Cancer Research

In vivo Significance of the G_2 Restriction Point

Floris Foijer, Elly Delzenne-Goette, Marleen Dekker, et al.

Cancer Res 2007;67:9244-9247.

Updated version	Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/67/19/9244

Cited Articles	This article cites by 27 articles, 8 of which you can access for free at: http://cancerres.aacrjournals.org/content/67/19/9244.full.html#ref-list-1
Citing articles	This article has been cited by 1 HighWire-hosted articles. Access the articles at: http://cancerres.aacrjournals.org/content/67/19/9244.full.html#related-urls

E-mail alerts	Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions	To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions	To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.

Downloaded from cancerres.aacrjournals.org on August 20, 2013. © 2007 American Association for Cancer Research.

In vivo Significance of the G_2 Restriction Point

Floris Foijer, Elly Delzenne-Goette, Marleen Dekker, and Hein te Riele

Division of Molecular Biology, The Netherlands Cancer Institute, Amsterdam, the Netherlands

Abstract

Loss of activity of the retinoblastoma pathway is a common event in human cancer. Mouse models have revealed that tumorigenesis by loss of *Rb* was accelerated by concomitant loss of the cell cycle inhibitor $p27^{KIP1}$. This has been attributed to reduced apoptosis and weakening of the G_1 checkpoint. However, the role of $p27^{KIP1}$ in a recently identified G_2 restriction point may offer an alternative explanation for this synergy. Here, we have investigated the significance of the G_2 restriction point in *Rb*-deficient pituitaries. We show that *Rb* loss in the pituitary gland activated the G_2 restriction point, as evidenced by the appearance of cyclin B1–$p27^{KIP1}$ complexes. Somewhat unexpectedly, these complexes remained present in *Rb*-deficient tumors. These results indicate that the G_2 restriction point does operate *in vivo*. However, in the pituitary gland, this mechanism seems to retard rather than to prevent tumor growth. [Cancer Res 2007;67(19):9244–7]

Introduction

The retinoblastoma gene family, which is composed of *Rb, p107* and *p130*, plays an essential role in regulating the cell cycle and is commonly affected in human cancer (1). Its gene products, the so-called pocket proteins, inhibit E2F transcription factors, whose activity stimulates DNA synthesis and cell cycle progression (2–5). Several forms of stress, such as DNA damage, high cell density, loss of anchorage or mitogenic signaling, or cell differentiation, result in arrest of cells in the G_1 phase of the cell cycle. This G_1 arrest is completely abrogated by ablation of the three pocket proteins (6, 7). However, pocket protein–deficient mouse embryonic fibroblasts (triple knockout, *TKO* MEF) do not completely lack cell cycle control. We recently found that mitogen starvation of *TKO* MEFs led to a robust arrest in the G_2 phase of the cell cycle (8). This arrest was associated with accumulation of the cyclin-dependent kinase inhibitors $p27^{KIP1}$ and $p21^{CIP1}$ that became strong inhibitors of the mitotic cyclins A and B1 through direct interaction. G_2 arrest also significantly contributed to cell cycle arrest in mitogen-starved $Rb^{-/-}$ MEFs and became increasingly evident upon concomitant inactivation of *p107* or *p130* (8).

Monoallelic mutation of *Rb* in humans strongly predisposes to retinoblastoma, a tumor of the developing retina (9, 10). In mice, however, *Rb* hemizygosity led to tumors originating from the intermediate lobe of the pituitary gland with an average latency of 40 to 50 weeks. These tumors consistently showed loss of the wild-type *Rb* allele, thus emphasizing the critical role of *Rb* in pituitary tumor suppression (11, 12). Furthermore, in chimeric mice composed of wild-type and $Rb^{-/-}$ cells or in mice engineered to inactivate *Rb* exclusively in the anterior and intermediate lobe of the pituitary gland, the latency time of tumor development was strongly reduced (12–14). Interestingly, concomitant loss of $p27^{KIP1}$ in *Rb* heterozygous animals accelerated tumor development to 20 weeks, and the tumors were more aggressive. Although the synergistic effect of $p27^{KIP1}$ and *Rb* loss has often been explained by progressive loss of G_1 control, the observed synergy may also suggest $p27^{KIP1}$ and pRb to function in different pathways, which both need to be disrupted or attenuated for pituitary tumor growth. Indeed, we and others have recently identified a role for $p27^{KIP1}$ in G_2 control that may provide an alternative explanation for the observed synergy (8, 15–17).

