IN THE SPOTLIGHT

Drugging RB1 Deficiency: Synthetic Lethality with Aurora Kinases

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Summary: Two recent reports describe pharmacologic approaches to specifically treat RB1-mutant cancers. The basis of this approach is a synthetic lethal relationship between RB1 mutations and inhibition of Aurora kinases A or B.

See related article by Oser et al., p. 230 (1).
See related article by Gong et al., p. 248 (2).

Targeted cancer therapies, directed against specific oncogenic driver mutations, have been successfully deployed in the clinic. These agents are inhibitors of enzymes that are mutationally activated and directly antagonize the underlying molecular etiology of the cancer. They often cause rapid regression of cancer with limited side effects, and for this reason have become first-line treatment options for many patients. A conceptual challenge in broadening this approach is the relative scarcity of cancers with actionable driver mutations. Frequent loss-of-function mutations in tumor suppressor genes such as RB1 and TP53 have historically been untargetable. The best example of targeting a cancer-specific vulnerability that is created through tumor suppressor gene loss is that of BRCA1 mutations and PARP inhibitors. Loss of BRCA1 compromises DNA damage repair through the homologous recombination pathway and creates a dependence on nonhomologous end joining (NHEJ) to repair DNA breaks. Consequently, inhibition of NHEJ by PARP inhibitors is specifically toxic to BRCA1-mutant cancer cells. Two articles in this issue of Cancer Discovery describe agents and their molecular targets for a similar synthetic lethal approach to treating RB1-deleted cancers (1, 2).

APPROACHES TO TARGETING DEFECTIVE RB1 FUNCTION IN CANCER

Loss of function of the RB1 pathway is a common event in cancer (3). Typically, activating mutations in G1 cyclin-dependent kinase (CDK) complexes phosphorylate and inactivate the RB1 protein. Alternatively, inactivating mutations of CDK inhibitors, such as CDKN2A, can have a similar effect on activation of G1 CDKs, leading to constitutively phosphorylated RB1. Direct mutation of the RB1 gene itself is less common than upstream RB1 pathway mutations. RB1 deletion is common in retinoblastoma and small cell lung cancer (SCLC), and deep deletion of RB1 is found sporadically in most disease sites (1, 2). Loss of RB1 function creates a number of distinct but interrelated cellular phenotypes including deregulated proliferation, altered metabolism, and genome instability. Each of these phenotypes can be induced by RB1 pathway mutations leading to hyperphosphorylated RB1 and deregulated E2F transcription. Using fruit fly genetics and mammalian cell culture, Du and colleagues have identified synthetic lethal connections between RB1 pathway disruption and mutation of TSC2 and TORC1, as proliferation and stress from disrupted energy metabolism are incompatible (4). Approaches to exploit misregulated E2F transcription and its effects on mitochondrial biogenesis and function have also been suggested to create an RB1 pathway–dependent vulnerability in cancer. This has been noted by studies that observed synergy between CDK4/6 inhibitors and inhibitors of PI3K, IGF1, and others (4). Efforts to find compounds that selectively kill or inhibit proliferation of RB1 pathway–disrupted cancer cells have also identified PLK1 and CHK1 as targets (5). This study identified misregulated transcription as fueling replication stress and mitotic checkpoint abnormalities that create potentially targetable vulnerabilities.

In a related but distinct manner, rational approaches to treating retinoblastoma have been developed. A genomic study suggested that epigenetic alterations occur in response to RB1 loss (6). This study highlights elevated expression of SYK and demonstrates that SYK inhibitors can slow progression in a patient-derived xenograft model. In a similar effort, Sangwan and colleagues identified deregulated E2F transcription and abnormal CDK2 activity as downstream consequences of RB1 mutation in a mouse model of retinoblastoma (7). Pretreatment of pregnant murine mothers was shown to prevent retinoblastoma formation in offspring, suggesting a means by which targeting E2Fs and CDK2 can offer a cancer prevention approach. Collectively, these studies highlight ongoing efforts to exploit RB1 loss in new cancer treatments.

IDENTIFICATION OF AURORA KINASES FOR TREATING RB1-DEFICIENT CANCERS

As highlighted above, there are many similarities between RB1 pathway mutations that misregulate CDKs and genetic
Figure 1. Model of synthetic effects of RB1 deletion and Aurora kinase inhibition. **A,** In cells with a functional RB1 gene and Aurora A and B kinases, normal mitotic progression and checkpoints ensure high fidelity of chromosome separation and cell division. **B,** In the absence of RB1, chromosome condensation and cohesion defects lead to misshapen centromeres, misaligned chromosomes in metaphase, syntelic and merotelic microtubule attachments, and an activated spindle assembly checkpoint. In this circumstance, defective mitosis is tolerated, in part, through augmented activity of Aurora kinases. **C,** When RB1 is deleted and Aurora A or B kinase is inhibited pharmacologically, cells attempt to transit mitosis, but fail to enter anaphase and rapidly undergo apoptosis. **D,** Xenografted SCLC tumors treated with Aurora kinase inhibitors display evidence of mitotic catastrophe and regress in response to treatment.
deletion of the RB1 gene. Remarkably, the studies by Oser and Gong in this issue identify vulnerabilities that are specific for RB1 gene deletion (1, 2). This emphasizes that loss of RB1 causes additional targetable vulnerabilities beyond those that are created by CDK misregulation. CDK-independent noncanonical functions of RB1 in mitosis and chromosome structure have been reported, but remain relatively unexplored (3). In the article by Gong and colleagues, the authors devise a synthetic lethal screen approach in which a panel of cell lines with known genetic alterations is challenged by a collection of 36 targeted inhibitors, and agents that cause cytotoxicity are selected for further evaluation. This approach specifically identified RB1-mutant cell lines, but not those with RB1 pathway mutations, as being sensitive to compounds known to target functions in mitosis. In particular, compounds that inhibit Aurora kinases A and B had a much more pronounced effect than others in this mitotic category. Oser and colleagues searched for synthetic lethal vulnerabilities by devising a cell line system in which an RB1-mutant SCLC line, NCI-H82, was used. Conditional reexpression of RB1 in these cells did not inhibit proliferation, and as a consequence the authors searched for RB1-dependent vulnerabilities using a CRISPR/Cas9 screen approach in which they focused on hits from non-RB1-expressing cells compared with those that reexpress RB1. They used a custom gRNA library that was enriched for cell-cycle, epigenetic, and cancer-related genes, and their screen identified Aurora kinase B (the library did not contain guides to Aurora A).

