Perspective

Retinoid Chemoprevention Trials: Cyclin D1 in the Crosshairs

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Successful clinical cancer chemoprevention with retinoids was promised by compelling preclinical and early clinical evidence. Indeed, the concept of cancer chemoprevention came largely from studies showing retinoid suppression of epithelial carcinogenesis (1–4). Yet, randomized phase III intergroup chemoprevention trials with the classic retinoid isotretinoin did not reduce second primary cancers in patients with early stages of non–small cell lung cancer (5) or head and neck squamous cell carcinoma (6). Absence of preventive activity in these trials may be attributed to frequent silencing of the retinoic acid receptor-β, which can confer resistance to classic retinoids (7). Several hypothesis-driven approaches for circumventing loss of retinoic acid receptor-β signaling have been developed, including the use of nonclassic retinoids that act through the retinoid X receptors or that have receptor-independent activities.

The nonclassic retinoid N-(4-hydroxyphenyl)retinamide (or fenretinide) was investigated in two studies by the same group, William and colleagues (8) and Papadimitrakopoulou and colleagues (9), reported in this issue of the journal. The authors are commended for completing hypothesis-driven trials with a nonclassic retinoid. Fenretinide at elevated concentrations can induce apoptosis (10). William and colleagues (8) describe a trial of high-dose fenretinide in oral leukoplasia patients with the rationale of achieving serum levels sufficient to induce apoptosis anticipated from preclinical studies. Yet, this study was closed early by design because of an insufficient response rate in the first cohort of patients. Papadimitrakopoulou and colleagues (9) treated moderate-to-severe laryngeal dysplasia patients with a regimen that combined daily 13-cis-retinoic acid and α-tocopherol with twice weekly α-IFN for 1 year. Nonprogressing patients were randomized to maintenance low-dose fenretinide or placebo for 2 years. Despite a previous positive trial of a regimen similar to that used in this induction phase (11), the present trial showed no apparent effect of either induction or maintenance therapy on laryngeal cancer development (9).

A prior study from this group found an intriguing association between shorter cancer-free survival and presence of the G/A870 cyclin D1 polymorphism (12). In the current study, these investigators confirmed this earlier finding and found additionally that high intralesional cyclin D1 protein expression (measured immunohistochemically) was also linked to shorter overall cancer-free survival (9). This work implicates cyclin D1 genotype and protein expression as molecular cancer risk factors and highlights cyclin D1 as a molecular pharmacologic target. Although many interesting aspects of these chemoprevention trials warrant comment, this perspective focuses on the therapeutic and preventive potential of targeting cyclin D1.

Cyclin D1 has been associated with carcinogenesis since it was cloned in 1991 as the gene rearranged in a subset of parathyroid tumors (13). Also known as bc11, the cyclin D1 genomic locus at chromosome 11q13 is altered in multiple cancer types. This region is amplified in a subset of human breast cancers and squamous cell cancers and at the breakpoint of the chromosomal translocation t(11;14)(q13;q32) in certain B-cell neoplasms. Murine studies also identified the cyclin D1 locus as a frequent hotspot for proviral insertions resulting in lymphoma (14–17).

The D-type cyclins are activating regulators of cyclin-dependent kinases (CDK) 4 and 6 (18). These CDKs phosphorylate the retinoblastoma protein, which releases E2F transcription factors allowing expression of genes required for S-phase entry and cell cycle progression. Cyclin D1 levels are elevated in many cancers through diverse mechanisms (19). In both engineered H-ras and c-neu/erbB-2 murine breast cancer models, tumors do not form in the absence of cyclin D1. This finding elegantly shows the key role cyclin D1 plays in carcinogenesis (20). Considerable attention has turned to targeting both CDK activity and associated mitogenic pathways that deregulate cyclin D1 levels (21).

Mitogenic signaling pathways maintain sufficient cyclin D1 levels to permit cell cycle progression predominantly through transcriptional regulation of cyclin D1. Signals that activate transcription from the cyclin D1 promoter include those mediated by ErbB2, Ras, Wnt, and signal transducer and activator of transcription (22). Cyclin D1 levels are regulated by multiple mechanisms. When a rapid decrease in cyclin D1 is required, a pathway of accelerated ubiquitin-mediated proteolysis is engaged. This pathway is activated at the end of S phase and in response to cellular stress. Cyclin D1 is phosphorylated at residue Thr286, is exported from the nucleus, and is recognized by a specific ubiquitin ligase that targets it for degradation by the proteasome (21, 23). Glycogen synthase kinase-3β (GSK-3β) is the most extensively studied kinase that phosphorylates cyclin D1 at Thr286 (24), although p38SAPK2 and extracellular signal-regulated kinase 2 can also target this site (23).

Cyclin D1b is a naturally occurring alternatively spliced cyclin D1 species, which lacks the COOH terminus, including residue Thr286, present in wild-type cyclin D1. It is localized predominantly in the nucleus and exhibits oncogenic activity (25). A specific polymorphism of cyclin D1 (G/A870) at the

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splice junction of exons 4/5 is proposed to influence the relative amounts of this spliced isoform. Individuals homozygous or heterozygous for the A allele are hypothesized to have an increased cancer risk due to increased cyclin D1b levels (25). Truncated cyclin D1 transcripts lacking a large portion of the 3’ untranslated region are translated more efficiently than wild-type cyclin D1 transcripts (26, 27). Several microRNAs have been identified that bind the long form of cyclin D1 mRNA and repress its productive translation (28–32). Therefore, both cyclin D1b mRNA and protein are resistant to several mechanisms that tightly regulate wild-type cyclin D1 levels. The alternative primary structures of cyclin D1 and cyclin D1b transcripts and protein products are displayed in Fig. 1.

