CHAPTER 2

pRb in the Differentiation of Normal and Neoplastic Cells

Deborah Pajalunga, Grazia Camarda and Marco Crescenzi*

Abstract

This chapter deals with the role played by the retinoblastoma protein (pRb) in a variety of differentiation processes. After broadly reviewing the current knowledge on this issue, it points at two common themes. The first is the exclusive involvement of pRb in the final maturation stages of each lineage, so that the functional ablation of the protein produces relatively subtle differentiation defects. The second is that, at least in the cell types more thoroughly investigated, pRb exerts its pro-differentiation potential by enhancing the activities of transcription factors that are key regulators of tissue-specific differentiation.

Finally, the hypothesis is put forward that pRb plays a role in the final differentiation stages of a much wider range of cell types than currently recognized. It is proposed that one reason for the well-know, poorly-understood, inverse relationship between differentiation and malignancy is the functional impairment of pRb and possibly its family members in the vast majority of human cancers.

Introduction

Tumor cells are uniformly characterized by unchecked proliferation on one side and impaired or arrested differentiation on the other.[1] In general, the degree of differentiation of tumors correlates inversely with their malignancy, the more undifferentiated neoplasias being also the more aggressive. The mechanisms through which altered proliferation and impaired differentiation are linked are only partially understood.

The general rule that all malignant tumors show altered differentiation applies even to malignancies ostensibly comprised of highly or even terminally differentiated cells. A case in point is that of multiple myeloma. In this disease, the vast majority of tumor cells are terminally differentiated plasma cells, while the malignancy grows through the expansion of a minor compartment of cells whose differentiation is arrested at a stage compatible with proliferation.[2] Conversely differentiation, when allowed to proceed in a tumor cell, can take over cell cycle control and bring the neoplastic cell to a halt. It is well known that differentiation tends to oppose cellular transformation. In extreme but not uncommon cases, terminal differentiation coexists with malignant transformation. Although terminally differentiated cells are unable to proliferate by definition and cannot possibly be transformed, spontaneous terminal differentiation often takes place even in highly aggressive cancers in a fraction–sometimes the majority–of the tumor cells. Such cells cease proliferating, thus demonstrating that differentiation can suppress the transforming events that lead to tumorigenesis. By way of example, in the

*Corresponding Author: Marco Crescenzi—Department of Environment and Primary Prevention, Istituto Superiore di Sanitá, Rome, Italy. Email: crescenz@iss.it

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solid tumor rhabdomyosarcoma, most cells are often undifferentiated and morphologically uncharacteristic. However, a variable percentage of cells derived from the malignant clone terminally differentiate into muscle fibers.[3] Understanding the molecular underpinnings of the conflict between neoplastic transformation and differentiation is a fundamental question in cancer biology. As it is true of all basic questions in cancer, finding answers should provide us with potential targets for therapeutic interventions. Indeed, it should be stressed that differentiation of some tumors can also be elicited both in vitro and in vivo by chemical treatments. We must understand the molecular mechanisms through which differentiation can force a tumor cell to stop as in the instances of differentiation therapies for myeloid leukemias and neuroblastomas.

The universal character of the transformation/differentiation antithesis suggests that one or very few mechanisms underlay it. While it is known that a large number of genetic and epigenetic alterations concur to cell transformation in a seemingly endless variety of combinations, we suggest that most or all transforming mechanisms share a common theme entailing impaired differentiation. Specifically, we hypothesize that one fundamental reason for the inverse correlation between differentiation and malignancy is the near–universal inactivation of the retinoblastoma protein (pRb) pathway in human tumors. Such inactivation alters the control of the cell cycle and contributes to determine the unchecked proliferation of tumor cells. At the same time, we contend, it impairs cell differentiation in a much wider variety of cell types than currently appreciated. Impaired differentiation is far from being a marginal byproduct of Rb pathway inactivation. On the contrary, it is a necessary condition for sustained proliferation of tumor cells in those cases in which their normal counterparts terminally differentiate into nonproliferating or postmitotic cells. This is very frequently true, as for example in the cases of most epithelial and hematopoietic cells which are almost always postmitotic in the final stages of their differentiation. Even when the differentiation of a given cell type is nonterminal (e.g., hepatocytes, thyrocytes), it is still accompanied by very low proliferation rates which are hardly compatible with neoplastic transformation.

