The Biology Behind

Cancer Chemoprevention by Targeting Proteasomal Degradation


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Cyclin D1 and other cyclins activate cyclin-dependent kinases to promote cell growth, and their overexpression has been associated with cell transformation and tumorigenesis (1, 2). In this issue of Clinical Cancer Research, Dragnev et al. (3) report that promoting proteasomal degradation of cyclin D1 and cyclin E, which results in cell cycle arrest at the G1 phase, is a mechanism of cancer chemoprevention by all-trans-retinoic acid (RA) and some other structurally unrelated agents. This research group has previously shown that RA prevents carcinogenic transformation of immortalized human bronchial epithelial cell line BEAS-2B by causing G1 cell cycle arrest and triggering cyclin D1 degradation via the ubiquitin-proteasome pathway (4–6). In the present study, the authors demonstrate further that cyclin E is also targeted for degradation by RA treatment. Treatment of BEAS-2B cells with N-(4-hydroxyphenyl) retinamide (4HPR), a nonclassical retinoid and 2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid (CDDO) also results in increased proteasomal degradation of G1 cyclins and cell growth suppression. The degradation is inhibited by a proteasomal inhibitor, N-acetyl-leucyl-leucyl-norleucinal (ALLN). However, RO-24-5531 (a vitamin D analog), resiglitazone (a peroxisome proliferator-activated receptor γ agonist), and indomethacin (a cyclooxygenase inhibitor) have no effect on cyclin levels, even though they inhibit cell growth. A variant BEAS-2B-R1 cell line, which is partially resistant to RA, remains responsive to 4HPR and CDDO with respect to inhibition of cell growth and promotion of G1 cyclin degradation.

Phosphorylation of the retinoblastoma tumor suppressor protein (Rb) is crucial for the progression of the cell cycle from G1 into S phase. (Fig. 1). This phosphorylation releases Rb-mediated inhibition of E2F transcription activity, which is necessary for the transcription of genes essential for entry into S phase. The phosphorylation state of Rb, a process governed by the cyclin D and E families together with their respective phase. The phosphorylation state of Rb, a process governed by essential for the transcription of genes essential for entry into S phase. The phosphorylation state of Rb, a process governed by essential for the transcription of genes essential for entry into S phase. The phosphorylation state of Rb, a process governed by essential for the transcription of genes essential for entry into S phase. The phosphorylation state of Rb, a process governed by essential for the transcription of genes essential for entry into S phase. The phosphorylation state of Rb, a process governed by essential for the transcription of genes essential for entry into S phase. The phosphorylation state of Rb, a process governed by essential for the transcription of genes essential for entry into S phase. The phosphorylation state of Rb, a process governed by essential for the transcription of genes essential for entry into S phase. The phosphorylation state of Rb, a process governed by essential for the transcription of genes essential for entry into S phase. The phosphorylation state of Rb, a process governed by essential for the transcription of genes essential for entry into S phase. The phosphorylation state of Rb, a process governed by essential for the transcription of genes essential for entry into S phase. The phosphorylation state of Rb, a process governed by essential for the transcription of genes essential for entry into S phase. The phosphorylation state of Rb, a process governed by essential for the transcription of genes essential for entry into S phase. The phosphorylation state of Rb, a process governed by essential for the transcription of genes essential for entry into S phase. The phosphorylation state of Rb, a process governed by essential for the transcription of genes essential for entry into S phase. The phosphorylation state of Rb, a process governed by essential for the transcription of genes essential for entry into S phase. The phosphorylation state of Rb, a process governed by essential for the transcription of genes essential for entry into S phase. The phosphorylation state of Rb, a process govern by the cyclin E-dependent kinases followed by the cyclin E-dependent kinases seems to be necessary for the orchestrated phosphorylation of Rb and derepression of E2F-dependent genes. The present work shows convincingly that RA induces degradation of cyclins D1 and E, but the mechanism is not known. This research group has previously identified UBE1L, an E1-like ubiquitin-activating enzyme, as an RA target gene in promyelocytic leukemia cells, and the induction of UBE1L triggers retinoic acid receptor α degradation (7). It is not known whether the degradation of other proteins is also enhanced in the presently studied BEAS-2B cells.

Other investigators have reported that CDDO activates peroxisome proliferator-activated receptor γ and causes mammary cancer cell growth arrest (8). It also induces proteasomal degradation of cyclin D1 protein and enhanced p21WAF1 expression (8); both events may be important in CDDO-mediated growth inhibition. It is possible that RA, 4HPR, and CDDO also inhibit cell growth or transformation by mechanisms other than promotion of the proteasomal degradation of G1 cyclins. To demonstrate that proteasomal degradation is the major mechanism for the chemopreventive activity of RA, 4HPR, and CDDO, it would be interesting to know whether the suppression of cell growth and transformation by these agents could be abolished by specific inhibitors that block the degradation.

Ubiquitin ligation is a multistep process accomplished by three enzymes (9). A ubiquitin-activating enzyme (E1) activates ubiquitin in an ATP-dependent manner, and the ubiquitin is then transferred to a ubiquitin-proteasome complex. A general activation of the proteasomal activity may not be a useful chemoprevention mechanism. There is ample evidence that activation of the proteasome may contribute to the carcinogenic process. The bile acid deoxycholic acid is reported to stimulate proteasome-mediated degradation of p53 resulting in impaired p53 transactivation and response to DNA damage (10). Many publications deal with the inhibition of the proteasome as a cancer chemotherapeutic mechanism. For example, bortezomib (Velcade) has recently been approved for the treatment of multiple myeloma (11). Proteasome inhibitors are active against many tumor types, both as a single agent and in combination with standard chemotherapy drugs such as gemcitabine and irinotecan. Several potential mechanisms have been proposed for the antitumor activity of this proteasome inhibitor. p21 and p27 (inhibitors of cell cycle progression), Rb and p53 (tumor suppressors), and inhibitor kB are all substrates for the proteasome. Inhibition of the degradation of these substrates
would result in the accumulation of proteins that are negative regulators of the cell cycle and cell proliferation and would induce apoptosis (10). Inhibition of the proteasome by green tea polyphenol (-)epigallocatechin-3-gallate could also result in increased levels of the tumor suppressor genes p53, pRB, p21, and Bax, any of which may inhibit cell proliferation and/or induce apoptosis, and this has also been proposed as the cancer prevention mechanism (12).

Because 4HPR and CDDO are also effective in promoting G1 cyclin degradation in BEAS-2B-R1 cells, which are partially resistant to RA, Dragnev, et al. (3) suggest that these compounds might be useful in overcoming clinical resistance to classical retinoids by virtue of their ability to activate pathways downstream of retinoic acid receptor β. Dragnev, et al. (3) also advocate the use of combination chemoprevention with agents activating non-cross-resistant pathways. The concept of using combinations of agents to increase efficacy and decrease toxicity in cancer therapy and chemoprevention is well accepted. If the resistance of BEAS-2B-R1 cells is due to hypermethylation of retinoic acid receptor β, then the combination of RA with a demethylating or chromatin-remodeling agent might reverse the resistance. It remains to be demonstrated whether a combination of RA and 4HPR or CDDO can improve the therapeutic index. According to Dragnev, et al. (3), if there is a mixed population of RA-responsive and non-RA-responsive cells, 4HPR and CDDO should be effective against both cell types. RA and 4HPR (or CDDO) are proposed to induce the same molecular event, i.e., degradation of cyclin D1 and cyclin E. Combination of these agents with agents that work through different mechanisms may be even more beneficial.

References

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