Ras and pRb: The Relationship Gets Yet More Intimate

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Ras and pRb are key regulators of a plethora of cellular processes, including proliferation, differentiation, and tumorigenesis. Adding to several previously established lines of communication between the two, in this issue of *Cancer Cell*, Shamma et al. resolve a new signaling network involved in cellular senescence and tumor suppression.

The *ras* family of proto-oncogenes and the retinoblastoma tumor suppressor gene, *Rb*, have been the subjects of thousands of studies, yet not all of their secrets have been revealed. The general message emanating from these studies is that each gene acts as a key regulator of several cellular processes, most notably proliferation and differentiation. Most likely as a consequence of these involvements, they also correspond to common human cancer genes, with *ras* frequently acting as an oncogene upon activation by point mutation and *Rb* representing a classical tumor suppressor gene contributing to cancer upon (functional) loss of both of its alleles. Ras resides near the cell membrane, where it transduces extracellular signals through various cascades to the nucleus. pRb is a nuclear protein that regulates proliferation and differentiation by controlling transcriptional programs, at least in part by engaging in physical interactions with transcription factors like E2Fs, MyoD, and C/EBPβ.

In spite of their geographical distance, intimate communication takes place between Ras and pRb, through various signaling channels. First, pRb acts as a critical effector for Ras. Specifically, whereas Ras activity is required for progression through the G1 phase of the cell cycle in normal mouse embryo fibroblasts, *Rb*-deficient cells continue to divide irrespective of Ras activity (Peeper et al., 1997). Second, their communication is bidirectional: in turn, Ras activity is regulated as a function of pRb (Figure 1A). *Rb* deficiency sharply increases the ability of Ras to bind guanine nucleotides, resulting in the activation of the latter (Lee et al., 1999). Third, in addition to this mutual negative feedback loop, a genetic interaction between Ras and the *C. elegans* ortholog of *Rb* has been shown to underlie vulval cell fates (Lu and Horvitz, 1998).

In the mouse, work from Ewen and coworkers in particular has revealed strong genetic interactions between these factors (Figure 1B). During embryogenesis, inactivation of *N-ras* rescues several developmental defects incurred by *Rb* loss (Takahashi et al., 2003). Furthermore, heterozygosity for either *N-ras* or one of its cousins, *K-ras*, reduces the expansion of pituitary adenocarcinomas in $Rb^{+/-}$ mice, thereby prolonging survival (Takahashi et al., 2004). In contrast, in another tumor setting, thyroid C cell adenomas, deficiency for *N-ras* in the context of *Rb* loss allows tumors to acquire metastatic potential (Takahashi et al., 2006), indicating that the role of *N-ras* in the context of *Rb* loss-driven carcinogenesis is cell type dependent.

What has emerged from these and other studies is a complex interplay between Ras and pRb, impacting on proliferation, differentiation, development, tumorigenesis, and metastasis. As always, these studies have triggered new questions. Most notably, although a corner of the veil has been lifted, it has remained unclear exactly how the intimate communication between these two proteins is relayed. In this issue of *Cancer Cell*, Takahashi and colleagues unmask an unanticipated mechanism by which Ras and pRb functionally interact (Shamma et al., 2009). They also portray a physiological setting, cellular senescence, in which this new type of communication is highly relevant.

Senescence can be elicited by various cellular stress conditions, including the unscheduled activation of oncogenes and the inactivation of tumor suppressor genes. Whereas it was initially considered a phenomenon limited to cells in culture, over the past five years, several laboratories have demonstrated that, in fact, "oncogene-induced senescence" (OIS, a collective term) acts next to various cell death programs, constituting a strong and separate line of defense against cancer (Prieur and Peeper, 2008). OIS operates during early tumorigenesis and involves the activation of several tumor suppressor networks, including the $p16^{INK4A}$/pRb and ARF/p53 pathways. They keep incipient tumor cells in check and thereby prevent them from progressing toward malignancy.

Shamma et al. (2009) studied the progression of murine *Rb*-deficient C cell adenoma to adenocarcinoma. As already mentioned, this group had shown previously that, whereas loss of both *Rb* alleles is required for onset of the adenomas, inactivation of both *N-ras* alleles causes malignant conversion and metastasis. In their current paper, they show that loss of N-Ras expression is associated with increased proliferative activity. A steep drop in several DNA damage-associated specific phosphorylation events involving histone H2AX, ATM kinase, and p53 accompanied the conversion from adenoma to adenocarcinoma. Interestingly, *N-ras*-proficient (*Rb*-deficient) adenomas displayed several markers of cellular senescence such as elevated expression of histone H3 trimethylated at lysine 9 (H3K9me3), heterochromatin protein 1γ (HP1γ), and $p16^{Ink4a}$ and increased senescence-associated β-galactosidase activity. These markers were