The pRb Pathway in Normal and Neoplastic Cells

The tumor suppressor protein pRb is a central regulator of cell homeostasis, involved in the control of such critical functions as proliferation, differentiation, and programmed cell death. In the cell cycle, pRb exerts its activity in close proximity to the restriction point, regulating the decision to enter S phase. In its cell cycle regulatory capacity, pRb is primarily regulated through phosphorylation. Un- or hypo-phosphorylated pRb is conventionally regarded as "active" and prevents entry into S phase. During G1 phase of an unperturbed cell cycle, pRb is progressively phosphorylated by the cyclin D-dependent kinases cdk4 and cdk6 and the cyclin E/cyclin A-dependent cdk2. Phosphorylation of pRb "inactivates" it, thereby allowing advancement into S phase. pRb phosphorylation allows cell cycle progression mainly by releasing transcription factors of the E2F family. The E2F factors, when bound by hypophosphorylated pRb, form complexes that bind target promoters bearing E2F binding sites and actively repress transcription. Upon phosphorylation, pRb releases the E2F factors, that promote transcription of a large number of genes, many of which are essential regulators or direct effectors of DNA replication. The kinases that phosphorylate pRb are controlled by a variety of mechanisms at different levels. One prominent regulation is exerted by two groups of inhibitory molecules, the INK4 and KIP families. The INK4 family consists of four members, commonly indicated as p15, p16, p18, and p19 from their molecular weights. The INK4 inhibitors have binding specificity for the cyclin D-dependent cdk4 and cdk6 kinases and, when bound to them, prevent their forming complexes with the activating cyclins. The KIP inhibitors include p21, p27, and p57, have the ability to bind all cyclin/cdk complexes or their cyclin moieties alone, either way inhibiting cdk activity.

This highly simplified view of cell cycle regulation in G1 is summarized in Figure 1. It includes only those players whose alterations are frequently involved in pRb pathway inactivation in the course of neoplastic transformation. It is designed solely to serve this discussion,

