Cyclin D1 and Pancreatic Carcinoma: A Proliferative Agonist and Chemotherapeutic Antagonist

Commentary on Biliran et al., p. 6075

J. Alan Diehl and Sharon Benzeno

Cyclin D1, the regulatory partner of the G₁ cyclin-dependent kinases, CDK4/6, plays a critical role in promoting cell proliferation by virtue of its capacity to trigger cell cycle progression through the first gap phase, G₁. Given the central role for this enzyme in regulating growth factor–dependent cell cycle progression, it is not surprising that overexpression of cyclin D1 is frequently associated with neoplasia where it is thought to play critical role in tumor initiation and progression. By analogy with the c-myc oncprotein, which can induce proliferation or apoptosis depending upon the cellular environment, cyclin D1 expression has been paradoxically associated with either induction of apoptosis or in some instances resistance to apoptosis. If cyclin D1 indeed plays an integral role in promoting tumor cell proliferation, elucidating its function in either promoting or inhibiting commitment of these cells toward apoptosis will critically effect the response of tumors to therapeutic intervention. In this issue of _Clinical Cancer Research_, Biliran et al. address this notion by investigating the influence of cyclin D1 overexpression on pancreatic tumor cell sensitivity to cisplatin (1).

All three D-type cyclins are responsive to growth factor–mediated signaling; growth factors induce transcription, translation, and subsequent assembly of the penultimate D-type cyclin-CDK4/6 complex during the G₁ phase of the cell cycle (2). This kinase complex is subsequently transported into the nucleus where it initiates the phosphorylation-dependent inactivation of the retinoblastoma family of proteins, Rb, p107, and p130 defining a critical step in the irreversible commitment of a cell to a single round of cell division (3). Functional inactivation of Rb and related proteins relieves repression of the E2F family of transcription activators, thereby permitting expression of genes whose products determine S-phase entry and progression.

Cyclin D1 is also negatively regulated via proteasome-dependent degradation and nucleocytoplasmic shuttling. Upon entry into the S phase, cyclin D1 is phosphorylated at a single threonine residue, Thr²⁸⁶, which in turn triggers association with the CRM1 nuclear exportin, an event essential for nuclear export of the cyclin D1/CDK complex during the S phase (4). Once cyclin D1 is localized to the cytoplasm, it is a substrate for an as yet unidentified E3 ligase that triggers cyclin polyubiquitination and proteosomal degradation (5). Whereas residues that direct cyclin D1 nuclear export are conserved in cyclins D2 and D3, it has not yet been established whether these D-type cyclins are similarly regulated.

Although cyclin D1 is frequently overexpressed in cancer, the precise role it plays in cancer genesis remains unclear. Despite accumulating data spanning 15 years of extensive research describing the overexpression of cyclin D1 in cancer, the cyclin D1 protein does not fit the classic definition for an oncogene. For example, overexpression of cyclin D1 will decrease cell cycle transit times, and in some cell types reduce growth factor requirements; however, overexpression of wild-type cyclin D1 by itself is not sufficient to induce cellular transformation (6). Recently, however, it was shown that cyclin D1 mutants that are refractory to nuclear export are in fact capable of driving cellular transformation (3, 7). These findings suggest that in cancers wherein cyclin D1 is the initiating oncogene, mere overexpression of the wild-type protein is unlikely to be the initiating event.

In addition to its function in cancer initiation, cyclin D1 overexpression can be associated with induction of either programmed cell death (8, 9) or cell survival due to insensitivity to cytotoxic drugs (10, 11). In the latter scenario, cyclin D1 is thought to provide a critical but undefined prosurvival function. Specifically in the context of pancreatic cancer, overexpression of cyclin D1 is associated with poor prognosis and insensitivity to chemotherapy (12). Thus, its expression might contribute to cancer cell survival following treatment with chemotherapeutic agents. Indeed, previous work from the Korc laboratory revealed that antisense ablation of cyclin D1 in cells derived from a pancreatic carcinoma (Panc-1) increased cellular sensitivity to cisplatin (10). To directly assess the contribution of cyclin D1 in the sensitivity of pancreatic cancer cells to chemotherapeutic treatment, Biliran et al. used a pancreatic tumor cell line derived from elastase-myc transgenic mice (Ela-myc). The established cell line was previously shown to contain low levels of endogenous cyclin D1 protein, making it a suitable model for the direct manipulation of cyclin D1 levels. To determine the consequence of cyclin D1 overexpression in these cells, the authors engineered additional cell lines following transfection of cyclin D1–encoding constructs into parental Ela-myc cells.

