

PERSPECTIVE

Targeting the Neighbor's Pool

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What to do on a hot summer afternoon? Sneak over and dip into the neighbor's pool! This seems to be the story for the newest inhibitors of the Cdc25 phosphatases. Brisson et al. (2004), in this issue, present one of the most promising reversible competitive Cdc25 inhibitors to date, blocking cell cycle progression and causing growth arrest of cancer cell lines. Before considering the benefits of these latest inhibitors, we first need to consider why Cdc25 phosphatases are important but difficult drug targets and what swimming pools have to do with this problem.

Cancer kills an increasing number of people every year, with an estimated 10 million new diagnoses in the year 2000 alone. Although each type of cancer, even each individual case of cancer, can arise from diverse causes, all cancers share the common feature of a disordered cell cycle and irregularities in the molecules that control this cycle. Thus, the molecules that regulate the cell cycle have become attractive anticancer targets (Toogood, 2002). Primary among these are the cyclin-dependent kinases (Cdk/cyclins). These protein kinases are responsible for driving cell cycle progression (Nigg, 1995). For example, in promoting S-phase, one set of Cdk/cyclins phosphorylates proteins that initiate DNA synthesis and replication. In late G₂, a different set of Cdk/cyclins phosphorylates and activates the proteins required for the structural reorganizations involved in chromosome separation and actual cell division. In essence, all small molecule inhibitors of Cdk/cyclins and other protein kinases bind in the ATP pocket, a large groove between the N- and C-terminal lobes of these enzymes. Surprising specificity has been achieved in protein kinase inhibitors despite the similarities in the ATP binding pockets among different protein kinases.

Cdc25 phosphatases are key activators of the Cdk/cyclins and thus they too have become anticancer targets. The three

human Cdc25 phosphatases (Cdc25A, Cdc25B, and Cdc25C) are responsible for dephosphorylating pThr14 and/or pTyr15 on the Cdk/cyclins. This dephosphorylation triggers the final activation of the Cdk/cyclins during normal cell cycle progression and is prevented in the checkpoint response to DNA damage (Nilsson and Hoffmann, 2000; Iliakis et al., 2003). Cdc25A controls the G₁/S and G₂/M transitions, whereas Cdc25B and Cdc25C are regulators of G₂/M. In response to DNA damage by ionizing irradiation, ultraviolet light, replication inhibitors, or other DNA-damaging agents, the Chk1 and Chk2 kinases, activated by the ATM and/or ATR kinases, phosphorylate the Cdc25s. This phosphorylation leads to G₁/S or G₂/M blocks in the cell cycle by subsequent proteasome-mediated degradation of Cdc25A or 14-3-3-mediated sequestration of Cdc25C. Confirming an important role for the Cdc25 phosphatases specifically in cancer, Cdc25A and Cdc25B, but not Cdc25C, are found overexpressed in 20 different reports of 14 different primary human cancers (Kristjánsdóttir and Rudolph, 2004). Thus, studies of cancer and our understanding of cell cycle regulation point strongly to Cdc25 phosphatases as good targets for anticancer development.

However, Cdc25 phosphatases have proven difficult in high-throughput screening, medicinal chemistry, and cellular proof-of-principle studies. As noted originally upon the discovery that they were phosphatases (Kumagai and Dunphy, 1991), Cdc25s have extremely poor reactivity with small molecule substrates, peptidic ones in particular. The discovery that *O*-methyl fluorescein phosphate (mFP) is a good fluorescent substrate for the Cdc25 phosphatases greatly facilitated high-throughput screening, although numerous efforts in the late 1990s in both biotech and big pharmaceutical companies failed to generate a viable clinical candidate. The reasons for these failures probably lie in two obstacles presented by the active sites of Cdc25 phosphatases, the highly reactive cysteine and the lack of a deep active-site pocket.

Like other dual-specificity phosphatases, a subclass of the protein-tyrosine phosphatases, the Cdc25s contain the CX₅R

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motif, where C is the catalytic cysteine and the amide backbones of the five X residues form a phosphate binding loop along with the arginine R. This active site loop creates a special environment for the active site cysteine that primes it for its catalytic role as a nucleophile in the first step of the reaction, formation of a phosphocysteine intermediate. At the same time, the catalytic cysteine is also highly reactive with other molecules, such as hydrogen peroxide (Sohn and Rudolph, 2003) and quinone-containing inhibitors (Tamura et al., 2000). Whereas oxidation of the catalytic cysteine may be important for the regulation of the Cdc25s in response to oxidative stress, the pharmaceutical industry has tended to avoid reactive compounds that form covalent adducts, hampering and limiting their screening efforts.

The anticipation of the first crystal structure of the catalytic domain of Cdc25A turned out to be a great disappointment for medicinal chemistry (Fauman et al., 1998). The active site was not well ordered, contained no bound ligands, and did not reveal a sufficiently significant active-site pocket for structure-based drug design. The structure of the catalytic domain of Cdc25B was revealed a year later, now showing a well-ordered active site with a bound sulfate and an additional α -helix flanking the active site cysteine (Reynolds et al., 1999). However, it still did not yield a deep active site pocket. One of the largest cavities on the surface of Cdc25B is the "swimming pool", thus named for the abundance of well ordered water molecules it contains (Fig. 1). The swimming pool is adjacent (7–16 Å) to the active site. The swimming pool is also close to the unstructured C-terminal helix that is involved in protein substrate recognition (Wilborn et al., 2001) and 14-3-3 binding (Chen et al., 2003).