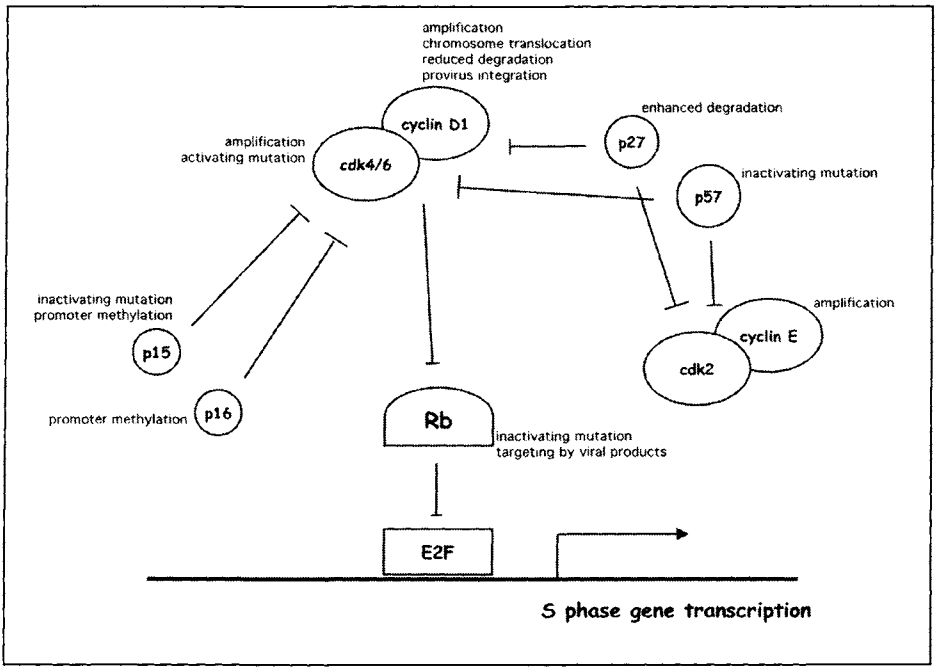

Figure 1. The pRb pathway. A simplified schematic of the pRb regulatory pathway showing some of the main factors. Mechanisms altering the function of each regulator in human cancers are indicated.

with no attempt to approximate a full description of our current understanding of G1 phase regulation.

In the last ten years it has been shown that the Rb pathway is impaired or altogether inactivated in virtually all human tumors. This remarkable discovery suggests that it is nearly impossible for a human cell to undergo neoplastic transformation without losing the restraint provided by pRb. However, pRb is directly inactivated via mutations or deletions or is targeted by viral proteins in a minority of neoplasias. Most often it is one of the components of the pRb pathway shown in Figure 1 to be altered in a number of different ways. A partial list of pRb pathway alterations in tumor cells includes reference 4 and references therein:

- amplification of the cyclin D1, -D2, -E, the cdk4, or the cdk6 gene
- chromosomal translocation of the cyclin D1 locus generating overexpression of the gene product
- activating mutations of cdk4
- inactivating mutations of the p16 or p57 cdk inhibitor
- methylation-mediated silencing of the p15 or p16 promoter
- accelerated degradation of p27
- reduced degradation of D cyclins
- activation of D cyclins by provirus integration

The main functional outcome of these alterations is constant: pRb inactivation and E2F release. Thus, the cell cycle regulatory function of pRb is suppressed in tumor cells by direct inactivation of the Rb gene or its product or by increased phosphorylation due to alterations in its regulatory pathway.

Most of the Rb-inactivating events that concur to neoplastic transformation also impinge on the two other pRb family members, p107 and p130. Whereas direct Rb gene mutations or deletions clearly affect only pRb, viral oncoproteins such as human papillomavirus E7 target all

three family members. Likewise, the three pRb family proteins are targets of the same kinases[5] that presumably, when hyperactive, similarly affect all of them. Thus, current knowledge allows us to assume that the impairment of the pRb cell cycle functions is mirrored by related alterations in the capacities of its cousins.

Impairment of pRb cell cycle-related functions does not automatically translate into repression of its differentiation promoting capabilities. Indeed, these two sets of functions can be genetically separated,[6] as it has been found that some Rb mutants unable to stably bind E2F and induce cell cycle arrest can still promote differentiation. However, differentiation is tightly associated with pRb hypophosphorylation in numerous cell types.[7] In addition, naturally occurring "low risk" pRb mutants that retain differentiation-promoting capacity are mostly associated with benign retinomas,[6] suggesting again that differentiation counteracts full neoplastic transformation. To our knowledge there is no instance in which abnormal, ectopic phosphorylation of pRb is compatible with unimpaired differentiation. Thus, for the rest of the discussion, we will assume that any reduction in the ability of pRb (or its family members) to control the cell cycle is reflected in an overt or subtle modification of its differentiating properties.

pRb Is Involved in the Differentiation of a Growing Number of Cell Types

It is very instructive to summarize briefly the chronology of the discovery of the differentiation role of pRb. Rb knockout (KO) mice and their derivatives led to the conclusion that the differentiation of several cell types depends on pRb. In 1992, three groups independently reported that Rb KO mice die in utero displaying defective maturation of erythrocytes and neurons.[8-10] Two years later, closer examination revealed that in Rb KO mice lens cell differentiation was also impaired, being characterized by excess proliferation, reduced expression of differentiation markers, and apoptosis.[11] Examination of Rb KO mice brought us so far. However, the phenotype of these mice does not tell the whole story. For example, the severe impairment of skeletal muscle differentiation was first discovered in vitro in 1994[12] by examining MyoD-converted Rb KO fibroblasts and confirmed in vivo only two years later in partially rescued Rb KO mice.[13] Likewise, pRb has been shown to be essential for adipocyte differentiation in vitro,[14,15] while a fat phenotype in the KO mice has not been reported.