In this study, we show that loss of $p27^{KIP1}$ accelerated tumorigenesis in *Rb*-deficient pituitaries. In the presence of $p27^{KIP1}$, loss of *Rb* resulted in an increase of cells arrested or delayed in the G_2 phase of the cell cycle and accumulation of cyclin B1–$p27^{KIP1}$ complexes. However, by comparing the levels of cyclin B1–$p27^{KIP1}$ complexes in tumors in mice of different ages, we did not find selection for loss of this interaction during tumor development. Therefore, $p27^{KIP1}$ may decelerate rather than block the growth of *Rb*-deficient pituitary gland tumors.

Materials and Methods

Mouse strains. $Rb^{f/f}$ mice (18) were bred with Oluc-Cre mice (19) to obtain Oluc-Cre $Rb^{f/f}$ mice. In addition, Oluc-Cre $Rb^{f/f}$ mice were bred with $p27^{KIP1-/-}$ mice (20) to obtain Oluc-Cre $Rb^{f/f}p27^{KIP1-/-}$ mice. All mice were in an FVB-129Ola mixed background.

Histologic analysis. Pituitary glands were removed immediately after euthanasia and fixed in 4% formaldehyde (Sigma) in PBS (Invitrogen-Life Technologies) for at least 24 h. For histologic analysis, formaldehyde-fixed pituitaries were embedded in paraffin, cut into 5-μm sections, and stained with H&E.

Fluorescence-activated cell sorting analysis. Pituitary glands were removed immediately after euthanasia and homogenized to single-cell suspensions through mechanical disaggregation. Single-cell suspensions were fixed in 70% ethanol in PBS and stained for DNA content by propidium iodide. Cell cycle distributions were analyzed by fluorescence-activated cell sorting using "Cell Quest" software (BD Biosciences) and "FACS Express" software (De Novo Software).

Immunoprecipitations, immunoblots, and antibodies. Pituitary glands were removed immediately after euthanasia and homogenized to single-cell suspensions through mechanical disaggregation. Subsequently, cells were lysed for 30 min in ELB [150 mmol/L NaCl, 50 mmol/L HEPES (pH 7.5), 5 mmol/L EDTA, 0.1% NP40] containing protease inhibitors (Complete; Roche) and phosphatase inhibitors (5 mmol/L NaF, 0.5 mmol/L sodium vanadate, and 20 mmol/L β-glycerolphosphate). Protein concentrations were determined using the Bradford assay (Bio-Rad). For immunoprecipitations, 50 μg of protein was incubated with 0.5 μg of immobilized antibody overnight at 4°C while rotating. The antibodies used were mouse anti-$p27^{KIP1}$ (BD Transduction Laboratories), tubulin-α (Sigma), rabbit polyclonal cyclin E (M20), mouse monoclonal CDK1 (17), rabbit polyclonal CDK4 (C22), rabbit polyclonal cyclin B1 (H433) and cyclin B1 (GNS1; Santa Cruz). Secondary antibodies used were horseradish peroxidase (HRP)–conjugated goat anti-mouse and HRP-conjugated goat anti-rabbit (Dako).

Note: Current address for F. Foijer: Department of Systems Biology, Harvard Medical School, 200 Longwood Avenue, Boston, MA 02115.

