2005). Thus, tumors that have circumvented the requirement of p63-mediated survival may exhibit resistance to common cancer treatments (Rocco et al., 2006).

In contrast, upregulation of bcl-2 through alternative mechanisms may identify tumors that are resistant to the proapoptotic effect of these treatment modalities regardless of p53 family member status. Specific bcl-2 inhibitors show promise as cancer therapeutics in lung and other cancers. Other agents hold promise for increasing wild-type p53 levels, thus abrogating the p63 survival function. It would be interesting to explore whether novel biologic treatments that abrogate EGFR signaling in combination with DNA damaging agents can also overcome this resistance. A recent study showed that the addition of an EGFR antibody to local radiation therapy and platinum treatment markedly improved patient survival. Understanding the status of all p53 family members in HNSCC is crucial for developing more individualized combinations with standard DNA damaging agents and newer molecular therapies.

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Is Cyclin D1-CDK4 kinase a bona fide cancer target?

Previous studies have demonstrated that mice lacking Cyclin D1 were refractory to mammary tumor development induced by the c-*neu*/*erbB-2* oncogene, the rodent ortholog of the HER-2 receptor frequently overexpressed in human breast carcinomas. Two new studies in this issue of *Cancer Cell* provide additional evidence on this issue. Knockin mice expressing a mutant form of Cyclin D1 that binds to Cdk4/6 but cannot activate their catalytic activity are resistant to c-*neu*/*erbB-2* tumorigenesis in spite of undergoing normal epithelial cell expansion during pregnancy. Moreover, knockdown of Cdk4 in mammary tumor cells abrogates tumor formation. These observations provide new compelling evidence that inhibition of Cyclin D1-Cdk4/6 kinases might be beneficial for cancer therapy.

In 2001, a seminal paper by Sicinski and coworkers (Yu et al., 2001) reported that mice lacking Cyclin D1 were refractory to tumorigenesis induced by MMTV-driven Ha-*ras* and c-*neu*/*erbB-2* oncogenes. c-*neu*/*erbB-2* is the rodent ortholog of the human *HER-2* receptor gene frequently overexpressed in human breast carcinomas. Indeed, HER-2 is one of the few oncogenes already targeted in the clinic by means of specific monoclonal antibodies (reviewed in Hynes and Lane, 2005).

Earlier studies by Sicinski et al. (1995), then in the Weinberg laboratory, and by Fantl et al. (1995) in the Dickson laboratory had described that ablation of *cyclin D1* in the germline prevented the breast epithelial compartment of adult female mice to undergo the massive proliferative changes associated with pregnancy despite normal levels of ovarian steroid hormones. In their 2001 study, Yu et al. (2001) concluded that c-*neu*/*erbB-2* (and the Ha-*ras* oncogene) induced tumorigenesis by activating the *cyclin D1* promoter. Hence, ablation of Cyclin D1 expression prevented oncogenic signaling (Figure 1). In contrast, other oncogenes, such as c-*myc* and *wnt*-1, that could activate expression of other effectors, such as the related *cyclin D2*, efficiently induced mammary tumors in these Cyclin D1-defective mice (Yu et al., 2001). Intriguingly, c-*neu*/*erbB-2* and Ha-*ras* oncogenes induced other types of tumors (mainly salivary gland tumors) and efficiently transformed *cyclin D1* null fibroblasts in culture (Yu et al., 2001), indicating that the selective role of Cyclin D1 in mediating c-*neu*/*erbB-2* and Ha-*ras* oncogenesis is unique to mammary epithelial cells (Figure 1).

Cyclin D1 is not an obvious druggable target. Yet, one of the main biological activities of Cyclin D1 involves activation of its partners Cdk4 and Cdk6, two kinases whose catalytic activity is absolutely dependent upon binding of any of the D-type Cyclins (reviewed in Malumbres

