# Twists in views on RB functions in cellular signaling, metabolism and stem cells

# Review Article

# Twists in views on RB functions in cellular signaling, metabolism and stem cells

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One-quarter of a century ago, identification of the human retinoblastoma gene (*RB*) loci proved Knudson's 'two-hit theory' that tumor suppressor genes exist. Since then, numerous works delineated crucial roles for the RB protein (pRB)-E2F transcription factor complex in G1-S phase transition. In addition, discovering the relationship between pRB and tissue-specific transcription factors enabled a better understanding of how cell cycle exit and terminal differentiation are coupled. Recent works provoked many exciting twists in views on pRB functions during cancer initiation and progression beyond its previously well-appreciated roles. Various mitogenic and cytostatic cellular signals appeared to modulate pRB functions and thus affect a wide variety of effector molecules. In addition, genetic studies in mice as well as other creatures incessantly force us to revise our views on pRB functions. This review will focus particularly on the roles of pRB in regulating intracellular signaling, cell metabolism, chromatin function, stem cells and cancer stem cells. (*Cancer Sci* 2012; 103: 1182–1188)

*R*B mutation is found prevalently in retinoblastomas, osteosarcomas and small-cell lung carcinomas, and such a spectrum of tumors is reproducible in *Rb*-deficient mice.[1] These findings suggest that in these tumors *RB* mutation occurs when tumors initiate. However, in the majority of cancers, including prostate, breast, bladder, esophageal, hepatic cancers, glioma or chronic myelogenous leukemia, inactivation of pRB functions caused by either mutation, gene deletion, promoter methylation, deregulated phosphorylation or decreased protein level usually occurs during cancer progression.[2] Some evidence proposes that pRB is even 'required' for tumor initiation in these cancers due to its anti-apoptotic function or cooperation with Ras-transformation.[3,4] Despite having redundant functions for controlling G1-S transition, other 'pocket protein' family members *p107* and *p130* are rarely mutated in cancers. These findings, as well as other discoveries (discussed later), suggest that pRB might possess many more multifaceted and unique functions than previously thought. Compared with its smaller protein abundance (in empirical terms), pRB appears to possess 'too many' functions, and some of them cause both positive and negative effects on the same biological event. This implies that a particular pRB function (or an effect of pRB inactivation) is selected depending on the cellular context or cancer stage.

## Upstream Signals and Downstream Effectors of pRB

Various mitogenic signals (e.g. receptor tyrosine kinases/Ras, Akt, NF-κB, Shh, Hippo, Wnt, Myc, Jak/STAT) merge more or less on the upregulation of D-type cyclins, initiating pRB phosphorylation executed sequentially by cyclin D-CDK4/6 and cyclin E-CDK2. This prevents pRB from suppressing E2F function to transactivate the targeted genes. In addition, in response to DNA damage, ATM and Chk1/2 directly phosphorylate pRB.[5] AMP-activated kinase (AMPK) also directly phosphorylates pRB; this contributes to energy control favoring neural progenitor cell growth.[6] Furthermore, pRb phosphorylation induced by AMPK leads to E2F1-dependent cell death that occurs in inner ear cells when they sense mitochondrial defect (discussed later).[7] Oxidative stress resets pRB phosphorylation via protein phosphatase 2A (PP2A).[8] As is well documented, the CDK inhibitors (CDKI) suppress pRB phosphorylation from genetic upstream by attenuating the catalytic activity of cyclin-CDK. However, the clinical outcome of CDKI inactivation is not always equivalent to that of pRB inactivation.[9] pRB inactivation upregulates p16$^{Ink4a}$ by elevating Ras activity and its tumor suppressor role is taken over by p130.[10] This, as well as previously described findings,[9] indicate that the genetic interaction between CDKI and pRB is not linear. Furthermore, in addition to phosphorylation, many other types of post-translational modification regulate pRB activity. For instance, pRB is acetylated by p300/CBP, PCAF and Tip60, deacetylated by Sirtuin1 (SIRT1), and methylated by Set7/9 and SMYD.[2,11] These modifications may alter the susceptibility of pRB to undergo CDK-dependent phosphorylation or its binding affinity to other partners. Furthermore, pRB is sumoylated and is also catabolized following MDM2-mediated ubiquitination or caspase 3 and 8-mediated cleavage at the C-terminus (Fig. 1).[12–14] Mouse models demonstrated that E2F are crucial downstream mediators of the tumor suppressor function of pRB. The continuous advancement in our understanding of the E2F functions increases the number of possible functions that pRB might possess (discussed later). However, some mutant forms of pRB are defective in E2F binding and transcriptional repression, although the proteins partially retain their tumor suppressor activity.[15] This fact led researchers to focus on different downstream effectors. pRB directly binds to Skp2, which allows APC-Cdh1 to ubiquitinate Skp2. Thereby *RB* loss allows SCF$^{Skp2}$ E3 ligase complex to bind to and then ubiquitinate phosphorylated p27$^{Kip1}$. This nexus appears to be crucial in carcinogenesis since *Rb*$^{+/−}$;*Skp2*$^{−/−}$ mice are completely free of tumor.[16] In addition, EID1 and KDM5A/Jarid1a/RBP2 are recognized as molecules likely to be involved in E2F-independent functions of pRB (discussed later).[17–19] The pRB family can interact with many enzymes that remodel histones to generate repressive chromatin (Fig. 1 and discussed later).[20]