In a recent meta-analysis of 60 epidemiologic case-control studies (data from 18,411 cases and 22,209 noncancer controls) that assessed the cyclin D1 G/A870 polymorphism, individuals homozygous for the A allele had a small but significantly increased overall cancer risk (odds ratio, 1.23; ref. 33). Cancer risk also was increased (but less so) in individuals heterozygous for the A allele (AG genotype). A single case-control study found that cyclin D1 genotype correlated with head and neck premalignancy risk [odds ratio, 1.91 (AG) and 2.38 (AA); ref. 34]. The relatively low odds ratios of the meta-analysis and the other case-control study do not support the usefulness of cyclin D1 genotyping for clinical risk assessments, but prospective clinical findings are more promising.

Clinical studies are providing important insights into cyclin D1 genotype and expression and their potential effect on tumor biology (e.g., on genetic instability and the severity of malignant disease) and, ultimately, on the selection of cancer therapy or prevention agents (9, 12, 35–37). Prospective clinical results suggest that cyclin D1 genotyping in conjunction with measuring cyclin D1 expression levels may be clinically useful for risk assessment in patients with a specific premalignant lesion (9). In clinical studies of head and neck premalignancy, the adverse cyclin D1 genotype (AA or AG) is associated with increased progression to cancer development and resistance to retinoic acid down-regulation of cyclin D1 expression or lesion response (12). The unfavorable genotype has also been associated with early onset (38), poor prognosis (39), and therapeutic resistance of head and neck and other cancers (40).

Learning whether a molecular target such as cyclin D1 is modulated in malignant or premalignant target tissue following treatment is clinically relevant. Papadimitrakopoulou and colleagues found that both cyclin D1 genotype and protein expression were associated with shorter cancer-free survival in patients with laryngeal dysplasia. Of note, increased nuclear cyclin D1 protein levels substantially elevated the already higher cancer risk of laryngeal dysplasia patients carrying the A allele, suggesting that additional factors are involved in elevating nuclear cyclin D1 levels. The adverse genotype, however, can also produce wild-type cyclin D1 protein, and the favorable GG genotype, although usually associated with wild-type protein, can produce cyclin D1b protein. Based on these data, future clinical studies should clarify the relationship of genotype with both the generation of alternative cyclin D1 transcripts and the expression of nuclear cyclin D1b protein. This could be achieved by reverse transcription-PCR and immunohistochemistry using recently developed cyclin D1b-selective antibodies (40). This additional information would establish the value of cyclin D1b for risk assessment, predicting response to therapeutic or preventive agents, and as a surrogate end point biomarker.

In short-term clinical trials with pretreatment versus post-treatment biopsy studies, we have shown that cyclin D1 protein down-regulation is also associated with favorable findings/outcomes and thus may be a potential surrogate end point biomarker. Short translational studies of this type are an appealing approach for evaluating chemopreventive agents and monitoring cellular response. Repression of cyclin D1 in posttreatment versus pretreatment biopsies was a key biomarker of response in a preoperative trial of the retinoid bexarotene in stage I and II non–small cell lung cancer patients (41). Another proof-of-principle, short-term clinical trial

![Fig. 1](image-url). The different domains and structures of transcripts and protein products for cyclin D1 (A) and cyclin D1b (B). Translation from cyclin D1b mRNA terminates in intron 4 (I4). The exonic (E) structure and location of key cyclin D1 regulatory domains (such as the retinoblastoma binding motif, pRB, cyclin box, and PEST site) for cyclin D1 and cyclin D1b are shown. Arrows depict important residues involved in the regulation of these cyclin D1 species, as discussed in the text.
showed repression of cyclin D1 expression and proliferation in tumors when sufficient intratumoral levels of erlotinib (an epidermal growth factor receptor tyrosine kinase inhibitor) were reached in aerodigestive tract cancers (42). Furthermore, in vitro studies show that erlotinib inhibits cyclin D1 expression in sensitive cancer cells, but not resistant ones (42). Biomarker response can be assessed in surrogate tissues when it is not possible to obtain both pretreatment and posttreatment biopsies of target tissue. For example, the ability of combined retinoid and erlotinib treatments to repress cyclin D1 in aerodigestive tract cancers was examined in pretreatment and posttreatment buccal swabs (43). Therefore, proof-of-principle trials could guide personalized therapy for cancer.

To accelerate the development of new drugs and regimens, proof-of-principle and phase 0 clinical trials are conducted to determine whether sufficient drug reaches the tumor and whether the drug target is affected and triggers an antitumor response (41–46). Phase 0 trials are designed to determine if a drug can be administered at a pharmacodynamically effective dose, with reduced requirements for the preclinical toxicology designed into phase I trials. This translational research requires the coordination of an interdisciplinary team of clinical and basic scientific investigators. There is strong evidence from Papadimitrakopoulou et al. and others that cyclin D1 is a promising target for cancer chemoprevention. Small-molecule inhibitors of cyclin D1/CDK4/CDK6 are in clinical development and may allow more selective targeting of cyclin D1 for cancer prevention and therapy. Perhaps the most critical of the many lessons learned from retinoid chemoprevention is the identification of molecular targets. In this regard, retinoid chemoprevention has helped put cyclin D1 in the crosshairs of molecular-targeted work that promises to help guide future translational research.

Disclosure of Potential Conflicts of Interest
E. Dmitrovsky serves on the scientific advisory board of NuRx but has no ownership or financial interests in NuRx.

References