Subtler defects have been found in lineages for which an in vivo phenotype is not described. The differentiation of hematopoietic lineages other than the erythroid is also influenced by Rb. A role for pRb in monocyte/macrophage differentiation has been recognized as early as 1996[16] and later confirmed by direct suppression of pRb.[17,18] Some evidence exists indicating the involvement of pRb in granulocyte differentiation, too.[17,19]

Further cell types have been found to require pRb for their normal differentiation processes. In 2001 a role for pRb in osteoblast differentiation was recognized,[20] providing a molecular basis for the old epidemiological observation that osteosarcoma is one of the most common cancers arising in survivors of familial retinoblastoma.[21] Conditional Rb KO mice allowed to unveil in 2003 a previously unrecognized role of pRb in keratinocyte differentiation. Finally, in 2003, Rb KO mice were belatedly found to bear placental defects. This finding explains, at least in part, the previous recognition that some differentiation defects in erythroid and central nervous system cells are noncell-autonomous.[22,23] The proposal that at least in part such differentiation defects stem from hypoxia[24] finds a plausible mechanistic explanation in the placental abnormalities of Rb KO embryos.[25] In addition, these abnormalities suggest that yet another cell type, the placental trophoblast, might derive differentiation aberrations from the lack of pRb function.

A few cell types have been specifically reported to differentiate normally in the absence of pRb. One instance is mammary epithelium, as Rb KO cells formed histologically and functionally normal mammary glands when transplanted into wild-type female mice.[26] A second example is provided by Rb KO prostate epithelium, which has been reported to give rise to

fully differentiated and morphologically normal prostate tissue when transplanted into nude mice.[27] However, as always with negative results, caution should be exerted. Very subtle differentiation defects might go undetected by histological examination and manifest themselves only in specific physiological or pathological circumstances. The presence of pRb had been deemed irrelevant for granulopoietic differentiation, a contention later disproved as discussed above. In addition, the potential role of the other pocket proteins in the differentiation of breast and prostate cells has not been investigated.

It is not easy to separate the effects of pRb on the cell cycle from those exerted on differentiation. The available data do not always allow to conclude that in a given cell type a differentiation defect is primary rather than being an indirect consequence of a perturbation of the cell cycle produced by the absence of pRb. For instance, the role of pRb in retinal rod photoreceptor differentiation might be mediated exclusively by the inability of Rb KO precursors to exit the cell cycle. However, for the examples provided above, good evidence exists suggesting an involvement of pRb in the expression of the differentiation program itself rather than exclusively in the cell cycle.

The pRb family members p107 and p130 are also involved in the differentiation processes of at least some cell types. p107/p130 double-KO mice show severe defects in limb development due to deregulated chondrocyte growth.[28] These mice also show defective keratinocyte differentiation and delayed hair follicle morphogenesis and tooth development. Whether the defects of double-KO mice should be attributed to pure cell cycle deregulation or at least partially to impairment of cell-specific differentiation programs remains to be determined. In addition, p107 and p130 single KO mice with a prevalent Balb/cJ genetic background display a variety of developmental defects.[29,30] Thus, whereas this review focuses mainly on pRb, the role of its cognate proteins should not be disregarded. The three pRb family members share regulatory mechanisms and functional properties and in many cases the specific role of each member in a biological process cannot be easily untangled from those of the others. In consequence, the reasoning here applied to pRb extends to its family proteins.

