Oncogenes in Cancer Diagnosis *39*, 125–134.

Flores, E.R., Tsai, K.Y., Crowley, D., Sengupta, S., Yang, A., McKeon, F., and Jacks, T. (2002). Nature *416*, 560–564.

Halevy, O., Michalovitz, D., and Oren, M. (1990). Science *250*, 113–116.

Lane, D.P., and Benchimol, S. (1990). Genes Dev. *4*, 1–8.

Marin, M.C., Jost, C.A., Brooks, L.A., Irwin, M.S., O'Nions, J., Tidy, J.A., James, N., McGregor, J.M., Harwood, C.A., Yulug, I.G. et al., (2000). Nat. Genet. *25*, 47–54.

Martinez, L.A., Naguibneva, I., Lehrmann, H., Vervisch, A., Tchenio, T., Lozano, G., and Harel-Bellan, A. (2002). Proc. Natl. Acad. Sci. USA *99*, 14849–14854.

Melino, G., De Laurenzi, V., and Vousden, K.H. (2002). Nat. Rev. Cancer *2*, 605–615.

Irwin, M.S., Kondo, K., Marin, M.C., Cheng, L.S., Hahn, W.C., and Kaelin, W.J., Jr. (2003). Cancer Cell *3*, 403–410.

Soussi, T., and Béroud, C. (2001). Nat. Rev. Cancer *1*, 233–240.

Strano, S., Fontemaggi, G., Costanzo, A., Rizzo, M.G., Monti, O., Baccarini, A., Del Sal, G., Levrero, M., Sacchi, A., Oren, M., and Blandino, G. (2002). J. Biol. Chem. *277*, 18817–18826.

Urist, M., and Prives, C. (2002). Cancer Cell *1*, 311–313.

Cdk2 dethroned as master of S phase entry

The prevailing view of cdk2 as a critical regulator of cell cycle progression and optimal therapeutic target in cancer cells is now challenged by the observation that tumor cells deficient in cdk2 protein and kinase activity are not impaired in proliferation.

Deregulation of cell cycle control mechanisms is an obligatory step in tumorigenesis. Myriad individual genetic events lead to circumvention of checkpoints that restrain the activity of cyclin/cyclin-dependent kinase (cdk) complexes that are responsible for managing cell cycle transitions. Indeed, it is now widely accepted that alteration of some component of the retinoblastoma protein (pRb) pathway, the core of which is depicted in Figure 1, occurs in virtually all human tumors. In some tumor cells, the RB gene is a direct target of inactivating mutations, but most often pRb is inactivated consequent to inappropriate activation of the cyclin D/cdk4(6) complex. This in turn can be achieved through overexpression or mutation of one of the subunits, or through loss of the negative regulator $p16^{INK4a}$ (Sherr and McCormick, 2002).

One of the most significant consequences of pRb inactivation is activation of cyclin E/cdk2 subunits, often as a result of increased cyclin E expression. Cyclin E/cdk2 complexes can themselves participate in maintained inactivation of pRb in tumor cells that express this protein, but also appear to have several other critical roles in cell cycle progression (Bartek and Lukas, 2001). As shown in Figure 1, cyclin E/cdk2 complexes are thought to play critical roles in centrosome duplication (Hinchcliffe and Sluder, 2002), replication origin firing (Krude et al., 1997; Takisawa et al., 2000), and histone protein expression (Ma et al. 2000; Zhao et al., 2000). Consistent with such crucial roles for cyclin E/cdk2 downstream of pRb, many tumor cells are exquisitely sensitive to inactivation of cyclin E/cdk2 whether or not they express pRb. This conclusion has been drawn from a multitude of experiments demonstrating antiproliferative effects of overexpression of $p27^{KIP1}$, a protein inhibitor of cdk2, or of dominant-negative cdk2 subunits. Further, injection of antibodies against cdk2 activators cyclin E and cyclin A blocks proliferation, as does treatment of many different cells with cdk2 inhibitors (Tetsu and McCormick, 2003; Knockaert et al., 2002).

The view that pRb pathway inactivation has cyclin E/cdk2 activation as its ultimate proliferative consequence is supported by observations of mice engineered to express cyclin E in place of cyclin D1. Animals lacking cyclin D1 have profound proliferative defects in a subset of tissues, and fail to activate cdk2 in those tissues. These phenotypes are significantly suppressed in a knockin animal that expresses cyclin E from the cyclin D1 locus (Geng et al., 1999), suggesting that loss of cyclin D-mediated inactivation of pRb is inconsequential if cyclin E synthesis is no longer dependent on pRb inactivation. Indeed, excess cyclin E/cdk2 subunits can also overcome ectopic expression of a nonphosphorylatable, and thus constitutively active, pRb (Bartek and Lukas, 2001). This tumorigenic role of cyclin E/cdk2 may be most clearly manifest in human breast cancer cells, where reduced $p27^{KIP1}$ expression or cyclin E overexpression correlates well with aggressiveness of the tumor (Catzavelos et al., 1997; Porter et al., 1997; Keyomarsi et al., 2002). All together, these studies suggest that cyclin E/cdk2 regulation is targeted directly and indirectly by multiple, collaborative mutational events in a wide variety of tumor cells and thus chemical inhibition of cdk2 might provide an insurmountable obstacle to continued tumor cell proliferation.