As expected, overexpression of cyclin D1 endowed these cells with a growth advantage characterized by a contracted G₁ interval and reduced but not abrogated growth factor requirements relative to parental controls. Overexpression of cyclin D1 also conferred increased propensity for growth in semisolid medium. Biliran et al. subsequently assessed the sensitivity of parental versus cyclin D1–overexpressing cells to the chemotherapeutic drugs such as cisplatin and gemcitabine. Strikingly, significant resistance to the cytotoxic effects of either cisplatin
or gemcitabine were observed in the cyclin D1–overexpressing cell lines, as assessed by either 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assays or colony formation assays. This result shows that cyclin D1–expressing cells do not merely survive treatment but retain their proliferative potential. Although the cyclin D1–expressing clones exhibited only a 10% to 30% increase in survival, this difference in survival represents a drug-insensitive tumor cell population characterized by unchecked viability resulting from unrestricted proliferation and a capacity to infiltrate and metastasize in a chemoinensitive fashion.

What is the nature of cyclin D1 activities that determines its capacity to direct cell survival versus cell death? One possibility is that the ability of cyclin D1 to sensitize cells to cell death could reflect its capacity to drive E2F activity via Rb inactivation. Promiscuous activation of E2F1 in particular is known to be proapoptotic (13). In addition, ectopic expression of cyclin D1 in MCF7 breast cancer cells promotes a p53-dependent translocation of Bax to the mitochondria thereby sensitizing cells to retinoic acid–induced cell death (8). However, because p53 is inactivated in pancreatic carcinoma (14), cyclin D1 expression should not induce bax translocation thus perhaps tipping the balance in favor of cell survival.

The current work highlights the relevance of tissue specificity in contributing to the consequence of cyclin D1 overexpression. In contrast to the observed association between cyclin D1 overexpression and apoptosis in breast cancer cells and neuronal tissue, Biliran et al. show a protective advantage conferred by cyclin D1 overexpression and apoptosis in breast cancer cells and neuronal tissue, Biliran et al. show a protective advantage conferred by cyclin D1 overexpression in pancreatic cancer cells. The current work suggests that cyclin D1 overexpression coincides in the context of pancreatic cancer for both BcL-2 and NF-κB in promoting cell survival, it will be important for future efforts to establish whether the increase in NF-κB activity and/or BcL-2/BcL-xL levels is a direct consequence of increased cyclin D1–dependent CDK4/6 activity or if it reflects a CDK-independent activity of cyclin D1. Whereas the mechanistic basis for BcL-2/BcL-xL accumulation remains unclear, it is of interest to note that a concurrent overexpression of cyclin D1 and BcL-2 has been observed in mantle cell lymphoma (15).

Interestingly, the genes encoding both cyclin D1 and BcL-2 family proteins are regulatory targets of NF-κB (16–18). The current work suggests that cyclin D1 expression, through as yet to be identified mechanism(s), can trigger a feedback loop resulting in constitutive activation of NF-κB. Such a feedback loop would represent elements of a perpetual motion machine wherein increased basal levels of NF-κB concomitantly induce expression of both the antiapoptotic BcL-2 family and cyclin D1. Whereas cyclin D1 is a documented target of NF-κB signaling, it is less clear how cyclin D1 itself might trigger increased NF-κB activity. In most cell types, NF-κB resides in latent inactive complexes by virtue of its association with its cellular inhibitor IκB. Phosphorylation-dependent proteolysis of IκB leads to nuclear accumulation of active NF-κB. One could consequently envision a model wherein deregulation of active cyclin D1–CDK kinase leads to degradation of IκB followed by release and activation of NF-κB (Fig. 1). Because the serines and threonines within the IκB degron do not fall within the known consensus phosphorylation motif of CDK4 (19), it is unlikely that IκB itself is a direct target of the cyclin D1–dependent kinase. However, expression of cyclin D1 may contribute to increased activation of the IKK complex that will in turn contribute to increased IκB destruction and constitutive activation of NF-κB. The current system provides a good model wherein to test these hypotheses.

**Fig. 1.** The cyclin D1–CDK4/6 kinase contributes to G1 cell cycle progression via phosphorylation-dependent inactivation of Rb and related pocket proteins, which contributes to expression of S-phase specific gene products. Resistance of cells to cytotoxic drugs in turn reflects the capacity of the cyclin D1 to promote constitutive NF-κB activity, which in turn promotes increased expression of BcL-2 and BcL-xL and cyclin D1.
The present work raises additional interesting questions. First, given the increased basal activity of NF-κB in pancreatic cancer in concert with cyclin D1 overexpression, should therapies target NF-κB or cyclin D1? The present work argues that therapies that target cyclin D1 or the cyclin D1/CDK kinase should affect both cyclin D1 and NF-κB. Moreover, is cyclin D1–dependent chemoresistance a consequence of cyclin D1 activation of its cognate CDK? If chemoresistance reflects CDK-independent functions of cyclin D1, therapeutics that target the cyclin D1-CDK4/6 kinase will fail to obtain the desired effects. Conversely, if chemoresistance is conferred by activation of the cyclin D1–dependent kinase, the advent of CDK4/6 small-molecule inhibitors (20) may represent an effective therapeutic modality for pancreatic cancer, particularly when used in combination with standard chemotherapeutic regimens. Finally, if NF-κB is downstream of cyclin D1, it will be critical to elucidate the molecular basis for this regulation, which will clearly effect future therapeutic design.

References

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