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Restoration of tumor suppressor functions by small-molecule inhibitors

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Abbreviations: BIN1, bridging integrator-1; BRCA1/2, breast cancer 1 and 2; CDK, cyclin-dependent kinase; CDKN, cyclin-dependent kinase inhibitor; HDM2, human homolog of double minute 2; PAR, poly(ADP-ribosyl)ation; PARP1, poly(ADPribose) polymerase 1; RB1, retinoblastoma 1 protein.

Over the last decades, accumulating data have advanced our understanding of the mechanism of action of tumor suppressor proteins and therapeutic strategies to restore tumor suppressor pathways have emerged as a promising approach for cancer therapy. Based on our recent findings on bridging integrator-1 (BIN1), we outline potential advantages and disadvantages of chemical activation of tumor suppressors.

Small-molecule inhibitors continue to be at the leading edge of cancer therapeutics. The discovery of Gleevec (STI-571), a tyrosine kinase inhibitor, was a milestone achievement in clinical oncology and this inhibitor has demonstrated remarkable efficacy in Philadelphia chromosome-positive (Ph^+) chronic myeloid leukemia.[1] Since then, mechanism-based approaches have been used to specifically target various kinases and/or downstream oncogenic pathways that are critically involved in cell cycle progression and tumorigenesis. However, in addition to this approach, a more recent and novel use of small-molecule inhibitors has emerged as a promising endeavor in the field of cancer chemotherapy. Here, we briefly review the mechanistic basis of restoration of a tumor suppressor and its potential complications for cancer therapy.

The tumor suppressor function mediated by the retinoblastoma 1 protein (RB1) is principally attributed to its interaction with the E2F transcription factor 1 (E2F1). The RB1/E2F1 complex represses a number of E2F1-dependent transcriptional target genes that are required for the transition from G_1 to S phase in the cell cycle. Because RB1 is inactivated by phosphorylation mediated by the G_1 cyclin-dependent kinases 4 and 6 (CDK4 and CDK6), restoring RB1 function by inactivating CDK4/6 is theoretically an obvious approach. Although structural similarities among a number of CDK family members hampered the development of a CDK4/6-specific inhibitor for many years, some agents, including palbociclib (PD-0332991), have recently demonstrated promising results in Phase I/II clinical trials for human malignancies, including breast cancer.[2]

Above and beyond RB1, another tumor suppressor that is critical for numerous growth inhibitory pathways is tumor protein p53 (TP53, best known as p53). The abundance of wild-type p53 protein is massively reduced as a result of ubiquitin-dependent and human homolog of double minute 2 (HDM2)-mediated degradation of p53. Therefore, dissociation of p53 from the p53/HDM2 complex is a reasonable strategy for rescuing p53 function. Based on the crystallographic structure of the p53/HDM2 peptide complex, small p53 peptides that mimic the region of p53 sufficient for HDM2 binding and small-molecule HDM2 antagonists have been shown to disrupt the p53/HDM2 interaction *in vitro* and *in vivo*. Some of these, including MI-219, Nutlin-3, and RG7112, have been found to be effective preclinically and have consequently moved into Phase I/II clinical trials.[3] Although proteasome inhibitors such as bortezomib (PS-341) may not be as specific for stabilizing p53 as these HDM2 inhibitors, other growth-inhibitory gene products, including the cyclin-dependent kinase inhibitor 1B (CDKN1B or p27, Kip1) protein, can also be degraded in an ubiquitin-dependent manner.[4] Therefore, it may be advantageous to re-establish a broad spectrum of growth-inhibitory functions by blocking the proteasome pathway.

Although the approach of re-establishing tumor suppressor function in tumors as a therapeutic option is mechanistically intriguing, there are potential dilemmas associated with the systemic restoration of tumor suppressor function. Tumor suppressor genes are frequently mutated or

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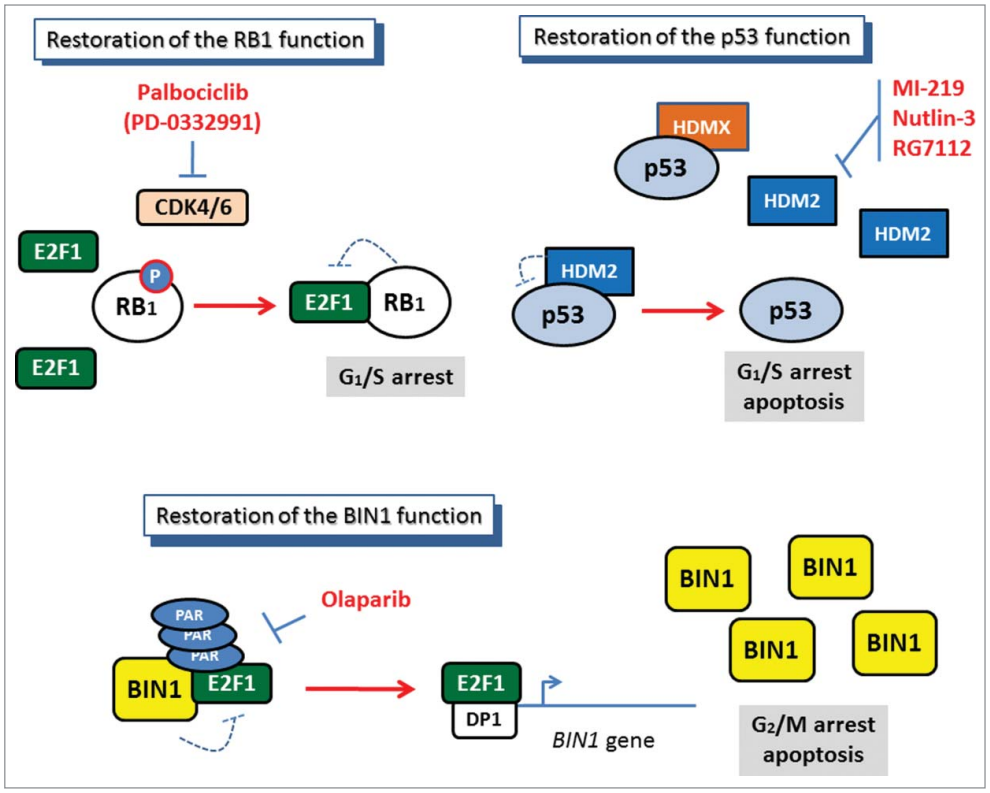