This view of cell cycle control in cancer is now challenged by work from Tetsu and McCormick reported in the March issue of *Cancer Cell*. Using primarily cell lines derived from colon cancers, Tetsu and McCormick have shown that direct chemical inhibition of cdk4(6) or indirect reduction of D cyclins and cdk4 by MEK inhibitors blocks proliferation, but multiple modes of cdk2 inhibition are without effect. For example, colon cancer cell lines proliferate without regard to $p27^{KIP1}$ or dncdk2 overexpression, but these same reagents do cause arrest in other cell lines previously shown to respond to cdk2 inhibition. Thus, colon cancer cells in general appear to evade the effects of cdk2 inhibition seen in other cancer cell types. The authors suggest that one reason for this is the ability of deregulated cdk4 to fully inactivate pRb as a consequence of eroded phosphorylation site specificity in tumor cells. Consistent with this, Tetsu and McCormick show that one form of dncdk4 can block proliferation of

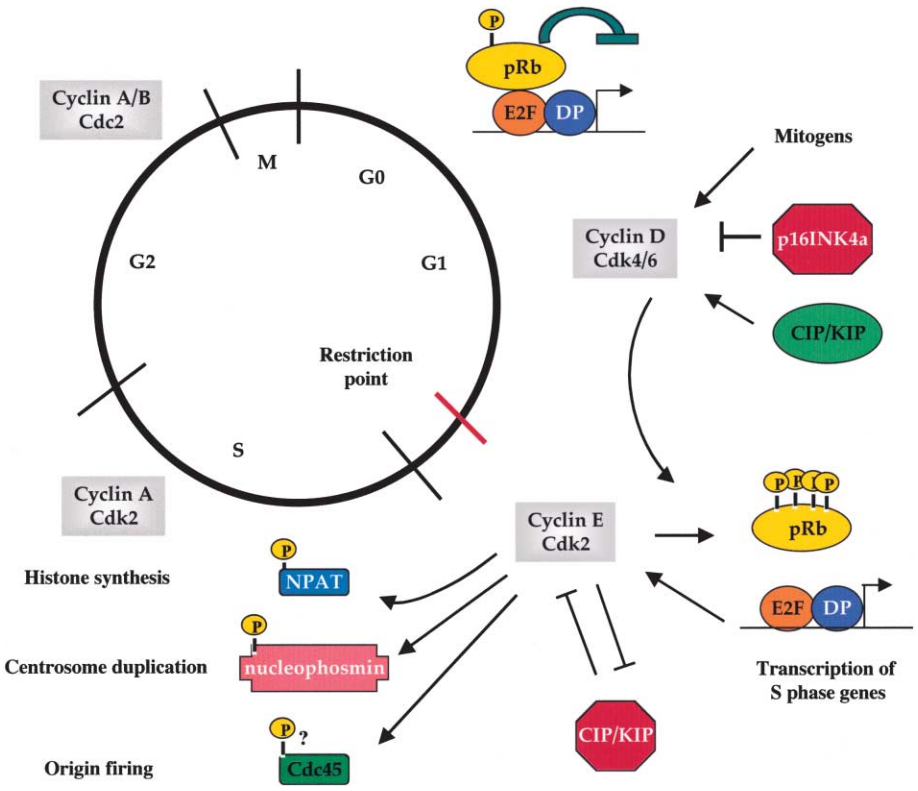

Figure 1. The RB pathway and the role of cdk2 in cell cycle progression

Inactivation of the transcriptional repressor function of the retinoblastoma protein is required for the G1-to-S phase transition. In normal cells, this is achieved through mitogen-induced activation of cyclin D/cdk4(6) complexes, the assembly of which is facilitated by $p21^{CIP1}$ and $p27^{KIP1}$, leading to phosphorylation and inactivation of pRb. This event correlates well with passage through the restriction point (R), after which cells remain committed to DNA replication even in the absence of mitogens (Pardee, 1989). Upon phosphorylation by D/cdk4(6), pRb loses its ability to inhibit E2F-dependent transcription and cyclin E levels increase. Subsequent activation of cdk2 leads to further phosphorylation of pRb and has recently been proposed to be critical for several other aspects of S phase entry. Cyclin E/cdk2 complexes can phosphorylate the transcription factor p220NPAT, leading to activation of histone gene promoters. Phosphorylation of nucleophosmin is one of several events in centrosome duplication that may be directly or indirectly controlled by cyclin E/cdk2, and cdk2 activity appears to contribute to cdc45 loading onto licensed origins of DNA replication, a prerequisite to origin firing. When conditions inappropriate for proliferation are sensed, these events can be blocked by $p16^{INK4a}$-mediated inhibition of cyclin D/cdk4(6) assembly and activation or by de novo synthesis of elevated levels of $p21^{CIP1}$ and $p27^{KIP1}$ that potently inhibit cdk2. Loss of pRb or $p16^{INK4a}$ or overexpression of cyclin D/cdk4(6) complexes is seen in most cancers and may synergize with enhanced degradation of $p27^{KIP1}$ or increased cyclin E level to promote unchecked entry into S phase.