## Function of pRB in Intracellular Signaling

**Ras signal.** Ras activity fluctuates in a cell cycle-dependent manner.[21] Epistasis studies in *Caenorhabditis elegans*

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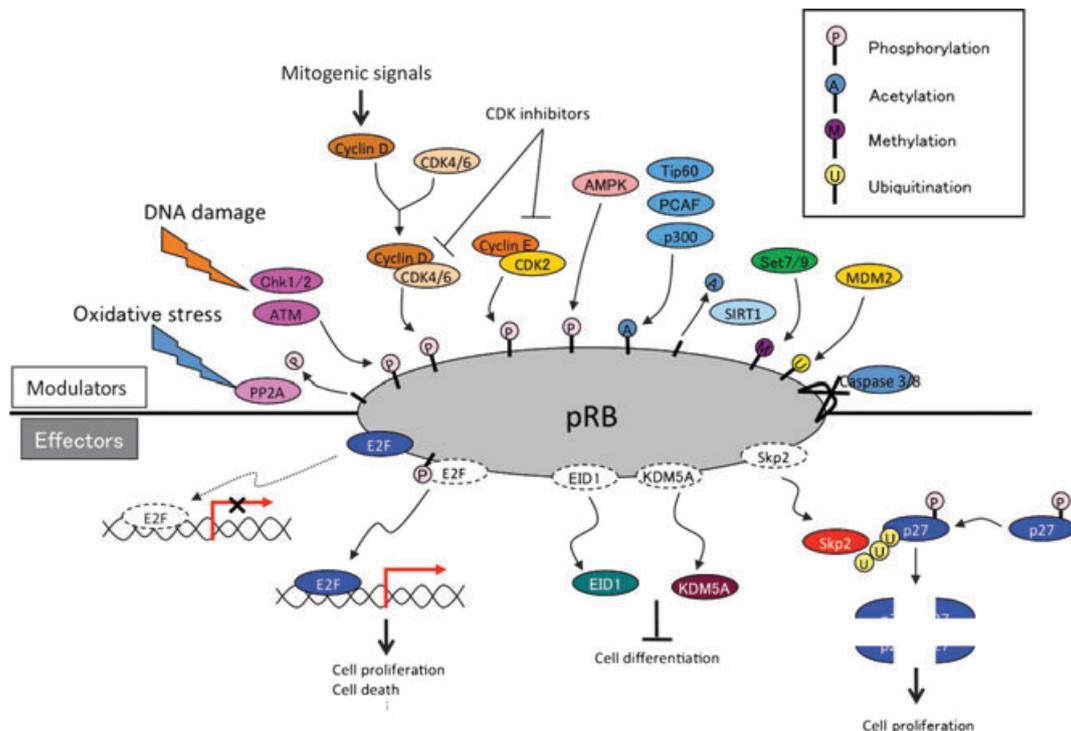
**Fig. 1.** Modulators and effectors of RB protein (pRB) functions. DNA damage induces phosphorylation of pRB by ATM and Chk1/2, whereas oxidative stress induces dephosphorylation of pRB through PP2A. AMP-activated kinase (AMPK) also phosphorylates pRB. pRB is acetylated by p300, PCAF, methylated by Set 7/9 and SMYD, and degradaded via MDM2-mediated ubiquitination and caspase 3 and 8 activation. Several molecules, Skp2, KDM5A and EID1, are activated after dissociation from pRB in an E2F-independent manner. Released Skp2 promotes ubiquitination and degradation of phospho-p27$^{kip1}$.