Requests for reprints: Hein te Riele, Division of Molecular Biology, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX, Amsterdam, the Netherlands. Phone: 31-20-512-2084; Fax: 31-20-669-1383; E-mail: h.t.riele@nki.nl.

©2007 American Association for Cancer Research.

doi:10.1158/0008-5472.CAN-07-1650

Downloaded from cancerres.aacrjournals.org on August 20, 2013. © 2007 American Association for Cancer Research.

Results

Loss of $p27^{KIP1}$ accelerates pituitary tumorigenesis. Both $p27^{KIP1-/-}$ mice (20, 21) and *Rb* heterozygous mice (11, 12, 14, 22) developed tumors originating from the intermediate lobe of the pituitary gland. Furthermore, $p27^{KIP1}$ loss accelerated pituitary tumorigenesis in *Rb* heterozygous mice (23). We wondered whether this acceleration was (partly) caused by alleviation of the G_2 restriction point. To address this issue, we examined pituitary glands from $p27^{KIP1}$ wild-type or knockout mice in which floxed *Rb* alleles could be inactivated by a pituitary gland–specific (POMC) Cre recombinase (18, 19). Consistent with previous findings (12–14), we did not detect macroscopically visible abnormalities in *Rb*-deficient, $p27^{KIP1}$ wild-type pituitary glands up to 8 weeks of age (Table 1), although histologic analysis revealed that the intermediate lobe of *Rb*-deficient pituitaries was much enlarged at 8 weeks and showed higher cell density (Fig. 1, compare *B* and *A*). Concomitant loss of $p27^{KIP1}$ dramatically changed this phenotype, causing hyperplasia in the intermediate lobe within 3 weeks (Fig. 1*C*) and full-blown pituitary gland tumors at 4 weeks of age (Fig. 1*D*; Table 1). Thus, tumorigenesis in the pituitary gland, as a consequence of *Rb* loss, is delayed by activity of $p27^{KIP1}$.

Rb-deficient pituitary glands show an increased G_2 population. Next, we examined whether the delay of pituitary tumorigenesis by $p27^{KIP1}$ is caused by arrest of *Rb*-deficient pituitary cells in G_2. Therefore, we homogenized *Rb*-proficient and *Rb*-deficient pituitaries and determined DNA content by flow cytometry. Figure 2*A* shows that the vast majority (~83%) of wild-type pituitary cells resided in the G_1 phase of the cell cycle. A similar profile was seen in $p27^{KIP1}$-deficient pituitaries (Fig. 1*C*). Loss of *Rb* alone resulted in an 8% decrease of G_1 cells and an increase of G_2 cells from 12% to 21% (Fig. 2, compare *A* and *B*), indicative of a partial G_2 arrest. The number of S-phase cells was unaffected (4% in wild-type and 3% in $Rb^{-/-}$ pituitaries).

In contrast, in $Rb/p27^{KIP1}$-deficient tumors, which arose at 4 weeks, the number of S-phase cells was increased up to 7%, whereas the number of G_2 cells was decreased (Fig. 2, compare *B* and *D*). These data suggest that *Rb* deficiency leads to increased G_2 arrest in the pituitary gland, which is alleviated upon concomitant loss of $p27^{KIP1}$.

Rb deficiency in pituitary glands leads to accumulation of cell cycle proteins. pRb is a well-established suppressor of E2F activity (1). Consequently, loss of *Rb* leads to activation of E2F and increased transcription of E2F target genes *in vitro* (24). We wondered whether *Rb* loss in the pituitary gland similarly affected the expression of E2F target genes. Therefore, we compared the protein levels of the E2F targets *cyclin B1* and *cyclin E* in *Rb*-proficient and *Rb*-deficient pituitaries harvested at 4 and 8 weeks of age. Figure 3*A* shows that both cyclin B1 (*top*) and cyclin E (*second row*) levels were strongly elevated in *Rb*-deficient pituitaries. Interestingly, cyclin accumulation coincided with increased $p27^{KIP1}$ levels (*third row*). Because we observed an increased number of G_2 cells in *Rb*-deficient pituitaries, and $p27^{KIP1}$ was shown to prevent G_2 progression through interaction with cyclin B1 (8, 15–17), we immunoprecipitated cyclin B1 from *Rb*-proficient and *Rb*-deficient pituitary glands harvested at 4 weeks of age, the stage at which we observed partial G_2 arrest. Consistent with the presence of

