trial
Breast, prostate, pancreatic, glioma, gastrointestinal stromal tumors	Abemaciclib, palbociclib, ribociclib	Inclusion criteria—tumors positive for pRB	Phase I, phase II	NCT03130439, NCT02806648, NCT03220646, NCT01907607, NCT03355794, NCT02607124, NCT03526250, NCT02555189
Breast	Palbociclib and endocrine therapy	Endpoint measurement—pattern of resistance	Phase II	NCT03184090
Breast	Chemotherapy	Endpoint measurement—correlation with chemotherapy sensitivity	Retrospective	NCT01514565

Note: Clinical trials that include pRB status as either inclusion criteria or an outcome measure.

is critical when defining biomarkers. Further, there is evidence to suggest that loss of pRB in SCLC is a later event whereas loss of pRB in prostate cancer is an early event, implying that the genomic and epigenomic context affects the timing of pRB loss. Overall, although pRB status alone may function as a prognostic marker in some tumor types, a better understanding of the biological consequences of pRB loss in combination with additional tumor suppressor loss may be required to be utilized as an effective marker.

Clinical Trials Using pRB Status as a Biomarker

Investigation into the clinical importance of pRB status across tumor types is ongoing, with several clinical trials focused on the applicability of pRB status as a prognostic or predictive marker (**Table 2**). As mentioned above, CDKs are the most commonly targeted proteins in the pRB pathway, and the use of CDKI in cancer therapy has increased over the recent years. A number of trials investigating CDK4/6 inhibitors across tumor types require pRB positivity as part of the inclusion criteria, designating pRB as a biomarker for drug response. Further, a trial investigating CDK4/6 inhibition in combination with hormone therapy in breast cancer is utilizing pRB status as an endpoint measure to investigate pRB loss as a mechanism of therapy resistance. Additionally, a retrospective study is exploring pRB status and patient recurrence-free survival and OS in response to chemotherapy to determine if pRB positivity correlates with chemotherapy sensitivity. Overall, whether investigating the efficacy of CDK4/6 inhibitors, hormone therapy, or chemotherapy, many studies have begun to consider the utility of pRB as a marker of response. These clinical studies, along with the biological studies of pRB function, are critical to expand the use of pRB status in the clinical setting.

Summary and Ongoing Questions

Technology advances have afforded significant new insight into the pleiotropic molecular functions of pRB and have identified activities beyond cell-cycle control that regulate tumor development and progression. pRB has been nominated as a putative prognostic marker, biomarker for response to therapy, and a context-specific marker of therapeutic response. Although much is known of pRB function and the consequence of pRB loss on disease progression and resistance to therapy, opportunities remain to connect the biological mechanism and clinical observation. Understanding the relative contribution of distinct pRB functions on tumor suppression and/or limiting progression will be critical for translating knowledge of pRB activity into clinical practice.

These novel biological functions of pRB along with clinical data predicting pRB function in response to therapeutics support a role for pRB status in the clinical setting. However, there remain key questions that have yet to be addressed. First, does pRB status have utility as a prognostic marker? Whether through use of a pRB loss gene signature or loss of the pRB protein, studies suggest that pRB status can inform clinical outcome. Further studies are required to test the use of pRB as a prognostic marker across priority human cancers. Second, can pRB status be used to predict outcome to clinically relevant therapeutics? Although novel functions of pRB have been identified and may be used to explain the response to current therapies, use of pRB status as a marker of therapeutic response directed at these pathways has yet to be rigorously investigated. Evidence for altered therapeutic response in tumors lacking pRB nominates pRB deficiency as a potential clinical subtype that is crucial specifically in disease models such as prostate cancer, where clinically actionable subtypes are only recently emerging (125, 126). Third, if pRB loss does promote resistance to therapy, what are the underpinning mechanism(s) of resistance? It will be critical to tie molecular understanding of pRB activity to therapeutic responsiveness, and to determine the relative impact across disease types and therapeutics. Developing a better understanding of the pRB-regulated pathways which underpin the aggressive features of pRB-deficient tumors will be essential for further developing pRB loss as a biomarker of disease progression and for stratifying pRB-deficient tumors into more effective treatment regimens.

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