proposed that class B *synMuv* genes including *Rb* (*lin-35*) can control *Ras* (*let-60*)-mediated vulval development even from genetic upstream.[22] Indeed, SV40 large T antigen-mediated pRb inactivation or loss of *Rb* induced elevated Ras activity in mammalian cells.[23,24] The genetic interaction of *Rb* and *Ras* has been analyzed extensively in mouse embryos simultaneously lacking *Rb* and one of the *ras* isoforms.[25] N- or K-*ras* deletion significantly prolonged the life span of *Rb*-null embryos. Their differentiation defects in different organs including muscle cells and erythrocytes were significantly rescued; however, aberrant cell proliferation and cell death persisted (results for erythrocytes are unpublished).[26,27] N- or K-*ras* deletion in *Rb*-deficient pituitary tumorigenesis attenuated tumor invasion with concomitant increase in differentiation degree without affecting tumor incidence.[27,28] These findings implicate that in addition to the previously appreciated pathway in which *Ras* is upstream of *RB*, *Ras* functions also downstream of *RB* in differentiation control and tumor progression (Fig. 2). Contrary to pituitary, N-*ras* deletion converts *Rb*-deficient calcitonin-producing cell (C cell) adenoma or low grade adenocarcinoma to highly metastatic adenocarcinoma.[28] The mechanism of this twist was explained in our later study[10] as follows: *Rb*-deficient C cell adenoma cells are sensitive to DNA damage response induced by mildly elevated N-Ras activity (at most 10-fold elevation in activity compared with that of *Rb*-positive cells, whereas Ras with oncogenic mutation exhibits an approximately 60-fold increase in activity). Subsequently, *Rb*-deficient C cells undergo 'paradoxical' cellular senescence with the aid of p16$^{Ink4a}$ and p130, which protects them from further malignant progression.[10] Consistent with this explanation, *Rb*-heterozygous mice simultaneously lacking any of the *Ink4a*, *Arf* or *Suv39h1* (senescence-inducing genes) alleles directly developed highly malignant C cell tumors and
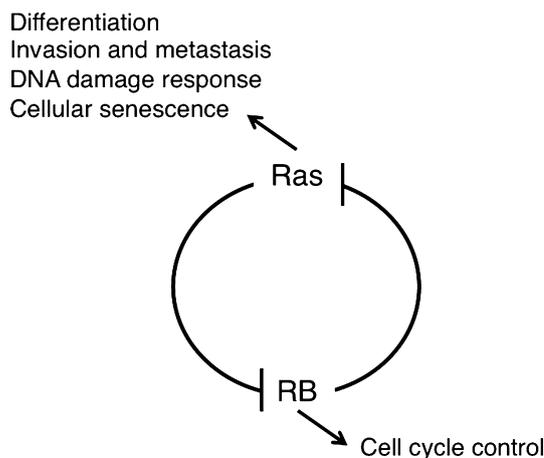


**Fig. 2.** The genetic interaction between Ras and RB. The Ras signal induces RB protein (pRB) phosphorylation by upregulating cyclin D1; this constitutes one of primary mechanisms by which mitogenic signals control the cell cycle. Inversely, RB status can influence Ras activation status by regulating sterol regulatory element-binding protein (SREBP) and isoprenylation-related genes. Thereby, Ras mediates pRB function to control the indicated biological events.

