CDK9 Inhibitors Push Cancer Cells over the Edge

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The trouble with CDK active-site inhibitors is their tendency to have off-target effects. This is not surprising, as the ATP binding sites of most protein kinases are very similar. Wang et al. (2010) have used some clever screening approaches to identify selective CDK9 inhibitors that drive cancer cells into apoptosis.

Cyclin dependent kinases (CDKs) were originally discovered through their role in cell cycle regulation and subsequently have been shown to act as key regulators of transcription. All thirteen members of the human CDK family share the same architecture and are activated by one of over ten possible cyclins. X-ray structures are now available for CDK1, CDK2, CDK4, CDK7, and CDK9. The strong link between CDKs and the cell cycle has led to considerable activity in developing specific CDK inhibitors as potential cancer therapeutics that would block cell proliferation in tumors where cell cycle control has been lost. However, it has become evident that there are a number of built-in parallel or redundant pathways possibly regulated by different cyclin/CDK complexes (CDK1 is the only essential cell cycle kinase) (Barriere et al., 2007). A major biological challenge is to tease out the roles of the various cyclin/CDKs. For example, two anticancer compounds now in clinical trials (R-roscovotine, selecilib from Cyclacel and flavopiridol, and alvocidib from Aventis) were originally developed as CDK2-specific cell cycle inhibitors, though it now appears that CDK9 inhibition and transcriptional repression plays a more important role (Fischer and Gianella-Borradori, 2005; Chen et al., 2005). The work presented by Wang et al. (2010) from the Cyclacel team makes an important contribution to this story by developing a set of chemical tools that show distinctive inhibitory profiles against CDKs that control transcription by regulating the phosphorylation state of RNA polymerase II. This is an impressively comprehensive piece of work covering chemistry, protein structure, cell biology, and in vivo testing.

A cell-based screening cascade was devised to distinguish "cell cycle CDKs (1,2,4)" inhibitors from "transcriptional CDK (7,9)" inhibitors (i.e., those that act via RNA polymerase II). By measuring the relative changes of concentration of p53 and MI (mitotic index, the ratio between the number of cells in mitosis and the total number of cells), three different classes of inhibitor were identified: class 1 (transcriptional inhibitors showing a decrease in MI and high levels of p53 caused by downregulation of the p53 regulator Mdm2); class 2 (mitotic inhibitors); and class 3 (cell cycle inhibitors). A clever analysis correlating antiproliferative effects with biochemical CDK selectivity showed that for class 1 compounds, CDK9 inhibition was necessary and sufficient to kill transformed cells. The emerging story is that the transcriptional inhibitors that block CDK9 and CDK7 induce apoptosis specifically in tumor cells by a mechanism that involves caspases.

These new biochemical results come on the heels of recently published structures of the heterodimeric complex of CDK9 with cyclin T, also known as PTEFb (positive transcription elongation factor b). In a twist to the CDK story, the X-ray structure of a complex of PTEFb with HIV-1 Tat has been solved, opening up new possibilities for the development of new families of anti HIV therapy with CDK9/CycT as a target (Tahirov et al., 2010). More relevant to this paper are the X-ray structures of the complexes of PTEFb with the small molecule inhibitors flavopiridol (Baumli et al., 2008) and 5,6-dichlorobenzimidazone-1-b-D-ribofuranoside (DRB), a widely used inhibitor of transcription elongation (Baumli et al., 2010). All PTEFb structures show a large rotation of the CycT domain when compared with other cell cycle CDK/cyclin complexes. Intriguingly, the structure of DRB crystallized with CDK2 showed surprising differences in the binding mode of the inhibitor in the ATP active site when compared with CDK9. These differences were attributed to an inherent flexibility of CDK9 in complex with CycT compared with the more rigid conformation of CDK2 in complex with CycA.

