Cell Cycle-Based Therapies Move Forward

Marcos Malumbres[1,*]
[1]Cell Division and Cancer Group, Spanish National Cancer Research Centre (CNIO), Madrid E-28029, Spain
*Correspondence: malumbres@cnio.es
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Targeted therapies directed against cell cycle regulators have been difficult to translate into the clinic. In this issue of *Cancer Cell*, Choi et al. and Sawai et al. rekindle the therapeutic value of inhibiting specific cyclin-dependent kinase complexes by demonstrating their requirements in the maintenance of breast tumors and leukemias.

> "*The real problem is to understand why cancer cells grow when their normal counterparts would not.*" (Hunt, 2008)

Tumor cells invariably display defects in the machinery that controls the cell division cycle. Yet, whether the cell cycle is a useful therapeutic target is still being intensely debated mostly due to the essential role of this process in tissue homeostasis and the difficulties of finding a therapeutic window (Malumbres and Barbacid, 2009). Progression through the cell cycle is driven by several protein kinases, including cyclin-dependent kinases (CDKs). Molecular analysis of tumor cells has provided strong evidence that the activity of these kinases is deregulated in human tumors suggesting their potential therapeutic use. However, the clinical benefit of the first generation of CDK inhibitors has been limited due to toxicity and lack of specificity, possibly derived from the critical function of CDKs in DNA replication and chromosome segregation.

The pioneering work by P. Sicinski's group in 2001 (Yu et al., 2001) showed that a specific interphase cyclin, cyclin D1, was required for RAS- and HER2-induced mammary tumors, but was dispensable for WNT- or MYC-induced mammary tumors. This work showed for the first time that the therapeutic value of a cell cycle regulator may be cell-type specific and dependent on specific oncogenic alterations. These studies were performed using germline cyclin D1 knockout mice, which raised some important questions at that time. Since cyclin D1 knockout mice displayed minor defects in mammary gland development, it was argued that the effect observed could be due to special requirements for cyclin D1 in the cell of origin of these tumors. Two studies in 2006 suggested that this was not the case, as a similar therapeutic benefit was observed using CDK4-deficient mice or knockin mice expressing a mutant cyclin D1, which does not bind CDKs but maintains other CDK-independent functions (Landis et al., 2006; Yu et al., 2006). None of these models displayed defects in mammary gland development; yet, they were resistant to HER2-induced mammary gland carcinomas. In addition, these studies suggested that CDK4, one of the kinase partners of cyclin D1, was the critical enzymatic activity to be targeted in HER2-positive mammary gland carcinomas. Subsequent studies by Barbacid and colleagues (Puyol et al., 2010) showed that CDK4, but not CDK2 or CDK6, was critical for the development of K-RAS-induced lung tumors, whereas lack of this interphase kinase did not affect the development of lungs in germline knockout mice.

Because the genetic modification was present since conception, the remaining question was whether the acute inhibition of cyclin D1-CDK complexes would be effective in already developed breast tumors. A new study in this issue of *Cancer Cell* by Choi et al. (2012) now shows that conditional genetic ablation of cyclin D1 in adult mice that bear tumors inhibits the growth of HER2-positive mammary carcinomas. A similar effect is obtained using a small-molecule inhibitor of CDK4/6 (PD 0332991) currently being studied in clinical trials. Importantly, this effect is not accompanied by toxicities associated with the acute inhibition of cyclin D1-CDK complexes in adult individuals (Puyol et al., 2010; Choi et al., 2012; Sawai et al., 2012), suggesting that side-effects of such treatments will be minimal.