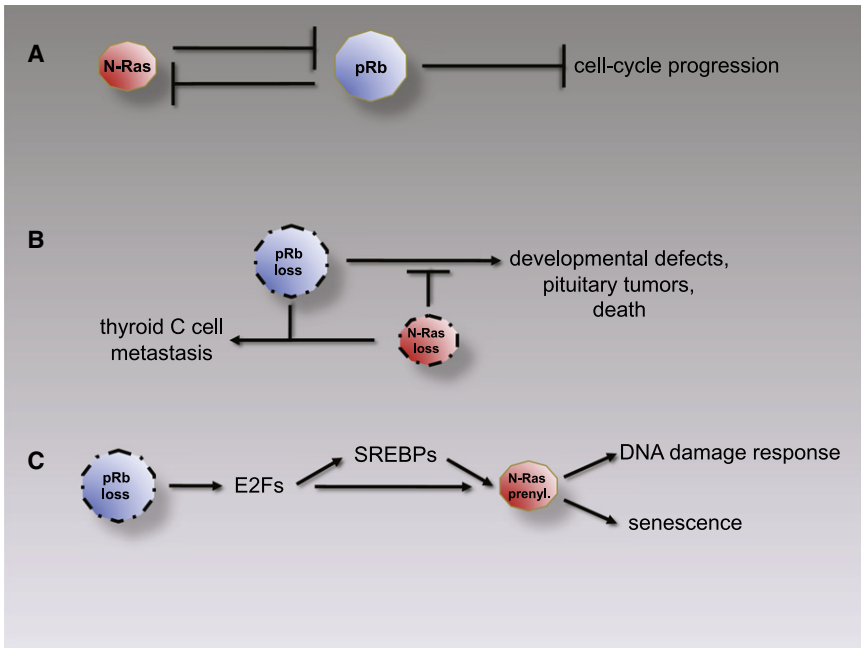

Figure 1. Interactions between N-Ras and pRb in Cell-Cycle Control, Development, Senescence, and Cancer

(A) Ras proteins, including N-Ras, and pRb communicate during cell-cycle progression through mutual negative feedback loops. Ras activation leads to pRb inactivation in a cyclin D-dependent manner. Conversely, *Rb* deficiency results in elevated Ras activity.

(B) In vivo, loss of *N-ras* rescues developmental defects resulting from *Rb* deficiency, thereby extending life span. At the same time, *N-ras* loss reduces aggressiveness of pituitary tumors. In contrast, combined deficiency for *N-ras* and *Rb* results in metastatic thyroid C cell adenocarcinomas.

(C) Shamma et al. (2009) demonstrate the existence of a pathway from pRb back to N-Ras, in which the latter is activated upon loss of the first through E2F- and SREBP-dependent stimulation of prenylation. Subsequently, activated N-Ras triggers a DNA damage response and cellular senescence, thereby blocking C cell carcinogenesis driven by *Rb* deficiency.

largely absent in proliferating *N-ras*-deficient adenocarcinomas.

OIS is sometimes associated with DNA hyperreplication and a DNA damage response (DDR) (Campisi and d'Adda di Fagagna, 2007). However, the relative kinetics of these processes are largely unclear. Shamma et al. effectively exploited the opportunity offered by their model system to look into this important issue. Monitoring various markers in early (6 months) and late (11 months) adenomas, they observed little difference in the expression patterns of DNA damage markers. In contrast, senescence markers were expressed at lower levels in early adenomas than in late adenomas. As expected, this was inversely correlated with the adenomas' proliferative capacity. A likely interpretation of these results is that, at least in this experimental model, a DDR is insufficient (or perhaps not even required) to boost a cytostatic response, and it is the senescence program that is responsible for the cell-cycle arrest of these adenomas. Shamma et al. confirmed the latter idea by a series of genetic inactivation experiments demonstrating a causal role for the senescence-associated genes *Ink4a*, *Arf*, and *Suv39h1* in antagonizing *Rb*-deficient C cell carcinogenesis.

These results suggest a model in which N-Ras proteins, in response to *Rb* deficiency, protect C cells from progressing to malignancy. This predicts that N-Ras somehow affects cell-cycle progression. Indeed, when Shamma et al. restored N-Ras expression in *Rb*/*N-ras*-deficient thyroid tumor cells established in culture, they elicited both a DDR and a senescence response. Reconstituted expression of pRb abolished the ability of N-Ras to induce senescence.