In conclusion, the differentiation of a fairly large number and broad variety of cell types is influenced by one or more of the pRb family members. We have showed that, as more and more cell types are examined closely, a differentiation role for pRb and its family proteins is recognized in far more tissues than initially suggested by the phenotype of Rb KO mice. Thus, lack of in vivo evidence for pRb differentiation activity does not necessarily rule it out. The number of cell types whose differentiation is affected by pocket proteins is still growing and we suggest that eventually most lineages will show some degree of pRb-dependence in their differentiation.

pRb-Regulated Differentiation: Common Themes

In our view, two facts recur in studies of pRb-influenced differentiation processes. First, pRb seems to be involved exclusively in the final stages of differentiation, as the development of precursor cells is generally normal numerically, morphologically, and functionally. Second, in the cases analyzed more in depth, pRb appears to exert its activities by functionally interacting with key transcription factors regulating differentiation.

The first generalization derives from the observation that in all cases in which a differentiation role for pRb has been recognized (see Table 1), differentiation defects are confined to the final cell types in each lineage, while precursor cells are ostensibly normal. For example, while skeletal Rb KO muscle fibers display impaired tissue-specific gene expression and persistent, ectopic DNA synthesis, myoblasts are seemingly normal.[12,13,31] Likewise, Rb KO erythrocyte precursors are capable of going normally through most of their differentiation pathway. A defect is present in the last differentiation stages, leading to reduced numbers of circulating erythrocytes, many of which show an immature phenotype with nucleus retention.[8-10] The contention that pRb is required for late differentiation steps in this lineage is also supported by

Table 1. Involvement of pRb in the differentiation of diverse cell types and interacting transcription factors

Cell Type	Transcription Factor Interacting with pRb	Physical Interaction	Key References
Neuron	?		8,10,24,39,40
Erythrocyte	?		8-10
Granulocyte	C/EBPε	yes	17, 19
Monocyte	NF-IL6	yes	16
Lens cell	?		11, 41
Skeletal muscle	MyoD, MEF2C	MyoD, controversial	12,31,42,43
Adipocyte	C/EBP		14,46
Osteocyte	CBFA1	yes	20
Keratinocyte	?		44
Retinal rod photoreceptor	?		45

in vitro data showing that suppression of pRb in an early culture of hematopoietic progenitor cells has no effect, while similarly treating a late culture produces a strong inhibition of erythroid colonies.[32] In an analogous fashion, central nervous system abnormalities in Rb KO mice are described as ectopic proliferation and excess apoptosis in mature neurons, but neurogenesis itself is grossly normal.[8-10,33] In the bone, pRb is dispensable for early osteoblastic differentiation, but required for the expression of such late differentiation markers as osteocalcin and for mineralization.[20] Finally, the role played by the other pocket proteins, p107 and p130, should not be forgotten. The combined absence of these two proteins severely alters limb cartilage development and late-stage chondrocyte differentiation.[28,34] The examples just cited illustrate the broad conclusion that pocket-protein deficient cell lineages are competent to initiate their differentiation programs, as indicated by their ability to express early markers, but fail to achieve a fully differentiated state.[33]