The absence of convincing proof-of-principle experiments from cell biology is the final difficulty faced in developing and

testing Cdc25 inhibitors. Elimination of individual Cdc25s using mouse knock-out studies have been reported. The *cde25C*^{-/-} mouse is viable, develops normally, and does not display any obvious abnormalities (Chen et al., 2001). The *cde25B*^{-/-} mouse is also viable and responds normally to DNA damage, although the female mice are sterile as their oocytes are unable to undergo meiosis (Lincoln et al., 2002). Here, the redundancy of the three human Cdc25s is apparently the root of the problem. Reducing the levels of all three Cdc25s, as a nonselective Cdc25 inhibitor would, with antisense DNA or RNAi or a triple-knock-out mouse has not been reported.

Most of the Cdc25 inhibitors to date have come from academic labs mining small public or selected combinatorial libraries (extensively reviewed in Pestell et al., 2000). Many of these previously described inhibitors are covalent, including the Vitamin K derivatives, the quinone-containing compounds discovered in the screening of the National Cancer Institute repository, and the newest inhibitors described by Ducommun's group (Brezak et al., 2004). Thus, it is particularly exciting that novel, noncovalent, yet selective compounds that target the Cdc25 phosphatases are finally emerging. The primary compound described in Brisson et al. (2004), 5169131, has a K_i of 4.5 μ M and is competitive with the small-molecule substrate mFP. How is this possible in the absence of a deep active-site pocket? Molecular modeling studies performed in this work suggest that these inhibitors bind into the swimming pool adjacent to the active site and that this swimming pool is also used in part by the substrate mFP. These observations suggest that binding occurs in the same site as has been demonstrated for the *para*-quinone inhibitors NSC95397 and NSC663284 (Lazo et al., 2002) and the indolylhydroxyquinones (Sohn et al., 2003). These latter compounds are reversible inhibitors that are also competitive versus mFP with K_i values of $\sim 0.5 \mu$ M. The new compound of Brisson et al. (2004), however, is more promising than others described previously as it leads to cell cycle arrest in synchronized cells, a hallmark expected of Cdc25-specific inhibitors. This arrest is accompanied by increased phosphorylation on the Cdk1 substrate of Cdc25, another favorable indication of specificity toward the Cdc25s. Compound 5169131 also inhibits proliferation of human PC-3 prostate and MDA-MB-435 breast cancer cells in the low micromolar range. The only concern with these, as with all Cdc25 inhibitors, is that many cellular insults ultimately lead to cell cycle arrest, and thus the observed phosphorylation of Cdk1 and cell cycle arrest may be indirect. However, this new class of reversible inhibitors may pave the way to compounds with increased potency and specificity that should resolve these questions with greater certainty. We look forward to future significant advances, including hopefully one day a crystal structure with an occupant enjoying the water in the swimming pool of a Cdc25 phosphatase.

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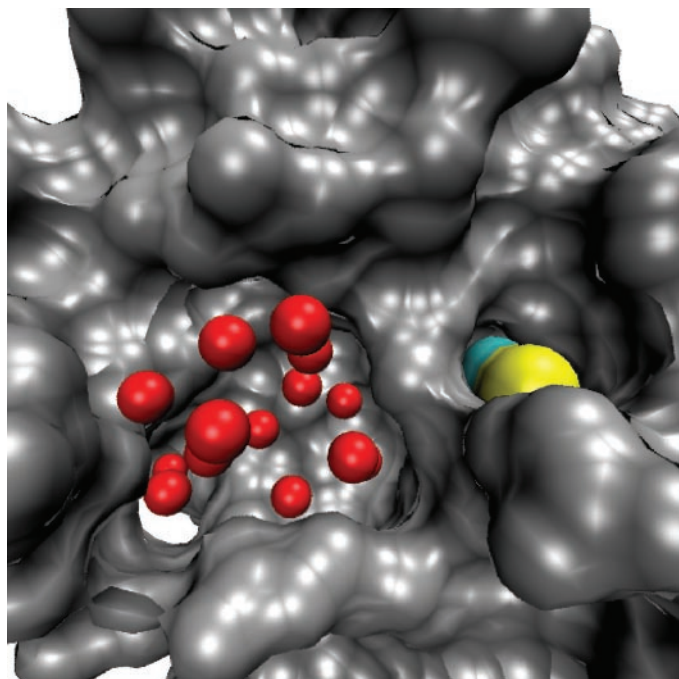


Fig. 1. The active site surface of Cdc25B showing the active site cysteine in yellow in a very shallow pocket and the adjacent deeper and larger swimming pool containing several water molecules in red (the radius of the oxygen atom has been reduced to 0.6 Å for clarity). The figure was created using VMD and the PDB file 1qb0.

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