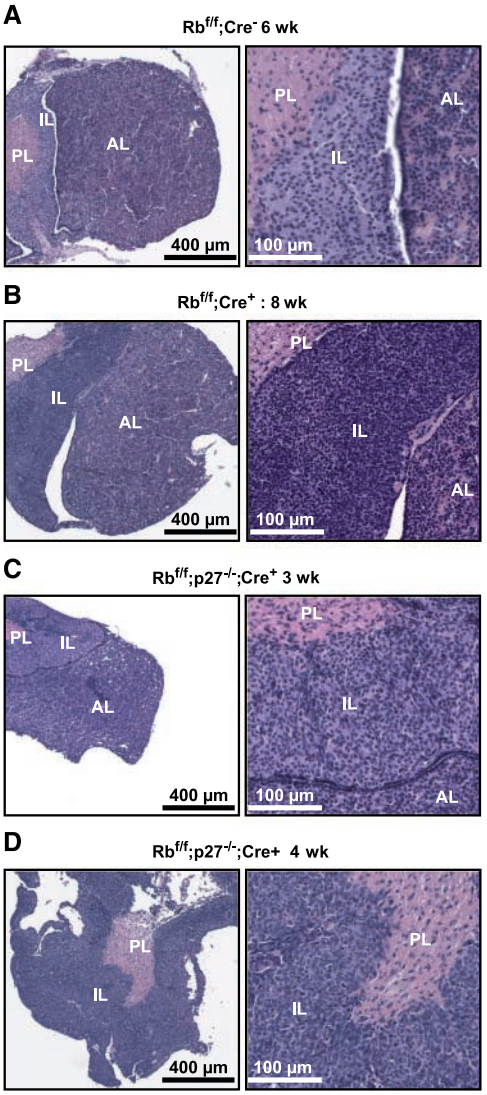

Figure 1. Loss of $p27^{KIP1}$ accelerates pituitary gland tumorigenesis. Histologic slides showing control pituitary tissue at 6 wk (*A*), premalignant pituitary tissue of an Oluc-Cre $Rb^{f/f}$ mouse at 8 wk (*B*), premalignant pituitary tissue of an Oluc-Cre $Rb^{f/f}p27^{KIP1-/-}$ mouse at 3 wk (*C*), and an intermediate lobe pituitary tumor in a Oluc-Cre $Rb^{f/f}p27^{KIP1-/-}$ mouse at 4 wk (*D*). Magnification, 10× (*left*) and 40× (*right*).

Table 1. $p27^{KIP1}$ loss accelerates pituitary tumorigenesis by loss of *Rb*

Genotype	Age (wk)	Tumors
$Rb^{f/f}$; Cre^-	3.5	0/8
	5	0/5
	8	0/3
	10	0/9
$Rb^{f/f}$; Cre^+	3.5	0/9
	5	0/8
	8	0/3
	10	8/10
$Rb^{f/f}$; $p27^{-/-}$; Cre^-	3	0/1
	4	0/1
$Rb^{f/f}$; $p27^{-/-}$; Cre^+	3	1/1 (enlarged)
	4	3/3

Downloaded from cancerres.aacrjournals.org on August 20, 2013. © 2007 American Association for Cancer Research.

