What's So Special about RB?

Christin E. Burd[1,2] and Norman E. Sharpless[1,2,3,*]
[1]Department of Genetics
[2]Curriculum in Toxicology
[3]Department of Medicine
The Lineberger Comprehensive Cancer Center, University of North Carolina School of Medicine, Chapel Hill, NC 27599-7295, USA
*Correspondence: nes@med.unc.edu
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RB, p107, and p130 are highly related proteins, each capable of regulating cellular proliferation. However, only RB is frequently mutated in cancer. In this issue of *Cancer Cell*, Chicas et al. shed new light on this conundrum, defining a "special," nonredundant role for RB in promoting cellular senescence.

Cellular senescence is a durable growth arrest that occurs in response to a variety of cellular insults including telomere dysfunction, DNA damage, oxidative stress, and/or oncogene activation (Campisi and d'Adda di Fagagna, 2007). In response to these stressors, the archetypal tumor-suppressor proteins p53 and $p16^{INK4a}$ promote cell cycle arrest and cellular senescence through inhibition of cyclin-dependent kinase (CDK) activity. In the absence of CDK phosphorylation, the RB family of proteins (RB, p107, and p130) binds E2F transcription factors and blocks S-phase entry. Intriguingly, although all three "pocket proteins" have the capability to block cellular proliferation at the G1-S checkpoint, only RB is mutated with high frequency in human cancers (see COSMIC, http://www.sanger.ac.uk/genetics/CGP/cosmic/). This observation has prompted many investigators to wonder what makes RB so "special," at least with regard to tumor suppression.

One hypothesis, based upon the selective binding of RB, p107, and p130 to specific E2F family members, has been that the antioncogenic properties of RB can be attributed to a unique set of transcriptional targets. Several groups have tested this model via candidate gene approaches in knockout murine embryo fibroblasts (MEFs) and cancer cell lines (e.g., Hurford et al., 1997; Lehmann et al., 2008). Although these studies suggested that the pocket proteins control specific subsets of E2F-regulated genes (e.g., *cyclin E*), the consequences of such selectivity were not fully understood. Moreover, the use of tumor-derived or germline knockout cell lines for these analyses failed to account for functional changes in the cell cycle machinery resulting from transformation or developmental compensation.

Against this background, Chicas and colleagues now provide additional insights into the nonredundant functions of RB family members through the use of unbiased technologies (Chicas et al., 2010). The authors evaluated cellular quiescence, cellular senescence, and gene expression after knockdown of each pocket protein individually in nontransformed human fibroblasts. They further used chromatin immunoprecipitation and unbiased next-generation sequencing (ChIP-Seq) to identify DNA sites bound specifically by each RB family member in cycling, senescent, or quiescent cells.

Supporting the dogma that pocket proteins control cell cycle progression primarily through E2F regulation, a large number of the differentially regulated genes identified in their screen were known E2F targets. In addition, knockdown of RB, p107, or p130 individually failed to abrogate quiescence induced by contact inhibition or serum starvation. Only upon targeting of all three genes at once were cells able to bypass the G1-S checkpoint in the setting of quiescence (Figure 1). These data are in accord with other recent publications (Reed et al., 2010; Stengel et al., 2009), demonstrating redundancy among the RB family members during normal growth and quiescence. However, when the authors examined RB family protein binding in the context of cellular senescence, they observed a distinction between RB and p107/p130.

To examine oncogene-induced senescence, Chicas and colleagues transduced fibroblasts with a retrovirus expressing oncogenic RAS in the presence or absence of shRNAs specific to each RB family member. Knockdown of no single RB family member was sufficient to fully abrogate RAS-induced growth arrest. Intriguingly, however, knockdown of RB, but not p107 or p130, did substantially overcome aspects of the senescence-promoting effect of RAS expression, increasing DNA replication and reducing expression of senescence markers such as senescence-associated (SA) heterochromatin formation and SA-β-galactosidase (Figure 1). Moreover, gene profiling experiments combined with ChIP-Seq data showed regulation of an interesting subset of genes in the setting of senescence by RB, but not p130 or p107. In accord with other studies of RB function, many of these senescence-associated, RB-specific targets were DNA replication factors or genes expressed as part of the senescence-associated secretory phenotype. The authors also observed a novel cluster of RB-specific targets that are largely uncharacterized in function. The only familiar member of this cluster was cyclin E1, a well-known RB target that induces CDK2 activity and further phosphorylation of RB family members (Herrera et al., 1996).