colon cancer cells that are immune to cdk2 inhibition.

Extending this work to obtain greater precision in assessing the role of cdk2 in proliferation of cancer cells, Tetsu and McCormick specifically reduced cdk2 expression using antisense oligonucleotides or siRNA techniques. As expected from results with $p27^{KIP1}$ and dncdk2 expression, this was without effect in colon cancer cells. Remarkably, however, several other tumor cell lines tested also showed no effect of severe cdk2 reduction, including those known to be sensitive to $p27^{KIP1}$ and dncdk2. This quite surprising result suggests that the cdk2 enzyme, long thought to be key to the G1-to-S phase transition in both normal and tumor cells, may not be solely responsible, nor indeed at all critical, for this event in human tumor cells. From this, the authors conclude that efforts to specifically target cdk2 with small molecule inhibitors as potential cancer therapeutics may be misguided.

Like any good, paradigm-shifting work, the paper by Tetsu and McCormick raises many crucial questions left for further exploration. Two of these center on the roles of $p27^{KIP1}$ and cyclin E (and perhaps cyclin A) as suppressor and activator of cell proliferation, respectively. First, as discussed above, it is clear that unlike the colon cancer cells studied here, many tumor cells are indeed arrested by $p27^{KIP1}$ and dncdk2. If cdk2 activity is not the specific target of this action, how might these inhibitors work? One explanation offered by the authors is inhibition of cdk4, which may occur on the one hand through an inhibitory action of high levels of $p27^{KIP1}$, or on the other hand through a titration of $p27^{KIP1}$ (or $p21^{CIP1}$) by dncdk2, leading to a reduction in the assembly of functional cyclin D/cdk4(6) complexes. This, however, is unlikely to explain the effect of $p27^{KIP1}$ and dncdk2 on pRb-minus cells that typically lack detectable cdk4(6) activity as a consequence of elevated $p16^{INK4a}$ expression.

An attractive alternative possibility is that a distinct kinase subunit other than cdk2 may be able to partner with cyclin E in a $p27^{KIP1}$-sensitive manner in such cells. A candidate for such a kinase in human cells is cdk3, which has been shown to be activated by cyclin E2 (Zariwala et al., 1998). However, if this is the case, the tumor cells tested in the Tetsu and McCormick study must be able to proliferate with very low levels of cyclin E-associated kinase activity, since this was undetectable in cells treated with cdk2 antisense oligonucleotides. A second possibility that is not mutually exclusive with this is that a non-cdk kinase might be able to drive all of the events ascribed to cdk2 in Figure 1, at least in tumor cells. A candidate here that has recently generated much interest is aurora kinase. Aurora kinase is overexpressed in some cancers and has been linked to centrosome duplication and cellular transformation (Duterte et al., 2002). Perhaps unscheduled activation of aurora kinase greatly limits the requirement for cdk2 in tumor cells—just as ectopic expression of cyclin E precludes the need for cyclin D1 in certain mouse tissues as described above. Indeed, given that cdk2 cannot be said for certain to be completely absent after siRNA or antisense treatment, minute amounts of activity may collaborate with kinases such as aurora to drive cancer cell cycles. Regardless, proliferation of cancer cells with such a paltry amount of cdk2 challenges the chemist targeting cdk2 in tumors.

Finally, the present study ups the

ante for those betting types who strive to predict the phenotype of mice lacking the E type cyclins or cdk2, or of normal human cells treated to lack these molecules. Based on current dogma, many would place their money on an absolute requirement for cdk2 or E cyclins in normal cell cycles. Given the work presented by Tetsu and McCormick, the payoff may go to those betting on less profound phenotypes, and such experiments will likely force the preparation of a new set of model slides for all those with interest in cdk2's role in mammalian cell cycle control.

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Selected reading

Bartek, J., and Lukas, J. (2001). FEBS Lett. *490*, 117–122.

Catzavelos, C., Bhattacharya, N., Ung, Y.C., Wilson, J.A., Roncari, L., Sandhu, C., Shaw, P., Yeger, H., Morava-Protzner, I., Kapusta, L., et al. (1997). Nat. Med. *3*, 227–230.