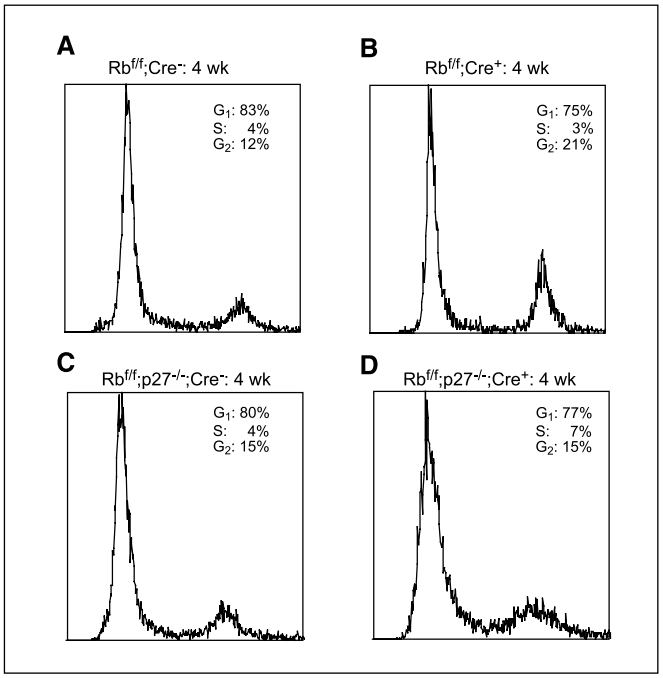

Figure 2. *Rb* deficiency leads to a partial G_2 arrest, which is abrogated upon concomitant loss of *p27^{KIP1}*. Histograms showing cell cycle distributions of single-cell suspensions from pituitary glands of an *Rb$^{f/f}$* mouse (*A*) and an Oluc-Cre *Rb$^{f/f}$* mouse (*B*) at 4 wk and of an *Rb$^{f/f}$p27$^{KIP1-/-}$* mouse (*C*) and an Oluc-Cre *Rb$^{f/f}$p27$^{KIP1-/-}$* mouse (*D*) at 4 wk.

G_2-arrested cells, we detected an interaction between cyclin B1 and p27^{KIP1} in *Rb*-deficient pituitaries (Fig. 3*B, top*). Additionally, we detected p27^{KIP1}-CDK2 complexes (Fig. 3*B, bottom*), which can be indicative for G_1 (25), but also G_2, arrest (8, 15–17).

These results suggest that *Rb* deficiency in the pituitary gland stimulates cell cycle progression by induction of the E2F targets cyclin B1 and E, which is counteracted by accumulation of the CKI p27^{KIP1}, resulting in G_1 and G_2 cell cycle arrest or delay.

Cyclin B1–p27^{KIP1} interaction persists in tumors. Because p27^{KIP1} loss accelerated pituitary tumorigenesis, we wondered whether p27^{KIP1}-mediated inhibition of cyclin B1 was attenuated or even abrogated during tumor progression. To address this issue, we first determined the levels of p27^{KIP1} and CDK1, the latter as a measure for E2F activity. Figure 3*C* (*middle*) shows that loss of *Rb* led to increased levels of CDK1, both in normal pituitary glands (3 weeks) and in full-blown tumors (10 weeks). However, also p27^{KIP1} was increased in *Rb*-deficient pituitary glands at ages ranging from 3 to 10 weeks (Fig. 3*C, top*). Furthermore, when we immunoprecipitated cyclin B1 from *Rb*-deficient pituitaries, we found that the cyclin B1–p27^{KIP1} interaction, rather than disappearing, persisted or became even increasingly evident upon aging (Fig. 3*D*, compare 3, 5, 8, and 10 weeks). These data indicate that *Rb* loss resulted in E2F activation and stimulation of the cell cycle, but also in elevated p27^{KIP1} levels and cyclin B1–p27^{KIP1} interaction.

