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Treating cancer when pRb and p53 cannot be reactivated

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Activation of oncoproteins and inactivation of tumor suppressors induces tumorigenesis. When these events happen upstream of pRb and p53, cancer therapies may initially succeed and then fail when pRb and p53 are activated and then re-inactivated. Therapies might succeed if they remain effective when pRb and p53 are genetically inactivated.

To conquer cancer is first and foremost to understand cancer. Although cancer researchers have solved a significant amount of the cancer puzzle (**Fig. 1A**) the puzzle as a whole has not been solved and most pieces are themselves puzzles. In the meantime, these puzzle pieces have guided the development of modern cancer therapeutics.

As one example, following the determination that the BRAF kinase in the "kinase networks" piece is frequently activated by the V600E mutation,[1] inhibitors for $BRAF^{V600E}$ were developed and showed remarkable effectiveness for patients with metastatic melanoma containing $BRAF^{V600E}$, but not for those without $BRAF^{V600E}$.[2] This exciting success provided the ultimate confirmation of the "driver" role of $BRAF^{V600E}$. $BRAF^{V600E}$ also exists in many nevi (up to 82%),[3] but most nevi remain as nevi for decades, exhibiting features of cellular senescence.[4] Thus, $BRAF^{V600E}$ by itself is unable to induce melanoma. Furthermore, effective $BRAF^{V600E}$ inhibitors quickly lose their effectiveness.[5] One could therefore ask the question, "How do $BRAF^{V600E}$ inhibitors produce their initial therapeutic benefits?"

Based on current literature, I suggest that oncogenic activation of BRAF by the V600E mutation in the "kinase networks" puzzle piece (**Fig. 1A**) might first activate the INK4A (also called $p16^{INK4A}$)-cyclin D1/Cdk4-pRb pathway, the ARF (also called $p14^{ARF}$)-MDM2/MDM4-p53 (also called TP53) pathway, or both pathways (**Fig. 1B**). The effectors of these 2 pathways, pRb and p53 respectively, are the 2 major tumor suppressors that together implement the most and the best antitumor mechanisms, such as cell cycle arrest (sometimes to the extreme of cellular senescence), cell death, and emerging mechanisms in cell metabolism, stemness, and epithelial or mesenchymal identity. These effects could prevent $BRAF^{V600E}$ from transforming cells.[4] Ensuing factors, many still undefined, might inactivate pRb, p53, or both (**Fig. 1C**), allowing tumorigenesis to progress. Inhibition of $BRAF^{V600E}$ in this context halts the mechanisms that inactivate pRb and/or p53, leading to their reactivation (**Fig. 1B**) to halt the cancer. Combining $BRAF^{V600E}$ inhibitors with inhibitors that target other kinases that interact with $BRAF^{V600E}$, or with inhibitors that directly reactivate the INK4A-cyclin D1/Cdk4-pRb pathway, the ARF-MDM2/MDM4-p53 pathway, or both pathways, could improve effectiveness and delay or overcome resistance until disease progression to genetic inactivation of pRb and/or p53. When genetically inactivated by DNA sequence deletions, insertions, or mutations in *RB1* and *TP53* (the genes encoding pRb and p53, respectively), pRb and p53 can no longer be reactivated (**Fig. 1D**).

The database of TCGA contains data showing frequencies of genetic inactivation of pRb and p53 in various cancer types. For urothelial bladder cancer, genetic inactivation of pRb and p53 co-occurred in 15% of 125 specimens. Prostate cancer progression from adenocarcinoma at primary sites to metastatic cancer at remote sites correlated with an increase in the proportion of cases containing genetic inactivation of both pRb and p53, from 1% to 18%. Thus, a significant number of cancers, especially late-stage cancers, have permanently lost the antitumor mechanisms provided by pRb and p53.

To determine the consequences of genetic inactivation of pRb and p53 in cancer therapy, Zhao et al. used *Skp2* deletion to inhibit pRb and p53 double knockout (DKO) tumorigenesis in mouse models.[6] Skp2 is best known as an E3 ubiquitin ligase for p27, and previous studies showed that *Skp2* deletion blocked *Rb1* (mouse homolog to *RB1*)-deficient,[7] *Arf*-, or *Pten*-deficient tumorigenesis.[8] Zhao et al. reported that p53 is a transactivator for the promoters of Pirh2 and KPC1 p27 ubiquitin ligases. Consequently, combined deletion of *Trp53*