at an earlier age (Fig. 3).[10] This provided a further explanation why *RB* mutation is a relatively infrequent event during tumor initiation; *RB* loss-induced carcinogenesis can be antagonized by cellular senescence in some types of cells. In a progenitor for human retinoblastoma, *RB* loss-induced carcinogenesis might be antagonized by p53-dependent apoptosis.[29] However, retinoblastoma progression could be antagonized also by
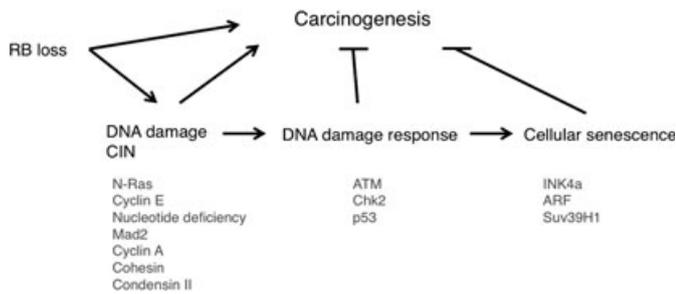
**Fig. 3.** RB loss-induced carcinogenesis is antagonized by cellular defense mechanisms such as DNA damage response or cellular senescence controlled by the indicated genes or events, depending on the cellular context.
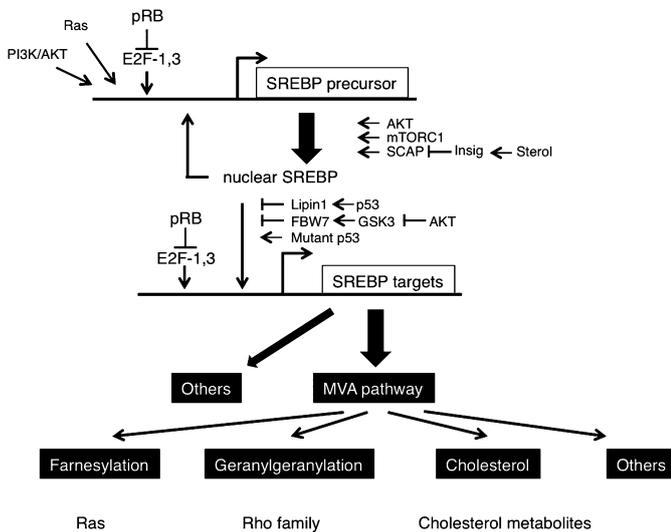


**Fig. 4.** Various oncogenic signals merged on sterol regulatory element-binding protein (SREBP) regulations (transcription, processing, nuclear localization, ubiquitination and transactivation). Because SREBP genes are dually regulated by E2F and SREBP, pRB gives rise to a different impact on genes involved in the mevalonate (MVA) pathway.

senescence since human *RB*-null retinomas express elevated p16[INK4a] and p130 expression, and lose such marks during progression presumably due to chromosomal instability (CIN) (discussed later).[30] To determine the mechanism that enables pRB to cease Ras activation status, pRb transcriptional targets were determined in *Rb*-deficient *N-ras*$^{-/-}$ mouse C cell tumor cells in which proliferation was not affected by the presence of pRB. This enabled the cell cycle-independent function of pRB to be determined. The study[10] detected many genes involved in protein farnesylation and geranylgeranylation (isoprenylation); these post-translational modifications are essential for Ras to be matured and activated. In addition, the study discovered that these genes are dually innervated by E2F and sterol regulatory element-binding protein (SREBP) transcription factors. The SREBP are also regulated by E2F (Fig. 4). Consistently, the same study demonstrated that the enhancement of pRb activity delays the trafficking of cytosolic N-Ras to Golgi for which isoprenylation is essential.[10] In addition to N-Ras, many other small GTPases, CENP-E and CENP-F those with CAAX motifs are possibly regulated by pRB (Fig. 4). We also observed RhoA activity was actually suppressed by pRB.[28]