Our second generalization states that pRb acts on differentiation programs by interacting functionally, and sometimes physically, with transcription factors that are key regulators of differentiation and facilitating their activities. Table 1 reports the cell types for which a differentiation role for pRb has been established. In several cases, it has been shown that pRb enables critical transcription factors or enhances their activity, as also reported in Table 1. The means through which pRb performs these functions are far from clear. For example, pRb binds the transcription factor NF-IL6 in the course of monocytic differentiation and it has been proposed that in this specific case pRb acts like a chaperone protein, enhancing the ability NF-IL6 to bind DNA and transactivate its target genes.[16] In skeletal muscle differentiation, pRb enhances the activity of MEF2 via a poorly understood, MyoD-dependent mechanism.[35] In addition, pRb has been proposed to promote skeletal muscle differentiation by disrupting the transcription-inhibitory MyoD-HDAC1 interaction.[36] Finally, pRb has been shown to mediate the degradation of the muscle-differentiation inhibitor EID-1.[37] It is also likely that the ability of pRb to bind a variety of chromatin-modifying proteins[38] is an important, possibly a crucial factor in determining its differentiation-modulating capacities. However, this possibility has not been thoroughly investigated. Altogether, a variety of mechanisms have been proposed for pRb-mediated enhancement of differentiation and no unifying features seem to emerge yet. However, it appears reasonable to propose that pRb might enhance the activity of even more transcription factors in the examples of differentiation in which such an activity has not yet been described. Thus, we suggest that Rb-mediated enhancement of tissue-specific transcription factors should be looked for and investigated in tissue types that require pRb for optimal differentiation.

pRb-Mediated Impairment of Differentiation in Cancer?

Although neoplasias are characterized by altered or blocked differentiation, this impairment is rarely such as to prevent identification of the normal counterpart of the tumor cell. Indeed, tumor classification has long been based on purely morphological grounds. The conservation of cell-type specific morphological features and tissue-specific markers characteristic of normal cells indicates that in the neoplastic ones differentiation is only partially arrested and mostly in its final steps.

The well-known, universal impairment of cell differentiation in tumors suggests that one or a few common mechanisms exist in all neoplasias, invariably producing limited but biologically significant alterations in their differentiation processes. Loss of pocket-protein function, a characteristic of most if not all cancers, impairs the final steps in differentiation. Thus, we propose that it might explain, at least in part, the ubiquitous differentiation phenotype of tumor cells. This proposal implies that pocket proteins significantly regulate differentiation in most cells, a plausible hypothesis since more and more cell types are found to depend on pRb family proteins for proper differentiation.

Since in most tumors the functional inactivation of pRb is mediated by kinase activities amenable to pharmacological intervention, it seems reasonable to suggest that even a partial inhibition of the relevant kinases would rescue pRb functions. Our proposal that this would result in the recovery of differentiation programs in a large number of tumors adds a new rationale for testing cyclin-dependent kinase inhibitors as anti-cancer drugs and suggests new endpoints to evaluate their activities.

References

1. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000; 100(1):57-70.
2. Seremetis S, Inghirami G, Ferrero D et al. Transformation and plasmacytoid differentiation of EBV-infected human B lymphoblasts by ras oncogenes. Science 1989; 243(4891):660-663.
3. Cotran R, Kumar V, Collins T et al. Pathologic basis of disease. Philadelphia: Saunders, 1999.
4. Bartek J, Bartkova J, Lukas J. The retinoblastoma protein pathway in cell cycle control and cancer. Exp Cell Res 1997; 237(1):1-6.
5. Grana X, Garriga J, Mayol X. Role of the retinoblastoma protein family, pRB, p107 and p130 in the negative control of cell growth. Oncogene 1998; 17(25):3365-3383.
6. Sellers WR, Novitch BG, Miyake S et al. Stable binding to E2F is not required for the retinoblastoma protein to activate transcription, promote differentiation, and suppress tumor cell growth. Genes Dev 1998; 12(1):95-106.
7. Lipinski MM, Jacks T. The retinoblastoma gene family in differentiation and development. Oncogene 1999; 18(55):7873-7882.
8. Clarke AR, Maandag ER, van Roon M et al. Requirement for a functional Rb-1 gene in murine development. Nature 1992; 359(6393):328-330.
9. Jacks T, Fazeli A, Schmitt EM et al. Effects of an Rb mutation in the mouse. Nature 1992; 359(6393):295-300.
10. Lee EY, Chang CY, Hu N et al. Mice deficient for Rb are nonviable and show defects in neurogenesis and haematopoiesis. Nature 1992; 359(6393):288-294.
11. Morgenbesser SD, Williams BO, Jacks T et al. p53-dependent apoptosis produced by Rb-deficiency in the developing mouse lens. Nature 1994; 371(6492):72-74.
12. Schneider JW, Gu W, Zhu L et al. Reversal of terminal differentiation mediated by p107 in $Rb^{-/-}$ muscle cells. Science 1994; 264:1467-1471.
13. Zacksenhaus E, Jiang Z, Chung D et al. pRb controls proliferation, differentiation, and death of skeletal muscle cells and other lineages during embryogenesis. Genes Dev 1996; 10(23):3051-3064.
14. Chen PL, Riley DJ, Chen Y et al. Retinoblastoma protein positively regulates terminal adipocyte differentiation through direct interaction with C/EBPs. Genes Dev 1996; 10(21):2794-2804.
15. Classon M, Kennedy BK, Mulloy R et al. Opposing roles of pRB and p107 in adipocyte differentiation. Proc Natl Acad Sci USA 2000; 97(20):10826-10831.
16. Chen PL, Riley DJ, Chen-Kiang S et al. Retinoblastoma protein directly interacts with and activates the transcription factor NF-IL6. Proc Natl Acad Sci USA 1996; 93(1):465-469.
17. Bergh G, Ehinger M, Olsson I et al. Involvement of the retinoblastoma protein in monocytic and neutrophilic lineage commitment of human bone marrow progenitor cells. Blood 1999; 94(6):1971-1978.