Discussion

Our findings confirm that loss of *p27^{KIP1}* strongly synergizes with loss of *Rb* in tumorous outgrowth of the pituitary gland. *Rb/p27^{KIP1}*-deficient tumors arose earlier and grew more aggressively than *Rb*-deficient tumors. We have investigated here whether tumor development by loss of *Rb* alone was delayed by p27^{KIP1}-mediated G_2 arrest by a mechanism that we have previously described *in vitro* (8). Taken together, our findings indicate that the G_2 restriction point also operates *in vivo*: upon loss of *Rb* in the pituitary gland, the number of cells in G_2 increased, and this was accompanied by the appearance of cyclin B1–p27^{KIP1} complexes.

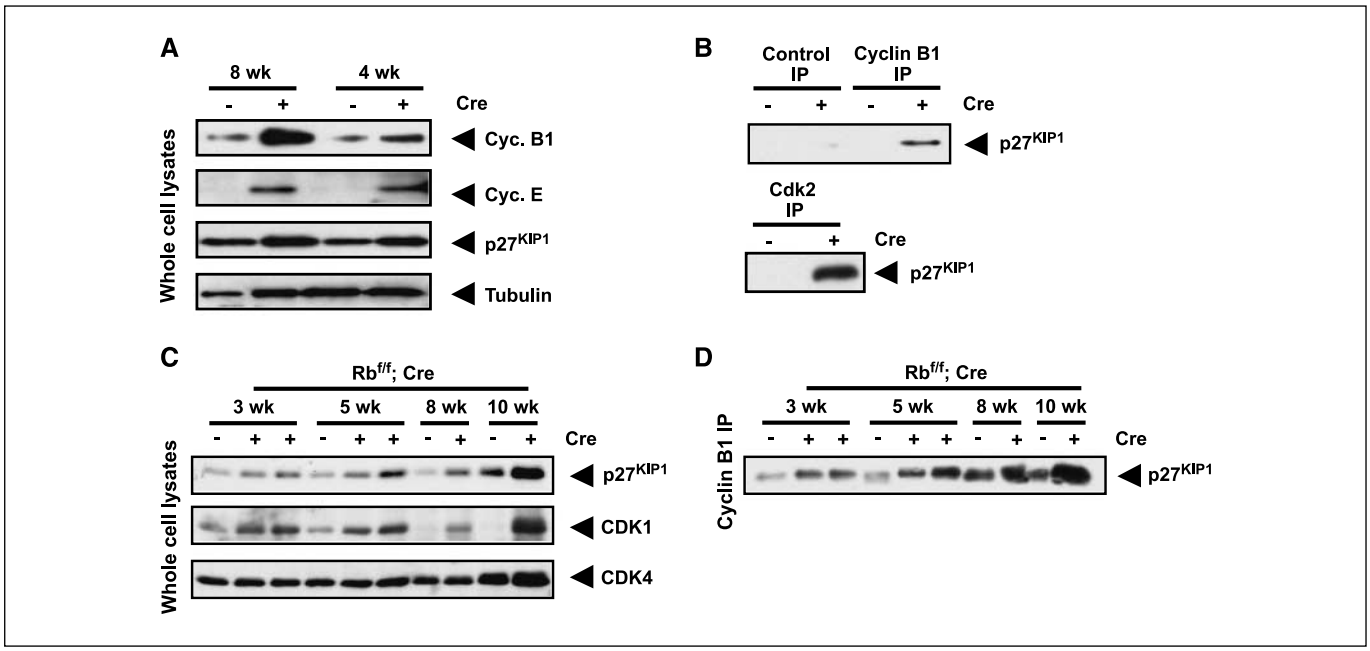

Figure 3. *Rb* loss leads to activation of several E2F targets that are involved in cell cycle progression and is associated with the formation of cyclin B1–p27^{KIP1} complexes. *A,* Western blots showing levels of cyclin B1, cyclin E, and p27^{KIP1} in pituitary glands in the presence (−Cre) or absence (+Cre) of pRb. Tubulin functions as a loading control. *B,* immunoprecipitation of cyclin B1 (*top*) or CDK2 (*bottom*) from *Rb*-proficient and *Rb*-deficient pituitary glands probed for the presence of p27^{KIP1}. *C,* Western blots showing CDK1 and p27^{KIP1} levels in pituitaries of *Rb*-proficient and *Rb*-deficient mice at different ages. CDK4 functions as a loading control. *D,* immunoprecipitation of cyclin B1 from pituitaries of *Rb*-proficient and *Rb*-deficient mice probed for the presence of p27^{KIP1} at different ages.