**Other signals.** A study by another group has demonstrated that AKT[Ser473] phosphorylation is specifically upregulated in cells lacking all *RB* family members.[31] Although the exact mechanism is still unclear, our own preliminary study demonstrated that the kinetics in which acute pRb inactivation activates AKT seems to be different from that in which pRb inactivation increases GTP-loaded Ras (Shunsuke Kitajima, unpublished data, 2011). In addition, elevated Ras activity reportedly prefers to induce phosphorylation at AKT[Ser308] rather than AKT[Ser473] via the PI3K pathway. Thus, mTORC2 function as well as Ras should be analyzed to discover more on this interaction. Of note, *RB* and *TSC2* (downstream of PI3K/AKT signal) are in a synthetic lethal relationship.[32] The genetic interaction between RB and PI3K/AKT/mTOR signal has just begun to be understood. Myc transcription factors are perhaps one of the most well-recognized pRB transcriptional targets. *N-myc* gene amplifications are found in retinoblastoma cases free from *RB* mutation, suggesting that Myc functions might be considerably overlapped with the signals induced by pRB inactivation.[33] In addition, pRB might modulate intracellular signaling by regulating extracellular signaling molecules including VEGF, FGFR, bFGF, matrix metalloproteinases, interleukin-8, hypoxia-responsive gene products and Cox-2; the mechanisms might involve E2F, Id2, Oct-1, HIF-1 or others.[34,35]

## Function of pRB in Cell Metabolism

**Metabolic pathways.** The aforementioned genetic interaction between *RB* and *Ras* mined a new genetic interaction between *RB* and *SREBP*; this further allocated a new role to pRB in lipid metabolism, since SREBP are master regulators of lipogenic and steroidogenic genes.[10,36] Indeed, in our initial study, pRb appeared to target many of the genes coding enzymes that participate in fatty acid and cholesterol biosynthesis. In their promoter, these genes possess either sterol regulatory elements (SRE) or E2F-binding consensus sequences or both (Fig. 4).[10] Recently, a new regulator of SREBP has emerged. Mutated p53 directly binds to SREBP-2 and enhances its transactivation potential, thus contributing to the invasive morphology of breast cancer cells in 3D culture probably due to enhanced geranylgeranylation.[37] SREBP transactivate most genes implicated in the mevalonate (MVA) pathway that governs farnesylation, geranylgeranylation and cholesterol synthesis (Fig. 4). This report, in addition to our own study, tightly linked two important tumor suppressors, pRB and p53, to the MVA pathway. Another regulator of SREBP is the PI3K/AKT signaling pathway. An activated AKT signal regulates the SCAP-mediated processing of SREBP precursors, and also attenuates ubiquitination of mature (nuclear) SREBP by inhibiting GSK3 function to phosphorylate mature SREBP.[38] A recent study demonstrated that Lipin1, which is a substrate for mTORC1 kinase activity, eliminates mature SREBP from the nucleus.[39] Lipin1 could also link SREBP and p53.[40] One of the SREBP targets, fatty acid synthase (FASN), has also been identified to be a pRB transcriptional target.[10] This product uses NADPH provided by the shunt from the glycolytic pathway (pentose phosphate pathway) and fuels carbon sources into the MVA pathway. An elevated FASN level during tumor progression might well explain the 'lipidogenic phenotype' in cancer cells.[41] This in conjugation with 'aerobic glycolysis (Warburg's effect)' constitutes two major metabolic perturbations featured in cancer cells. Current understanding is that these mechanisms synergize to efficiently produce and utilize NADPH for the synthesis of macromolecules including lipids and nucleotides. This occurs while avoiding ROS-producing oxidative phosphorylation (OXPHOS) in mitochondria and preventing ATP production (Fig. 5).[42] In addition, increased cellular cholesterol might suppress OXPHOS by altering the components of the mitochondria membrane.[42] NADPH is also required for the synthesis of glutathione, an
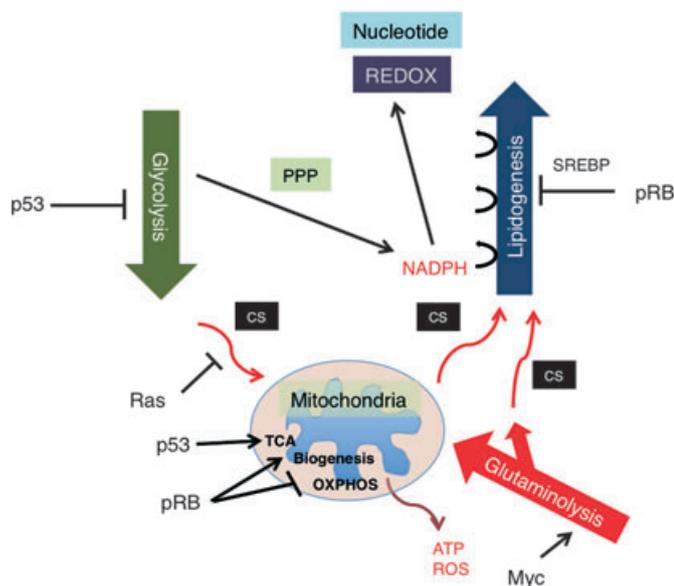
**Fig. 5.** Various oncogenic signals contribute to metabolic perturbation in cancer cells. Simultaneous inactivation of RB protein (pRB) and p53 might optimize coupling of glycolytic and lipidogenic phenotypes, which is facilitated by NADPH production through the pentose phosphate pathway (PPP). Red arrows indicate the flow of carbon sources (CS).