18. Ji Y, Studzinski GP. Retinoblastoma protein and CCAAT/enhancer-binding protein beta are required for 1,25-dihydroxyvitamin D3-induced monocytic differentiation of HL60 cells. Cancer Res 2004; 64(1):370-377.
19. Gery S, Gombart AF, Fung YK et al. C/EBPε interacts with retinoblastoma and E2F1 during granulopoiesis. Blood 2004; 103(3):828-835.
20. Thomas DM, Carty SA, Piscopo DM et al. The retinoblastoma protein acts as a transcriptional coactivator required for osteogenic differentiation. Mol Cell 2001; 8(2):303-316.
21. Fletcher O, Easton D, Anderson K et al. Lifetime risks of common cancers among retinoblastoma survivors. J Natl Cancer Inst 2004; 96(5):357-363.
22. Williams BO, Schmitt EM, Remington L et al. Extensive contribution of Rb-deficient cells to adult chimeric mice with limited histopathological consequences. Embo J 1994; 13(18):4251-4259.
23. Maandag EC, van der Valk M, Vlaar M et al. Developmental rescue of an embryonic-lethal mutation in the retinoblastoma gene in chimeric mice. Embo J 1994; 13(18):4260-4268.
24. MacPherson D, Sage J, Crowley D et al. Conditional mutation of Rb causes cell cycle defects without apoptosis in the central nervous system. Mol Cell Biol 2003; 23(3):1044-1053.
25. Wu L, de Bruin A, Saavedra HI et al. Extra-embryonic function of Rb is essential for embryonic development and viability. Nature 2003; 421(6926):942-947.
26. Robinson GW, Wagner KU, Hennighausen L. Functional mammary gland development and oncogene-induced tumor formation are not affected by the absence of the retinoblastoma gene. Oncogene 2001; 20(48):7115-7119.
27. Day KC, McCabe MT, Zhao X et al. Rescue of embryonic epithelium reveals that the homozygous deletion of the retinoblastoma gene confers growth factor independence and immortality but does not influence epithelial differentiation or tissue morphogenesis. J Biol Chem 2002; 277(46):44475-84.
28. Cobrinik D, Lee MH, Hannon G et al. Shared role of the pRB-related p130 and p107 proteins in limb development. Genes Dev 1996; 10(13):1633-1644.
29. LeCouter JE, Kablar B, Hardy WR et al. Strain-dependent myeloid hyperplasia, growth deficiency, and accelerated cell cycle in mice lacking the Rb-related p107 gene. Mol Cell Biol 1998; 18(12):7455-7465.
30. LeCouter JE, Kablar B, Whyte PF et al. Strain-dependent embryonic lethality in mice lacking the retinoblastoma- related p130 gene. Development 1998; 125(23):4669-4679.
31. Novitch BG, Mulligan GJ, Jacks T et al. Skeletal muscle cells lacking the retinoblastoma protein display defects in muscle gene expression and accumulate in S and G2 phases of the cell cycle. J Cell Biol 1996; 135(2):441-456.
32. Condorelli GL, Testa U, Valtieri M et al. Modulation of retinoblastoma gene in normal adult hematopoiesis: Peak expression and functional role in advanced erythroid differentiation. Proc Natl Acad Sci USA 1995; 92(11):4808-4812.