Duterte, S., Descamps, S., and Prigent, C. (2002). Oncogene *21*, 6175–6183.

Geng, Y., Whoriskey, W., Park, M.Y., Bronson, R.T., Medema, R.H., Li, T., Weinberg, R.A., and Sicinski, P. (1999). Cell *97*, 767–777.

Hinchcliffe, E.H., and Sluder, G. (2002). Oncogene *21*, 6154–6160.

Keyomarsi, K., Tucker, S.L., Buchholz, T.A., Callister, M., Ding, Y., Hortobagyi, G.N., Bedrosian, I., Kinckerbocker, C., Toyofuku, W., Lowe, M., et al. (2002). N. Engl. J. Med. *347*, 1566–1575.

Knockaert, M., Greengard, P., and Meijer, L. (2002). Trends Pharm. Sci. *23*, 417–425.

Krude, T., Jackman, M., Pines, J., and Laskey, R.A. (1997). Cell *88*, 109–119.

Ma, T., Van Tine, B.A., Wei, Y., Garrett, M.D., Nelson, D., Adams, P.D., Wang, J., Qin, J., Chow, L.T., and Harper, J.W. (2000). Genes Dev. *14*, 2298–2312.

Pardee, A.B. (1989). Science *246*, 603–608.

Porter, P.L., Malone, K.E., Heagerty, P.J., Alexander, G.M., Gatti, L.A., Firpo, F.J., Daling, J.R., and Roberts, J.M. (1997). Nat. Med. *3*, 222–225.

Sherr, C.J., and McCormick, F. (2002). Cancer Cell *2*, 103–112.

Takisawa, H., Mimura, S., and Kubota, Y. (2000). Curr. Op. Cell Biol. *12*, 690–696.

Tetsu, O., and McCormick, F. (2003). Cancer Cell *3*, 233–245.

Zariwala, M., Liu, J., and Xiong, Y. (1998). Oncogene *17*, 2787–2798.

Zhao, J., Kennedy, B.K., Lawrence, B.D., Barbie, D.A., Matera, A.G., Fletcher, J.A., and Harlow, E. (2000). Genes Dev. *14*, 2283–2297.

Connecting estrogen receptor function, transcriptional repression, and E-cadherin expression in breast cancer

A recent paper in *Cell* (Fujita et al., 2003) demonstrates that MTA3, a novel component of the Mi-2/NuRD transcriptional repression complex, is an estrogen receptor-regulated inhibitor of the Snail zinc finger transcription factor in breast cancer. Given the important role of Snail in repressing E-cadherin transcription and the function of E-cadherin as a tumor suppressor protein and regulator of epithelial architecture, the findings offer potentially significant new insights into cancer pathogenesis.

In the United States and much of the Western world, breast cancer rivals lung cancer as the most frequent cause of cancer-related death in women, with upwards of 12 percent of women diagnosed with breast cancer during their lifetimes. While breast cancer mortality appears to have shown some encouraging decreases in the recent past, presently, about 25% of women diagnosed with breast cancer will die of the disease (Baselga and Norton, 2002). Hormonal factors play a key role in normal breast development and in growth and progression of breast cancer.

Perhaps chief among the hormonal factors involved in breast cancer is the ovarian steroid hormone estrogen. The biological actions of estrogen are dependent on the cellular function of a high-affinity estrogen receptor (ER) (McDonnell and Norris, 2002). Two estrogen receptors—ERα and ERβ—have been identified, but most estrogenic responses appear to require ERα. Upon binding estrogen or other ligands, ER is released from its inhibition by a large heat shock protein complex. Following dimerization, ER activates transcription of specific cellular genes via direct and indirect interactions with their regulatory regions. In breast cancer cells expressing ER, estrogen has potent effects on cell proliferation, differentiation, and survival, perhaps in part via estrogen's ability to affect the cellular response to various growth factors and other cues from surrounding extracellular matrix and stromal cells. While ER expression in breast cancer is generally associated with a better clinical outcome, the clinical utility of ER as a prognostic marker is modest. Rather, the principal value of defining the ER status (and the progesterone receptor status) of a breast cancer is for prediction of the patient's likely response to systemic therapy, particularly adjuvant therapy with tamoxifen, a selective estrogen receptor modifier and antiestrogenic agent in the breast (Baselga and Norton, 2002).

Besides hormonal and other environmental factors, germline and somatic mutations and gene expression changes play key roles in breast cancer initiation and progression. There is great interest in defining how the various mutations and gene expression changes contribute to breast cancer development and its aggressive behavior, especially because recent studies have indicated that particular gene expression signatures in breast cancer are associated with good prognosis and other signatures are associated with poor prognosis (van de Vijver et al., 2002). A major challenge for workers pursuing gene expression