antioxidant. It is of note that wild-type p53 controls glycolysis in a bipolar manner, for example, by upregulating hexokinase 2, which promotes glycolysis, and TIGAR, which suppresses glycolysis. In total, inactivation of the p53 function is thought to result in the shift of cell metabolism to glycolytic (Fig. 5). Not only sequential regulation of SREBP expression and maturation, but also an astonishing level of cooperation in regulating cancer cell metabolism is becoming evident between pRB and p53 (Fig. 5). AMPKα2 was identified to be in a PI3K-sensitive gene group among E2F targets.[43] This molecule functions as a subunit of a system that senses the cellular level of AMP and antagonizes many metabolic perturbations in cancer cells mostly driven by mTORC1, TSC2 or SREBP. Therefore, together with the phopshorylation of pRB by AMPK, these findings suggest a mutually suppressive genetic interaction between pRB and AMPK.[6,7] AMPK also functions downstream of another tumor suppressor, LKB1.[44] Metformin, an AMPK agonist, was suggested to lower the cancer risk in individuals who were administered the drug.[45] Although E2F-AMPK genetic interaction was initially perceived in the context of apoptosis control, together with our knowledge on pRB genetic interaction with SREBP, Ras, AKT, Myc, p53, Oct-1 and HIF-1, this discovery will further our understanding of pRB functions in cancer cell metabolism.

**Mitochondrial function.** pRB controls mitochondrial biogenesis and function in dissimilar ways. For instance, pRB sustains mitochondrial biogenesis under particular stress conditions. *Rb* loss induces differentiation defects in cell cycle-exiting erythrocytes and myotube-forming muscle cells presumably by reducing the mitochondrial copy number.[46,47] The latter defect was associated with features of autophagy/mitophagy, and rescued by shifting cells to glycolytic status. This further prompted us to hypothesize that without glycolytic shift, pRB inactivation might induce a shortage of energy to support ATP-consuming differentiation processes (i.e. hemoglobin or myogenic protein synthesis) or even tumor growth. The mechanism linking pRB inactivation to mitophagy might involve a genetic interaction between E2F and Bnip3.[48] pRB also controls transcription of

genes involved in mitochondrial functions. This also could overlap with the mechanism by which pRB controls mitochondrial biogenesis. pRB, probably through its functional interaction with KDM5A, is likely to upregulate a number of genes encoding mitochondrial proteins in human monocytic cells.[49] Another study conducted in erythrocytes suggested that pRB affects mitochondrial biogenesis through regulating NRF1, NRF2a, PPARγ or PGC-1β in a slightly more complicated manner.[46] A recent twist in this field is that the pRB-E2F-1 complex appeared to suppress genes implicated in OXPHOS.[50] Currently, bifurcated roles of pRB in controlling mitochondrial biogenesis and OXPHOS are unexplained, but this is an intriguing conundrum to solve (Fig. 5). In addition, pRB seems to participate in the regulation of cellular reactive oxygen species (ROS) levels, and undergoes feedback from ROS through CDKI, PP2A, SIRT1 or seladin.[51] Various metabolic pathways controlled by pRB might counterbalance each other in order to keep homeostatic control of cellular metabolism.