Provocatively, cyclin E1 is also believed to stimulate prereplication complex (pre-RC) formation through the CDK-independent recruitment of minichromosome maintenance proteins (MCMs) to DNA replication origins (Geng et al., 2007). On the basis of the observation that cyclin E1 was potently induced by RB loss in the context of senescence, the authors proposed a two-pronged mechanism by which RB inhibits replication in the setting

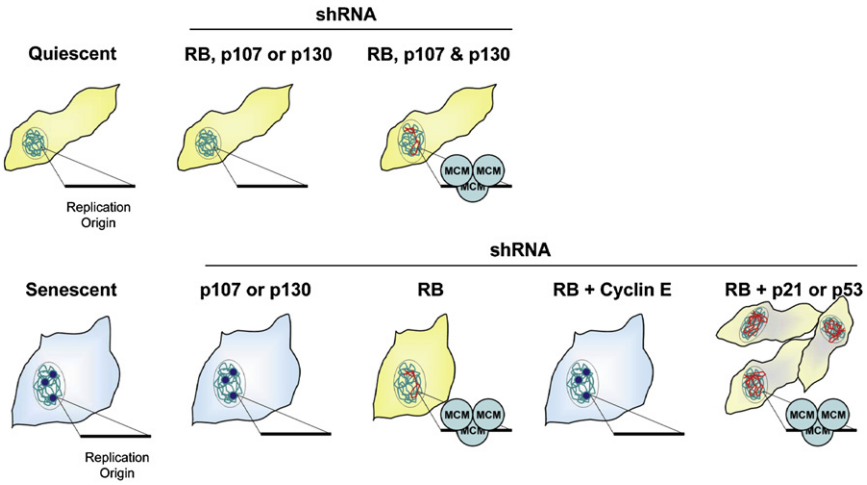

Figure 1. RB Is Critical to Cellular Senescence

Top: Knockdown of RB, p107, or p130 in quiescent normal human fibroblasts fails to influence proliferation or loading of the pre-RC complex onto replication origins. Only upon loss of all three proteins do cells begin to traverse the G1-S checkpoint.

Bottom: Normal fibroblasts senesce upon the expression of an oncogenic RAS showing increased SA-β-galactosidase expression (blue) and heterochromatin foci (blue circles). Knockdown of RB, but not p107 or p130, allows the cells to partially escape senescence through induction of DNA replication factors and cyclin E1. This process leads to pre-RC formation and limited endoreduplication (red) and requires cyclin E1. Additional loss of p53 or $p21^{CIP}$ provides further resistance to senescence.

of senescence: by repression of transcripts associated with DNA replication and by repression of cyclin E1, which participates in pre-RC formation. As predicted by this model, concomitant knockdown of cyclin E1 and RB in senescing fibroblasts inhibited pre-RC formation and reduced the aberrant DNA replication observed with RB deficiency alone (Figure 1). In addition, the reintroduction of shRNA-resistant wild-type and kinase-deficient cyclin E1 cDNAs caused the cells to revert to aberrant proliferation and endoreduplication. Therefore, deregulation of cyclin E1 alone appears to be responsible for the aberrant replication seen in senescing cells lacking RB.

Cells lacking RB escaped from oncogene-induced senescence only temporarily. After additional passaging, these cells arrested, suggesting the existence of additional proliferative barriers. Based upon previous work demonstrating the cooperativity of loss of p53 and RB in cellular transformation, the authors examined the role of p53 in the RB-deficient cell lines. Remarkably, knockdown of p53 or its transcriptional target, the $p21^{CIP}$ cyclin-dependent kinase inhibitor, allowed RB-deficient cells to undergo sustained proliferation, suggesting a critical interaction between these pathways in oncogene-induced senescence.

The finding that p107 and p130 fail to compensate for RB loss in the setting of oncogene-induced senescence raises several new questions. The molecular basis whereby p107 and p130 are able to sufficiently repress transcription of DNA replication factors and cyclin E1 in the setting of generic G1 arrest (quiescence), but not during senescence, is unknown. Moreover, it is unclear whether these nonredundant RB functions can be extended to other cell types. Recent publications have suggested tissue-specific roles for the pocket proteins in murine homeostasis. For example, although loss of RB and p130 function failed to increase hepatic proliferation (Reed et al., 2010), mutation of these two proteins in the intestinal epithelium led to hyperproliferation (Haigis et al., 2006). Moreover, the pocket proteins have recently been reported to regulate their own transcription in a tissue-specific manner (Burkhart et al., 2010). A tissue specificity in the ability of p107 and/or p130 to provide compensation for RB loss may in turn explain why RB inactivation is so strongly associated with transformation of some tissues (e.g., the retina), but not others.

As a whole, this work substantially clarifies our understanding of RB family function, identifying an unexpected, nonredundant role for RB in senescence but not quiescence. This function of RB is mediated by increased promoter affinity and gene regulation of several factors involved in DNA replication, as well as cyclin E1, which plays a newly discovered, noncatalytic role in DNA replication. Through these actions, RB appears unique among RB family members in its ability to fully and durably promote cellular senescence, arguably the most important of mammalian tumor-suppressor mechanisms. In this regard, RB appears truly to be special, proving that, at least when it comes to tumor suppression, three proteins are not better than one.

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