How, then, is N-Ras activated in response to pRb loss? The investigators noted that when reintroduced into these cells, pRb suppressed transcription of farnesyl diphosphate synthase (*Fdps*) and sterol regulatory element-binding protein (SREBP)-encoding genes as well as several of their effector genes, including prenyltransferases, in an E2F-dependent fashion. Correspondingly, SREBP-1 and -2 expression was increased in murine C cell adenomas. In keeping with this, it was shown that pRb deficiency increased N-Ras isoprenylation under certain conditions, leading to its activation and subsequent induction of senescence. Confirming this model, an isoprenylation-deficient N-Ras mutant failed to trigger a DDR and senescence, whereas treatment with prenyltransferase inhibitors suppressed these responses. Extrapolating these results to the human situation, negative immunostaining for pRb coincided with low levels of N-Ras in several sporadic medullary thyroid carcinomas, consistent with a requirement of N-Ras suppression during tumor progression initiated by *Rb* loss.

The work of Shamma et al. unmasks an original mechanism for the functional interaction between N-Ras and pRb in the context of a tumor progression model and resolves a signaling network in which genetic inactivation of *Rb* releases E2Fs to induce a wave of farnesylation. Modified proteins include N-Ras, which as a result mounts robust DNA damage and senescence responses (Figure 1C). These results also explain why selection can occur against *N-ras* in *Rb*-deficient tumors and, as such, offer a rationale for the previously noted tumor suppressor function of Ras (Zhang et al., 2001 and references therein). Whether these results also bear on novel clinical entry points, as the authors predict, remains to be determined.

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Unraveling the Complexities of Androgen Receptor Signaling in Prostate Cancer Cells

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Androgen signaling is critical for proliferation of prostate cancer cells but cannot be fully inhibited by current androgen deprivation therapies. A study by Xu et al. in this issue of *Cancer Cell* provides insights into the complexities of androgen signaling in prostate cancer and suggests avenues to target a subset of androgen-sensitive genes.

Prostate cancer (PCa) is the most frequently diagnosed cancer and the second leading cause of cancer-related death in Western men. Since the recognition of PCa as an androgen-sensitive disease in 1941, androgen deprivation strategies have been the principal treatment option for non-organ-confined PCa or PCa that recurs after initial surgery or radiation therapy. Androgen deprivation therapies (ADTs) target the action of the androgen receptor (AR), the transcription factor mediating the cellular effects of androgens, by reducing the circulating levels of its natural ligands and/or by administration of antiandrogens that compete for binding to the AR. Following initial remission, most PCas recur after ADT, giving rise to castration-recurrent PCa (CRPC), which is almost invariably lethal. Evidence from basic research and clinical studies indicates that the AR and AR-dependent transcriptional program remain activated in CRPC (Chen et al., 2008; Debes and Tindall, 2004; Mohler, 2008). This unexpected reactivation of the AR in CRPC, which highlights its validity as a therapeutic target also in CRPC, has been attributed to AR amplifications, gain-of-function mutations of the AR, and changes in the activity of regulators and signal transduction pathways that modulate AR activity. More recently, findings of local, intratumoral production of androgens in CRPC at levels sufficient to activate the AR have led to therapeutic approaches targeting in situ androgen biosynthesis (Attard et al., 2008; Mohler, 2008). While initial results from such clinical trials are encouraging, they also underscore the continued reliance of PCa on AR activation and the resourcefulness of PCa cells in evading ADT strategies. In-depth knowledge of the molecular mechanisms by which the AR regulates transcription of target genes that are critical to PCa disease may offer the rationale to design therapeutic alternatives targeting this critical regulator of PCa cell growth.

The AR is a member of the nuclear receptor superfamily of ligand-activated transcription factors. Its mechanism of action resembles that of other members of the steroid hormone receptor family. In the classical model of AR action, binding of androgens causes an inactive cytoplasmic AR to undergo a change in conformation, homodimerization, and relocation to the nucleus. There, the activated AR binds to specific recognition sequences known as androgen-responsive elements (AREs) located in or near androgen-regulated genes where it recruits the coregulators and the basal transcriptional machinery necessary to assemble a productive transcriptional complex and ultimately affect the transcription of target genes (Heemers and Tindall, 2007).

Over a decade of screening has identified approximately 200 proteins that interact with the AR and collaborate with it to execute its transcriptional program. At the same time, systems and bioinformatics approaches have identified hundreds of androgen-regulated genes and characterized genome-wide AR recruitment sites in PCa cells. The combined knowledge gained from these studies is starting to reveal a picture of daunting complexity underlying the activity of the AR transcriptional complex that far exceeds the simplicity of traditional models of androgen action.

Apart from general transcription factors, proteins recruited to DNA-bound AR can be divided into two classes: coregulators and specific transcription factors that interact with their consensus binding elements (Heemers and Tindall, 2007). The former class consists of proteins that associate either directly or indirectly with