## Function of pRB in Chromatin Functions

**CIN and DNA damage.** Increased CIN is one of the representative events seen during progression of cancers. pRB inactivation undermines genome stability through various mechanisms that are E2F dependent (e.g. via Mad2, cyclin E-driven hyper-replication, nucleotide deficiency) or independent (e.g. via pRB complex with cohesin and condensin II).[52,53] As discussed above, CIN can contribute to retinoblastoma progression. Mad2-induced CIN provides causality to not only tumorigenesis but also to tumor relapse.[54] In addition to such mechanisms, pRB inactivation can induce DNA damage response and senescence due to mildly elevated Ras activity.[10] Because of such diverged roles of pRB in genome stability, the nature of signals generated by the DNA damage response induced by pRB inactivation would be different from those induced by other stimuli, for example, oncogenic Ras or Raf. We are currently investigating how pRb status modulates the DNA damage response driven by ATM.

**Epigenetic control.** A recent study[55] reported that epigenetic changes rather than CIN induced by *RB* loss contributes more strongly to retinoblastoma development. Among the genes that are epigenetically regulated by pRB, spleen tyrosine kinase (*SYK*) appears to play the most critical role.[55] The exact role and mechanism of pRB in epigenetic control is still unclear. Many chromatin modifiers with the LxCxE motif, including DNMT1 (DNA methyltransferase), Suv39H1, Suv4-20H1 (methyltransferase), HP1 (histone H3me3-binding protein), Brm1, BRG1 (ATP-dependent helicases) and HDAC (histone deacetylases), and many of the histone demethylases, including KDM5A, bind to pRB.[20] In addition, new insights on the 'metabolic reprogramming' caused in part by pRB and p53 double inactivation has led us to investigate its indirect but noteworthy impact on the chemical modification of chromatin-modifying enzymes by altering the cellular levels of metabolites such as nicotinamide adenine dinucleotide$^+$ (NAD$^+$), flavin adenine dinucleotide (FAD) or α-ketoglutarate.

## Function of pRB in Cell Fate Decision

The role of pRB in terminal differentiation has been well documented by its ability of binding to or functioning with lineage-specific transcription factors including MyoD, C/EBPα, GRα, GATA-1, PU1, CBFA-1, Pdx1, Runx2 and NF-Il6. In addition, pRB suppresses Id2, KDM5A and EID1, which disturb differentiation. pRB has a crucial role in cell fate decision. For instance, pRB status determines whether p53-mutated osteosarcoma cells accept osteogenic or fat fate.[56] This used to be explained by the functional interaction of pRB with

RUNX2 and PPARγ. Nevertheless, the impact of 'metabolic reprogramming' on the differentiation program that stems from the synergistic p53 and pRB inactivation is also a possible explanation. Furthermore, the pRB-p53 alliance appears to determine tumor subtypes through an unknown mechanism. The destruction of this mutually aiding partnership appears to shift breast and lung cancers to more primitive or epithelial-mesenchymal transition (EMT)-like types.[57,58]

## Function of pRB in Stem Cells and Cancer Stem Cells

**Tissue stem cells.** The intrinsic role of pRB in tissue stem cells has long been debated because studies on the extrinsic role of pRB in hematopoietic stem cells (HSC) for fetal–maternal nutrient exchange through placenta or bone marrow niche –hematopoietic stem cell adhesion had previously had a prevailing impact on the field.[59,60] However, recently the intrinsic role of pRB in HSC for oxidative stress response or mitochondrial biogenesis is also appreciated.[46,51] A surprising twist came from plant studies. *Arabidopsis thaliana* lacking a *RB* ortholog showed expansion in the root stem cell pool without losing its self-renewal capacity; this again stimulated a debate on the role of pRB in stem cells.[61,62]