Downloaded from cancerres.aacrjournals.org on August 20, 2013. © 2007 American Association for Cancer Research.

We have previously shown that this interaction inhibits cyclin B1–dependent kinase activity (8). The cyclin B1–$p27^{KIP1}$ interaction was induced upon loss of *Rb*, but strikingly, was not lost during tumor progression. We envisage that in *Rb*-deficient pituitary tumors, $p27^{KIP1}$ contributed to slower tumor growth by interacting with cyclin B1 and that within the time frame tumor development and behavior was followed, no selection occurred for loss of this interaction. We do realize that the contribution of the cyclin B1–$p27^{KIP1}$ interaction to slower tumor growth was only modest. Our earlier *in vitro* experiments have shown that the G_2 restriction point is particularly important in *TKO* cells that are devoid of all three pocket proteins. In the presence of one or two of the pocket proteins, the major mechanism of cell cycle control operated in G_1 whereas the contribution of G_2 arrest was less prominent. Thus, the presence of p107 and p130 and the interaction of $p27^{KIP1}$ and CDK2 (Fig. 3*B*) likely explain the modest level of G_2 cells in *Rb*-deficient pituitary glands. Furthermore, it should be noted that activation of the G_2 restriction point in pocket protein–deficient cells *in vitro* only occurred in response to mitogen deprivation. It is therefore possible that inhibition of CDK2 and CDK1 by $p27^{KIP1}$ was confined to a subset of tumor cells that was deprived of mitogens and that only these cells were arrested in G_1 or G_2. Alternatively, $p27^{KIP1}$ may have inhibited CDK activity in the majority of tumor cells but rather than imposing cell cycle arrest, this only caused a slower progression through the cell cycle. Either mechanism may explain that loss of $p27^{KIP1}$ strongly synergizes with loss of *Rb* in tumorigenesis and that loss of $p27^{KIP1}$ expression in human cancers is associated with poor prognosis (26, 27).

Acknowledgments

Received 5/4/2007; revised 6/27/2007; accepted 7/9/2007.

Grant support: Dutch Cancer Society grants NKI 2000-2232 and NKI 2002-2634.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank our colleagues of the Te Riele laboratory for fruitful discussions and the people of the animal facility for taking care of our mice.