Even with the availability of such detailed structural information on different CDKs, it is still difficult to design or predict isoform-specific inhibitors because of the highly conserved ATP binding site. The structure activity relationship (SAR) developed by Wang et al. (2010) suggests that the few amino acid differences along the sequence linking the CDK N-terminal domain and C-terminal domain may play an important role: for CDK2, this sequence is 81EFLHQDLK**K**89 and is highlighted in Figure 1. Importantly, the fourteen or so 2-anilinopyrimidine inhibitors described in the paper show a wide range of specificities and activities. An X-ray structure of the most potent CDK9-specific inhibitor (compound 14) complexed with CDK2 shows that the weaker interaction with CDK2 is likely caused by an unfavorable electrostatic and steric interaction between the piperazine group and Lys89. The equivalent residue to this lysine is valine in CDK7 and glycine in CDK9 (Figure 1), providing more space for the large piperazine substituent. Interestingly, Lys 89 in the CDK2 structure is situated on the first turn of a short helix; changing this to glycine in CDK9 will also have an effect on the flexibility of this inter-domain hinge region—a result that is also in keeping with the structural data from Baumli et al. Thus inhibitor specificity for a particular CDK isoform is likely to be governed by flexibility as well as shape and charge complementarity.

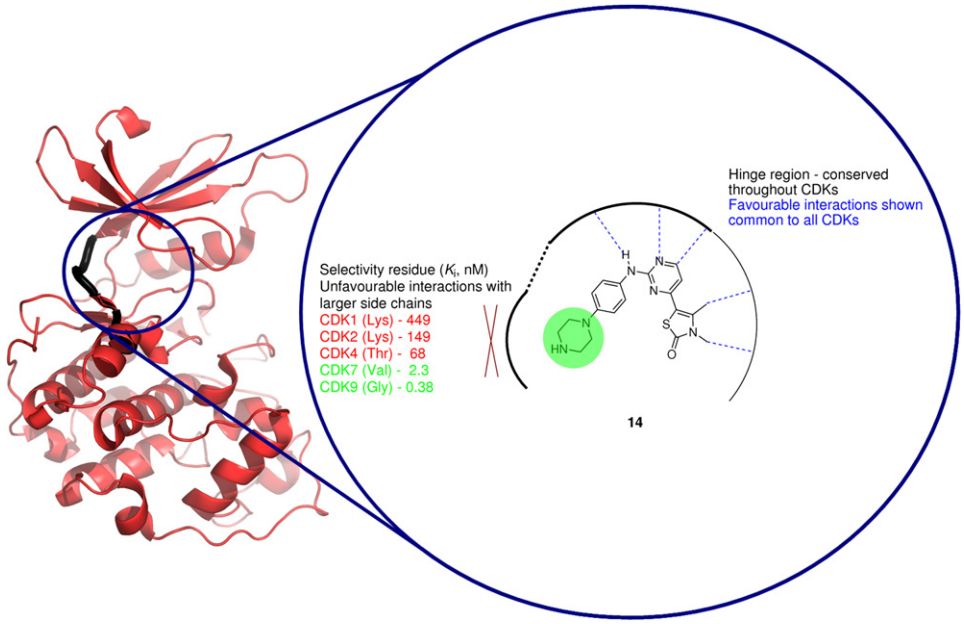

Figure 1. General Overview of CDK Nucleotide Binding Site with Inhibitor 14 Bound
Conserved interactions with all CDKs are shown as dotted blue lines. Differences along the linking sequence between the N- and C-terminal domains (depicted as thick black lines in the scheme and the protein structure) are thought to play an important role in inhibitor specificity. This sequence is EFLHQDLKK in CDK2, DFMETDLEV in CDK7, and DFCEHDLAD in CDK9. The final residue in these sequences seems crucial for the specificity of inhibitor **14**. The piperazine moiety (highlighted in green) occupies space allowed by small side chains (e.g., Gly in CDK9), resulting in a low K_i; however it interacts unfavorably with larger side chains (e.g., Lys in CDK2), resulting in a lower affinity. Hence, it is selective between the CDKs.

Compound 14 was selected for further in vivo testing and it shows good antitumor activity with an IC_{50} value of 0.3 μM and up to 40-fold selectivity toward a range of transformed cell lines. This compound also shows an impressive activity in solid tumor xenograft models producing a 10 day delay in tumor growth (compared to one day for the control 5 flurouracil treatment). The results provided in this paper provide an encouraging advance on the development of specific transcriptional-type CDK inhibitors and suggests that downregulation of RNA-polII activity in transformed cells will provide a useful therapeutic approach against cancer. The next big step will be to test clinically whether these antitranscriptional inhibitors are more efficacious and less toxic than CDK inhibitors with a broader specificity.

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