**Embryonic stem cells.** Embryonic stem (ES) cell biology and induced pluripotent stem (iPS) cell technology indicated a requirement of carcinogenic signals for the induction of genome-wide chromatin remodeling and 'stemness'. Compared with adult somatic cells, ES or iPS cells exhibit an extraordinarily short G1 phase. The length of G1 could be one of the determinants of the fate of ES cells of whether to stay in a pluripotent state or to differentiate.[63] A short G1 is at least in part due to pRB hyperphosphorylation and suppression of p53 transcription.[64] Nanog seems to maintain pRB at the hyperphosphorylated status via CDK6 or CDC25A.[65] In contrast, loss of p53 promoted the efficacy of iPS cell induction.[66] The p53-mSin3a-HDAC transcription suppressive complex present on the Nanog gene promoter might be a cell cycle-independent mechanism of this.[67] While an early published report denied the contribution of pRB depletion to iPS cell induction, one of the later studies has demonstrated caspase 3- and 8-mediated pRB cleavage/inactivation facilitates iPS cell induction.[14,66] Since pRB is considered to be one of the acute targets of some of the pluripotent core factors, knockdown of RB might not show any more additive effect over acute gain of function of these factors.[65] The ES cells lacking KDM5A fail prematurely to maintain Oct4 or Nanog expression under differentiation-promoting conditions.[68] KDM5A might also mediate the ability of pRB to control mitochondrial biogenesis or functions.[49] Compared with somatic cells, ES and iPS cells contain fewer numbers of mitochondria; cells with far fewer numbers of organelle maintained high pluripotency but had lower teratoma-forming activity (which might be relevant to cell proliferation). In contrast, cells with a comparatively high number of mitochondria lose pluripotency but gain higher proliferation activity.[69] It would be intriguing to address whether the status of pRB affects mitochondria biogenesis and pluripotency in ES cells or in cancer stem cells.

**Cancer stem cells.** Previous studies have detected similarities between poorly differentiated cancer cells and ES cells.[70]

Core pluripotent genes, polycomb genes and Myc target genes might play pivotal roles in these shared features.[71] The simultaneous disruption of pRb function and contact inhibition of cells allowed mouse fibroblasts to form a 3D aggregate expressing a variety of core pluripotent genes, and cells derived from such aggregate were also shown to form teratoma-like tumors in immunodeficient mice.[72] We developed a similar experimental model, and subsequently identified additional genetic changes that are in fact required for cancer stem cell-like behaviors in *Rb*-deficient cells (Shunsuke Kitajima, unpublished data, 2011). Detection of core pluripotent gene transcripts in cancers is often quoted to be circumstantial evidence of 'cancer stemness'; however, its significance is totally unclear. Overexpression of Nanog transforms NIH3T3 fibroblasts and induces cancer stem-like cells from astrocytes.[73,74] Sox2 (as an esophageal 'oncogene') and Nanog have been implicated in the anchorage-independent growth of tumor cells.[75,76] Indeed, phenomenally, anchorage-independent growth is hard to distinguish from sphere formation or cancer transplantability to immunodeficient mice. However, it is also possible that such circumstantial evidence is non-specifically associated with a genome-wide change in chromatin structure in transformed cells. Attenuation of *Rb*-deficient pituitary cancers by deletion of the KDM5A loci implies *Rb*-deficient carcinogenesis might depend on chromatin remodeling.[68] Epigenetic mechanisms are known to affect the causality of human retinoblastoma progression.[55] We observed pRb cooperates with a histone methyltransferase Suv39h1 in suppressing carcinogenesis.[10] pRB inactivation induces cell cycle reentry or tumorigenicity in postmitotic fully differentiated cells while preserving or even reviving their differentiation potential.[77–80] Based on these observations, we might be able to establish new *in vivo* and *in vitro* models to address the exact role of pRB in stem cell-like behaviors seen in cancer cells.

## Conclusion

To achieve a greater understanding of the roles of pRB during tumor progression, future research should focus on the genome-wide impact of pRB inactivation, which could be mediated by altered cell signaling, cellular metabolism and chromatin remodeling.

## Disclosure Statement

The authors declare no potential conflict of interest.

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