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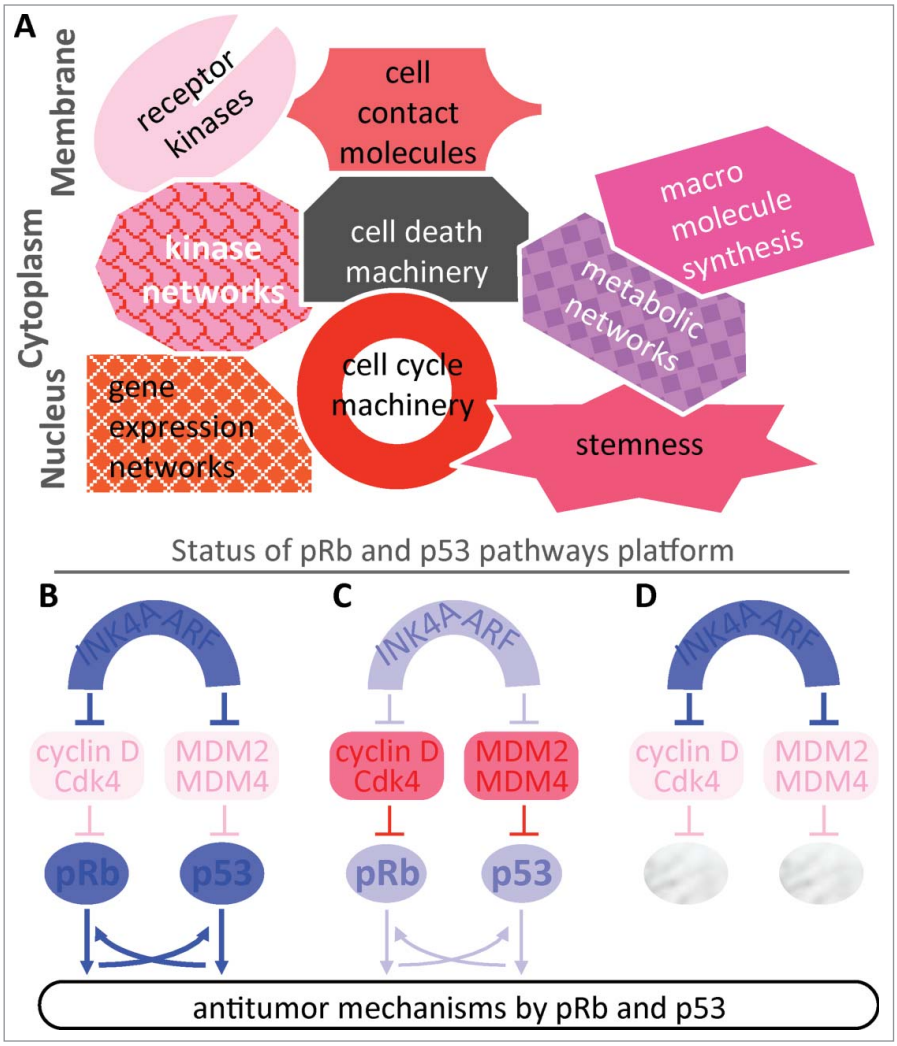

Figure 1. Current landscape of cancer therapy. (**A**) Cancer puzzle pieces as therapeutic targets. Major cancer puzzle pieces are organized according to their cellular location and their interplay as "work-in-progress." (**B–D**) Status of pRb and p53 pathway platforms. The first component in the pathways is encoded by an INK4A/ARF hybrid gene *CDKN2A*. Blue indicates tumor suppressive and red indicates oncogenic. Darker colors indicate stronger functions than lighter colors. Thus, (**B**) shows an active pRb and p53 platform; (**C**) shows functionally inactivated pRb and p53; and (**D**) shows genetically inactivated pRb and p53. See text for a full description of antitumor mechanisms of pRb and p53.

(mouse homolog of *TP53*) and *Skp2* increased p27 protein to much higher levels than could be achieved by *Skp2* deletion or *Trp53* deletion alone. The higher level of p27 resulted in activation of pRb, most likely by inactivating various cyclin-dependent kinases to reduce phosphorylation of pRb. Activated pRb inhibited DNA synthesis to the extreme of cellular senescence. After additional deletion of *Rb1*, DNA synthesis could not be inhibited. However, a high level of p27 was able to inhibit mitotic division independent of *Rb1* to block pRb and p53 doubly deficient tumorigenesis in the pituitary (no microscopic tumors) and prostate (no lesions beyond PIN), coexistent with ongoing DNA synthesis in the form of DNA re-replication. Apoptosis is also observed in DKO prostate tumorigenesis. In another study, deletion of *Rb1*, *Rbl1* (also called p107), and *miR-17-92* in mouse retina resulted in synthetic lethality.[9] When combined with *Skp2* deletion, *Rb1* deletion triggered the p53-independent apoptotic activity of E2F1.[10] Thus, a high level of p27 and apoptotic E2F1 could remain effective in blocking mitotic division and inducing apoptosis, respectively, in the absence of pRb and p53.

Based on these data, we envision the discovery of new therapeutics that remain effective when pRb and p53 are genetically inactivated. Could blocking the interaction of Skp2 with p27 by small-molecule inhibitors reproduce the tumor-blocking effects of *Skp2* deletion? Increased p27 protein levels following deletion of *Skp2* can relieve cyclin A repression of E2F1 on E2F target promoters, and one of the targets of miR-17-92 is E2F1 mRNA. Could small molecules be designed that produce these effects in cancer cells? Favoring cancer cells is that fact that, although *Rb1* and *Trp53* deletions are definitive, the additional oncogenic events that lead to DKO tumorigenesis vary and DKO prostate cancer can develop from only 1-2 focal lesions. It is possible that the nature of these additional events varies more widely in human cancer and thus will blunt the mechanisms that we have uncovered in mouse models. In short, will antitumor mechanisms that remain effective when pRb and p53 are genetically inactivated provide the ultimate rationale for cancer therapy? Only search and research will provide the answer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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