Based on the previous results, cyclin D1-CDK4 complexes seem to be critical targets in the proliferation of epithelial tumors, at least breast and lung carcinomas (Figure 1). What about other interphase CDK complexes? Previous studies established a critical role for cyclin D3 and, at least partially, CDK6 in lymphocytes and in the development of T cell leukemias (Hu et al., 2009; Sicinska et al., 2003). The relevance of these findings in cancer therapy has now been addressed using conditional knockouts or acute treatments with kinase inhibitors. Genetic ablation of cyclin D3 or inhibition of CDK4/6 complexes in Notch1-induced T cell acute lymphoblastic leukemia (T-ALL) results in tumor regression without causing major abnormalities in other tissues (Choi et al., 2012; Sawai et al., 2012; in this issue of *Cancer Cell*). Importantly, the response of leukemic cells to these treatments is dramatically different from that of epithelial cells. Both lung and breast cancer cells display characteristics of cell cycle arrest and senescence after genetic ablation or chemical inhibition of cyclin D1-CDK4 complexes (Choi et al., 2012; Puyol et al., 2010). Similar treatments, however, result in apoptotic cell death in mouse and human leukemic cells (Choi et al., 2012; Sawai et al., 2012). This response seems to be associated with the finding that Notch1-induced tumors display high levels of cyclin D2/D3 and cyclin D3-CDK6 complexes in agreement with the modulation of cyclin D3 expression by the Notch1 pathway (Choi et al., 2012; Joshi et al., 2009). In fact, Notch1-negative T-ALLs or other types of leukemic cells did not undergo apoptosis following CDK4/6 inhibition (Choi et al., 2012). Whether the

susceptibility to apoptosis is provided by the activation of Notch1 or the specific inhibition of cyclin D3-(and possibly CDK6) complexes is not clear at present. Interestingly, cyclin D2 cannot compensate for the lack of cyclin D3 when expressed from the *Ccnd3* locus suggesting intrinsic differences in the function of these two proteins (Sawai et al., 2012). The molecular basis for the differential response to CDK4/6 inhibition—senescence versus apoptosis—may be considered a new avenue of high interest in the therapeutic evaluation of these cell cycle regulators.

It is been a long road—20 years—since the initial generation of genetically-engineered mice with cell cycle mutations. The discovery that individual interphase cyclins or CDKs were dispensable for the development and homeostasis of most tissues was considered a demonstration of the developmental plasticity of mammalian tissues and raised doubts on the usefulness of inhibiting these activities in tumors (Malumbres and Barbacid, 2009). The recent studies in breast, lung, and T-ALL make a strong case for the identification of cellular contexts in which the inhibition of specific cyclin-CDK complexes may have therapeutic value (Figure 1). Based on these results, one could propose that many human tumors may be sensitive to specific cyclin-CDK complexes, as long as we identify the specific complexes that mediate the response to specific oncogenic pathways in each specific cell type. A long road in which difficult questions such as "why cancer cells grow when their normal counterparts would not" (Hunt, 2008) need to be addressed in a cell-type- and oncogene-specific manner. Cancer patients will undoubtedly benefit from these studies.

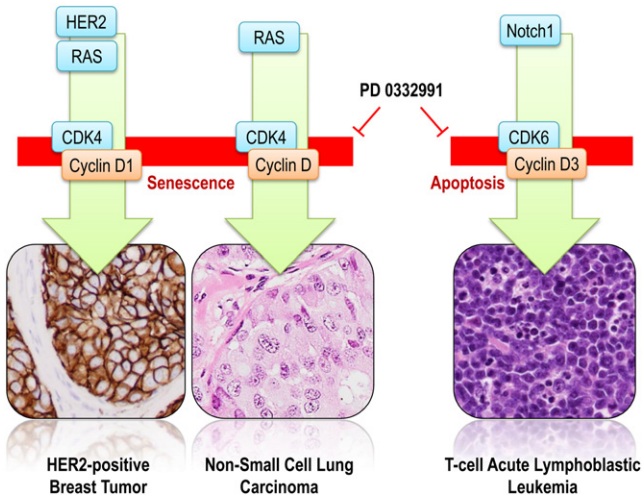

Figure 1. An Initial Road Map for the Treatment of Human Malignancies with Specific CDK4/6 Inhibitors

Since the small-molecule inhibitor PD 0332991 inhibits both CDK4 and CDK6 complexes, the participation of CDK4 in human leukemias cannot be discarded at this time. Similarly, it is not clear which specific cyclins activate CDK4 in lung tumors, although this information is likely to be dispensable for the selection of small-molecule kinase inhibitors. Further research will be necessary to evaluate the relevance of specific cyclin-CDK complexes in similar pathologies induced by other oncogenes (e.g., mammary gland or lung tumors without activation of the RAS pathway) or in other tumor types.

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