Figure 1. Small-molecule inhibitors used in cancer therapy restore the functions of various tumor suppressors in malignant cells. The tumor suppressor functions of retinoblastoma 1 protein (RB1), tumor protein p53 (TP53, known as p53), and bridging integrator-1 (BIN1) can be pharmacologically restored by small-molecule inhibitors, such as palbociclib (PD-0332991), MI-219, and olaparib, respectively. In the case of p53 restoration, a human homolog of double minute 2 (HDM2), named HDM4 (also known as HDMX), might interfere with the effectiveness of HDM2 antagonists, probably because of overlapping functions between HDMX and HDM2. P, phosphorylation; PAR, poly(ADP-ribosyl)ation; DP1 (or TFDP1), transcription factor DP1; E2F1, E2F transcription factor 1; CDK4/6, cyclin-dependent kinase 4 and 6; G_1/S: transition between G_1 (growth1/gap1) phase to S (DNA synthesis) phase in the cell cycle; G_2/M, transition between G_2 (growth2/gap2) phase to M (mitosis) phase in the cell cycle.

deleted in cancer patients, and given that some of the mutant genes acquire oncogenic potential, this approach may simply reboot a mutant (i.e., oncogenic) tumor suppressor. Even if a tumor suppressor gene is intact, its function should not depend on other cancer-susceptible proteins. For example, the *cyclin-dependent kinase inhibitor 2A* (*CDKN2A*) gene is not frequently deleted in cancer cells, but is inactivated by DNA methylation. However, epigenetic reactivation of the *CDKN2A* gene may not be an effective approach if RB1 and/or p53 are deficient, because the tumor suppressor functions of the products of the 2 alternative reading frames of *CDKN2A*—$p16^{INK4A}$ and $p14^{ARF}$ proteins—largely depend on RB1 and p53, respectively.[5] Therefore, for the tumor suppression approach to be fully effective, it will be important to identify a non-mutated (or non-deleted) tumor suppressor whose function does not rely on other tumor suppressors that might be already mutated or deleted.

Bridging integrator-1 (BIN1) was originally identified as a c-MYC oncoprotein-interacting tumor suppressor.[6] The *BIN1* gene itself is rarely mutated or deleted, but is frequently silenced in human cancer cells. Moreover, BIN1 acts as a tumor suppressor *in vitro* and *in vivo* in the absence of RB1 and p53.[7] We recently demonstrated that BIN1, whose gene promoter is activated by E2F1, directly interacts with E2F1 and represses its transcription, implying that a negative-feedback loop regulates *BIN1* gene expression.[8] Interestingly, we found that E2F1 is poly(ADP-ribosyl)ated by poly(ADP-ribose) polymerase 1 (PARP1) and that PARP1 inhibition unlocks the E2F1–BIN1 negative-feedback loop to vigorously activate the *BIN1* gene, which induces G_2/M arrest in the cell cycle and/or apoptosis.[8] Because of this so-called 'synthetic lethality,' PARP inhibitors have been actively used for clinical trials in breast cancer 1 and 2 (BRCA1/2)-deficient breast and ovarian cancers.[9] However, it was unclear why PARP inhibitors alone also show therapeutic efficacy, even in cancer cells expressing wild-type BRCA1/2. Based on our recent data,[8] the restoration of BIN1 by PARP inhibitors may offer a mechanistic rationale for expanding the clinical usage of PARP inhibitors over a wider range of tumor types, regardless of the status of *RB1*, *TP53*, and *BRCA1/2* genes (**Fig. 1**).

Chemotherapy and radiotherapy are conventional treatments for eradicating tumors, but cancer often develops therapeutic resistance over time. Given that many tumor suppressors are proapoptotic in response to DNA damaging agents, it would be clinically pertinent to increase the chemo- and radiosensitivities of cancer by combining standard treatments with agents that can restore the activity of silenced tumor suppressors, provided they are not mutated or deleted, in human malignancies.[10]

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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