References

1. Malumbres M, Barbacid M. To cycle or not to cycle: a critical decision in cancer. Nat Rev Cancer 2001;1:222–31.
2. Harbour JW, Luo RX, Dei Santi A, Postigo AA, Dean DC. Cdk phosphorylation triggers sequential intramolecular interactions that progressively block Rb functions as cells move through G_1. Cell 1999;98:859–69.
3. Harbour JW, Dean DC. The Rb/E2F pathway: expanding roles and emerging paradigms. Genes Dev 2000;14:2393–409.
4. Lundberg AS, Hahn WC, Gupta P, Weinberg RA. Genes involved in senescence and immortalization. Curr Opin Cell Biol 2000;12:705–9.
5. Ezhevsky SA, Ho A, Becker-Hapak M, Davis PK, Dowdy SF. Differential regulation of retinoblastoma tumor suppressor protein by G(1) cyclin-dependent kinase complexes *in vivo*. Mol Cell Biol 2001;21:4773–84.
6. Dannenberg JH, van Rossum A, Schuijff L, te Riele H. Ablation of the retinoblastoma gene family deregulates G(1) control causing immortalization and increased cell turnover under growth-restricting conditions. Genes Dev 2000;14:3051–64.
7. Sage J, Mulligan GJ, Attardi LD, et al. Targeted disruption of the three Rb-related genes leads to loss of G(1) control and immortalization. Genes Dev 2000;14:3037–50.
8. Foijer F, Wolthuis RM, Doodeman V, Medema RH, te Riele H. Mitogen requirement for cell cycle progression in the absence of pocket protein activity. Cancer Cell 2005;8:455–66.
9. Friend SH, Bernards R, Rogelj S, et al. A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. Nature 1986;323:643–6.
10. Lipinski MM, Jacks T. The retinoblastoma gene family in differentiation and development. Oncogene 1999;18:7873–82.
11. Jacks T, Fazeli A, Schmitt EM, Bronson RT, Goodell MA, Weinberg RA. Effects of an Rb mutation in the mouse. Nature 1992;359:295–300.
12. Robanus-Maandag E, 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;13:4260–8.
13. 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;13:4251–9.
14. Vooijs M, van der Valk M, te Riele H, Berns A. Flp-mediated tissue-specific inactivation of the retinoblastoma tumor suppressor gene in the mouse. Oncogene 1998;17:1–12.
15. Nakayama K, Nagahama H, Minamishima YA, et al. Skp2-mediated degradation of p27 regulates progression into mitosis. Dev Cell 2004;6:661–72.
16. Aleem E, Kiyokawa H, Kaldis P. Cdc2-cyclin E complexes regulate the G_1-S phase transition. Nat Cell Biol 2005;7:831–6.
17. Martin A, Odajima J, Hunt SL, et al. Cdk2 is dispensable for cell cycle inhibition and tumor suppression mediated by p27(Kip1) and p21(Cip1). Cancer Cell 2005;7:591–8.
18. Vooijs M, te Riele H, van der Valk M, Berns A. Tumor formation in mice with somatic inactivation of the retinoblastoma gene in interphotoreceptor retinol binding protein-expressing cells. Oncogene 2002;21:4635–45.
19. Vooijs M, Jonkers J, Lyons S, Berns A. Noninvasive imaging of spontaneous retinoblastoma pathway-dependent tumors in mice. Cancer Res 2002;62:1862–7.
20. Nakayama K, Ishida N, Shirane M, et al. Mice lacking p27(Kip1) display increased body size, multiple organ hyperplasia, retinal dysplasia, and pituitary tumors. Cell 1996;85:707–20.
21. Fero ML, Rivkin M, Tasch M, et al. A syndrome of multiorgan hyperplasia with features of gigantism, tumorigenesis, and female sterility in p27(Kip1)-deficient mice. Cell 1996;85:733–44.
22. Tsai KY, MacPherson D, Rubinson DA, et al. ARF mutation accelerates pituitary tumor development in Rb+/- mice. Proc Natl Acad Sci U S A 2002;99:16865–70.
23. Park MS, Rosai J, Nguyen HT, Capodieci P, Cordon-Cardo C, Koff A. p27 and Rb are on overlapping pathways suppressing tumorigenesis in mice. Proc Natl Acad Sci U S A 1999;96:6382–7.
24. Dannenberg JH, Schuijff L, Dekker M, van der Valk M, te Riele H. Tissue-specific tumor suppressor activity of retinoblastoma gene homologs p107 and p130. Genes Dev 2004;18:2952–62.
25. Toyoshima H, Hunter T. p27, a novel inhibitor of G_1 cyclin-Cdk protein kinase activity, is related to p21. Cell 1994;78:67–74.
26. Bloom J, Pagano M. Deregulated degradation of the cdk inhibitor p27 and malignant transformation. Semin Cancer Biol 2003;13:41–7.
27. Blain SW, Scher HI, Cordon-Cardo C, Koff A. p27 as a target for cancer therapeutics. Cancer Cell 2003;3:111–5.

Downloaded from cancerres.aacrjournals.org on August 20, 2013. © 2007 American Association for Cancer Research.