Both teams utilized highly specific inhibitors for Aurora A or B in their experiments and Gong and colleagues also described the generation of a novel Aurora kinase A–specific compound. Aurora kinase A is best known for its role in centrosome separation and mitotic spindle assembly, whereas Aurora kinase B coordinates microtubule attachments to centromeres and phosphorylates histone H3 in mitosis (8). Because Aurora kinase A can phosphorylate and activate B, it is possible that some interdependence of synthetic lethality exists. For this reason, it is important that both research teams generated drug-resistant mutant forms of their respective kinases to demonstrate on-target dependence of synthetic lethality with RB1 deletion.

It is difficult to conceptualize precisely why Aurora kinase inhibition is synthetically lethal with RB1 deficiency, and this will be an important area of future research. However, we envision the model in Fig. 1 to represent a possible explanation. In normal cells, RB1 contributes to mitotic fidelity through mechanisms that are both E2F-dependent and related to coordinating Cohesin and Condensin II function (4, 9). Aurora kinases also contribute to cell-cycle progression and mitotic fidelity through centrosomes, mitotic spindle formation, and chromosome attachment and separation (8). This suggests mitotic fidelity is simultaneously dependent on all of RB1, Aurora A, and Aurora B kinases (Fig. 1A). In RB1-deleted cells, the centromere structure and function is compromised, leading to misaligned chromosomes in mitosis as well as syntelic and merotelic chromosome attachments. Through an E2F-dependent effect of RB1 loss, numerous components of the spindle assembly checkpoint become overexpressed, leading to a hyperactivated checkpoint (10). Investigation of Aurora kinase expression and function in the current studies suggests that both Aurora A and B kinase activity and function are augmented in an RB1-deficient background, suggesting these kinases offer some compensation for effects caused by RB1 loss (Fig. 1B). Finally, when Aurora A or B kinases are inhibited, centrosome separation and initial spindle formation appear intact (1, 2), but the formation of a metaphase plate is difficult to observe and chromosomes disburse quickly followed by apoptosis (Fig. 1C). The distinct nature of Aurora A and B inhibitors, and the roles of these kinases in mitosis, suggests that slightly different effects on mitosis can elicit this synthetic lethal effect. Both groups sought further explanations for the mechanism through screening approaches. Oser and colleagues point out that there are also synthetic lethal hits with components of Condensin II and other mitotic components such as PLK1 and INCENP. Gong and colleagues carried out an shRNA loss-of-function screen to identify genes that rescue synthetic lethality between RB1 mutation and Aurora kinase A inhibitors. They identified mitotic checkpoint components BUB1B and BUB3 as essential for synthetic lethality, further underscoring the importance of a hyperactivated spindle assembly checkpoint. These findings collectively emphasize that RB1 loss and Aurora kinase inhibition converge on mitotic progression and fidelity.

PRECLINICAL EVALUATION OF AURORA A AND B KINASE INHIBITORS

The study led by Gong and colleagues from Eli Lilly developed and tested a lead compound, LY3295668, that they demonstrate is highly specific for Aurora A. Oser and colleagues make use of AZD2811, an inhibitor of Aurora B that was developed by AstraZeneca. Both groups tested these compounds in multiple cancer models ranging from patient-derived xenografts of SCLC to cell lines, and a genetically engineered mouse model. The inhibitors induced tumor regression in most cases and outperformed the standard-of-care for SCLC where evaluated. Oser and colleagues investigated the mechanism of action through histologic analysis of tumors from treated mice, and confirmed that treatment induced mitotic catastrophe in vivo (Fig. 1D). Historically, experimental treatment using Aurora kinase inhibitors has caused bone marrow toxicity that has limited dosing of these agents and compromised their performance in clinical trials. AZD2811 efficacy was greatly enhanced by new delivery using encapsulated nanoparticles, suggesting that past limitations with this class of inhibitor can be overcome. In addition, LY3295668 was tested for toxicity by culturing with bone marrow cells, which indicates that effective treatment doses with this new compound have limited toxicity. On the basis of these results, Eli Lilly and AurKa have initiated a phase I/II clinical trial for RB1-deficient solid tumors (NCT03092934).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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