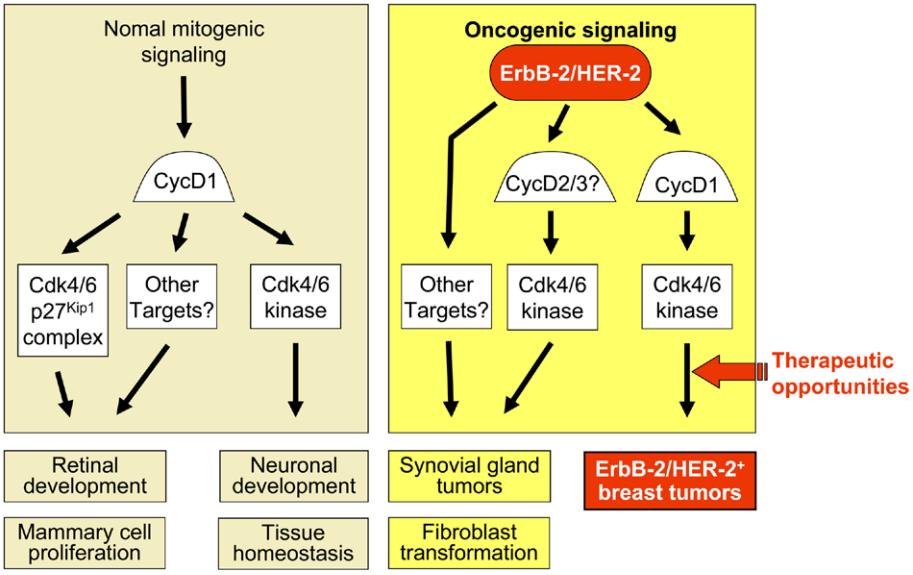

Figure 1. Therapeutic opportunities to block ErbB-2/HER-2 oncogenic signaling

Normal mitogenic signaling (left) and Erb-2 (and presumably HER-2) signaling (right) is mediated by Cyclin D1 or other D-type Cyclins through activation of either Cdk4/6 kinases, kinase-independent interaction with Cdk4/6 and $p27^{Kip1}$, or interaction with other targets. Boxes at the bottom indicate the main biological consequences of each pathway.

and Barbacid, 2005). Thus, the results of Yu et al. (2001) strongly argued that selective inhibitors of Cyclin D1-Cdk4/6 kinases should have therapeutic benefit, at least in HER-2-positive breast cancers. Industry did not seem too impressed by these findings, and currently there is only one Cdk4/6-selective inhibitor undergoing clinical trials.

In retrospect, the emerging concept of cancer stem cell provides an alternative explanation to the results described by Yu et al. (2001). It is possible that c-*neu*/*erbB-2* and Ha-*ras* oncogenes preferentially transform a subtype of lobuloalveolar breast precursor cell that is not present in *cyclin D1*-deficient mice. Instead, c-*myc* and *wnt*-1 may target either a more primitive precursor that can develop in the absence of Cyclin D1 or a distinct precursor for a related lineage. This scenario would imply that the failure of c-*neu*/*erbB-2* and Ha-*ras* oncogenes to induce mammary tumorigenesis may not be related to a signaling mechanism that requires Cyclin D1, but to a developmental defect caused by targeting the *cyclin D1* locus in the germline. If this happens to be the case, tampering with Cyclin D1 or its cognate kinases in breast cancer patients for therapeutic purposes may turn out to be not so promising.

Possibly, these and/or similar considerations may have deterred the pharmaceutical industry from developing Cdk4/6-selective inhibitors. Not to worry. Two papers appearing in this issue of *Cancer Cell* provide renewed and more convincing evidence that, indeed, Cyclin D1-dependent Cdk4/6 kinase activity is essential for tumorigenesis mediated by the c-*neu*/*erbB-2* oncogene (Landis et al., 2006; Yu et al., 2006). Landis et al. describe a new strain of gene-targeted mice that carries a germline mutation (replacement of lysine in position 112 by glutamic acid) within the *cyclin D1* locus. Previous studies have shown that Cyclin $D1^{K112E}$ binds with normal affinity to Cdk4 and Cdk6, as well as to other effectors, including various transcription factors. Yet, Cyclin $D1^{K112E}$ cannot activate the catalytic activity of Cdk4 or Cdk6, resulting in the generation of kinase-defective Cyclin D1-Cdk4/6 complexes. These knocked-in mice, designated as *cyclin* $D1^{KE/KE}$, share the same neurological defects (leg clasping), defective cerebellar development (in combination with Cyclin D2 deficiency), and reduced size as *cyclin D1* null animals. Surprisingly, *cyclin* $D1^{KE/KE}$ mice display normal retinal development and expansion of the mammary gland during pregnancy. These findings provide genetic evidence for a significant biological role of Cyclin D1 that is independent of its well-established role as the activating subunit of Cdk4/6 kinases.

Yet, it is possible that Cyclin D1 activity is not completely independent of Cdk4/6, at least as binding partners. Landis et al. (2006) report that the kinase-defective Cyclin $D1^{K112E}$-Cdk4/6 complexes retain the capacity to bind the cell cycle inhibitor $p27^{Kip1}$. Previous studies had demonstrated that $p27^{Kip1}$ is epistatic to Cyclin D1, at least in the developing retina and in pregnancy-driven proliferation of the mammary gland (Geng et al., 2001; Tong and Pollard, 2001). Thus, it is possible that the main difference between *cyclin* $D1^{-/-}$ and *cyclin* $D1^{KE/KE}$ mice resides in the ability of Cyclin D1 to form trimeric complexes with Cdk4/6 and $p27^{Kip1}$, independently of their kinase activity (reviewed in Sherr and Roberts, 1999). Interestingly, Yu et al. (2006) now show that transplanted Cdk4 null mammary glands develop normally during pregnancy, although $Cdk4^{-/-}$ females are refractory to c-*neu*/*erbB-2* oncogenesis. These observations suggest that Cyclin D1 can induce epithelial cell expansion during pregnancy by interacting with Cdk6 alone. Whether other Cyclin D1 partners, mainly transcription factors, also play a role in Cdk4/6 kinase-independent activities of Cyclin D1 awaits further studies.