33. De Bruin A, Wu L, Saavedra HI et al. Rb function in extraembryonic lineages suppresses apoptosis in the CNS of Rb-deficient mice. Proc Natl Acad Sci USA 2003; 100(11):6546-6551.
34. Rossi F, MacLean HE, Yuan W et al. p107 and p130 Coordinately regulate proliferation, Cbfa1 expression, and hypertrophic differentiation during endochondral bone development. Dev Biol 2002; 247(2):271-285.
35. Novitch BG, Spicer DB, Kim PS et al. pRb is required for MEF2-dependent gene expression as well as cell-cycle arrest during skeletal muscle differentiation. Curr Biol 1999; 9(9):449-459.
36. Puri PL, Iezzi S, Stiegler P et al. Class I histone deacetylases sequentially interact with MyoD and pRb during skeletal myogenesis. Mol Cell 2001; 8(4):885-897.
37. Miyake S, Sellers WR, Safran M et al. Cells degrade a novel inhibitor of differentiation with E1A-like properties upon exiting the cell cycle. Mol Cell Biol 2000; 20(23):8889-8902.
38. Morris EJ, Dyson NJ. Retinoblastoma protein partners. Adv Cancer Res 2001; 82:1-54.
39. Slack RS, El-Bizri H, Wong J et al. A critical temporal requirement for the retinoblastoma protein family during neuronal determination. J Cell Biol 1998; 140(6):1497-1509.
40. Marino S, Hoogervoorst D, Brandner S et al. Rb and p107 are required for normal cerebellar development and granule cell survival but not for Purkinje cell persistence. Development 2003; 130(15):3359-3368.
41. McCaffrey J, Yamasaki L, Dyson NJ et al. Disruption of retinoblastoma protein family function by human papillomavirus type 16 E7 oncoprotein inhibits lens development in part through E2F-1. Mol Cell Biol 1999; 19(9):6458-6468.
42. Gu W, Schneider JW, Condorelli G et al. Interaction of myogenic factors and the retinoblastoma protein mediates muscle cell commitment and differentiation. Cell 1993; 72:309-324.
43. Li FQ, Coonrod A, Horwitz M. Selection of a dominant negative retinoblastoma protein (RB) inhibiting satellite myoblast differentiation implies an indirect interaction between MyoD and RB. Mol Cell Biol 2000; 20(14):5129-5139.

44. Balsitis SJ, Sage J, Duensing S et al. Recapitulation of the effects of the human papillomavirus type 16 E7 oncogene on mouse epithelium by somatic Rb deletion and detection of pRb-independent effects of E7 in vivo. Mol Cell Biol 2003; 23(24):9094-9103.
45. Zhang J, Gray J, Wu L et al. Rb regulates proliferation and rod photoreceptor development in the mouse retina. Nat Genet 2004; 36(4):351-360.
46. Puigserver P, Ribot J, Serra F et al. Involvement of the retinoblastoma protein in brown and white adipocyte cell differentiation: Functional and physical association with the adipogenic transcription factor C/EBPalpha. Eur J Cell Biol 1998; 77(2):117-123.

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