Loss of Cdk4 has also been implicated in resistance to tumor development in other tissues. For instance, ablation of Cdk4 renders mice resistant to carcinogen and Myc-induced skin tumors without affecting keratinocyte proliferation (Rodriguez-Puebla et al., 2002; Miliani de Marval et al., 2004). Since Cdk4 expression was ablated in the germline, it is possible that tumor resistance might be, at least in part, due to developmental defects. Now, Yu et al. (2006) show that downregulation of Cdk4 expression by RNAi in c-*neu*/*erbB-2*-induced mammary tumor cells eliminates their oncogenic properties when reinoculated into mammary fat pads. These results, taken together, represent the strongest genetic evidence generated thus far in animal models to support the concept that inhibition of Cyclin D1-mediated Cdk4 kinase activity should have a therapeutic benefit for cancer patients.

As illustrated by Yu et al. (2006), about a quarter of HER-2-positive human breast tumors express abnormally elevated levels of Cyclin D1. Are we wasting a golden opportunity by not developing Cdk4/6-selective inhibitors to treat HER-2-positive breast cancers? Possibly. However, we must be cautious, since current animal models do not exactly recapitulate

the natural history of human tumors. First of all, the Cyclin D1 and Cdk4 mutations used in these studies were introduced in the germline, not in advanced tumors (RNAi results must be interpreted more cautiously, since other loci may be affected). More importantly, the etiology of experimental tumors differs significantly from that of human cancers. For instance, experimental mouse tumors often arise from tissues in which most cells carry the tumor-inducing mutation (MMTV-driven c-*neu*/*erbB-2* expression in the papers discussed here). In contrast, human tumors, especially solid tumors, result from mutations in single or few cells that accumulate additional mutations through a process of clonal selection. Thus, tumor development in human patients is likely to be less dependent on any given mutated gene than experimental tumors. Yet, these considerations should not be an excuse to further delay testing in patients suffering from HER-2-positive breast tumors the effectiveness of selective Cdk4/6 inhibitors, as suggested by these new studies (Landis et al., 2006; Yu et al., 2006).

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Fly Src: The Yin and Yang of tumor invasion and tumor suppression

The non-receptor tyrosine kinase Src is inactivated by the C-terminal Src kinase Csk. In a recent paper in *Developmental Cell*, Vidal et al. show that loss of *Drosophila* Csk (dCsk) in a large field of cells results in cell proliferation and disorganization of tissue architecture. In contrast, local inactivation of dCsk in a small field of cells results in loss of cells that are adjacent to normal tissue. This loss occurs by basal migration and death by apoptosis. These findings may shed light on mechanisms that restrain tumor initiation.

During the progression of many epithelial cancers, including breast and colon carcinomas, the expression and activity of the tyrosine kinase Src becomes progressively elevated. Src activity appears to be particularly important in tumor cell invasion and metastasis (Frame, 2002). Thus, inhibition of Src inhibits tumor metastasis in a number of xenograft models, as well as in transgenic mice overexpressing polyoma middle T or Her2. In cell culture, activated Src induces the appearance of invasive adhesions known as podosomes or invadopodia, sites of local matrix degradation. The pathway by which Src induces cell invasion through reconstituted basement membrane matrices involves the activation of the focal adhesion kinase (FAK) and the stress-activated Jun kinase (JNK), and the induction and local secretion of matrix metalloproteinases and other matrix-degrading proteases (Hsia et al., 2003). Activated Src is capable of inducing autonomous cell proliferation and cell transformation: viral *src* is a potent transforming gene. Yet mutations that activate Src are found rarely if at all in human cancers, and those that have been reported—the report has been disputed—occur in advanced metastatic cancers (Irby et al., 1999). The reason that activating mutations in Src do not appear to initiate human cancers is unknown.

One potential explanation for the rarity of activating mutations in Src is the ability of normal cells to suppress the malignant behavior of mutant cells within their midst. In cell culture, normal fibroblasts can suppress the transformed phenotype of Src-transformed fibroblasts, a phenomenon that does not appear to depend on junctional communication (Alexander et al., 2004). Similar tumor-suppressive mechanisms may operate in vivo. For example, normal liver tissue can induce the differentiation of injected hepatocarcinoma cells, provided that the tumor cells are present in small groups or single cells (McCullough et al., 1998). Similarly, the formation of tumors by grafted papilloma cells can be suppressed by admixture with normal keratinocytes (Strickland et al., 1992). Inhibition of tumor cell growth by adjacent normal cells has been postulated to represent a potent tumor suppression mechanism.

New light on these issues may now