Target identification, selectivity profiling and mechanistic insights of a Cdk9 inhibitor using complementary proteomics methods

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Institutes

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Introduction

In an effort to map the selectivity and understand the mode of action of a CDK9 inhibitor (Compound 1), we used several orthogonal proteomics methods. Our results show a clear selectivity against the CDK family of kinases, with the highest specific affinity towards CDK9. In addition, our multi-method approach allows us to also map the protein binding sites of the inhibitor, identify non-kinase (off-) targets as well as detect the cellular molecular responses to the added inhibitor. Here we describe the methods used, their strengths and weaknesses, how they can be used in the drug discovery pipeline and how they synergize to provide mechanistic insights of compounds of interest.

Methods

We have used Chemoproteomics, kinase affinity tools (kinobeads), Cellular Thermal Shift Assay (CETSA) and Limited Proteolysis (LiP). These techniques all rely on LC-MS/MS based analytical platforms, but they place different demands on the sample or require different sample handling protocols and subsequently also have different strengths and weaknesses for target deconvolution
Compound 1 was designed to be a potent and selective CDK9 inhibitor, predicted to occupy the proteins ATP-binding site.

Preliminary Data

The results obtained clearly show that CDK9 is the primary target of Compound 1, with affinity curves highly correlated between the different target deconvolution techniques.
The chemoproteomic approach rely on a compound derivate, able to bind to a sepharose bead. Subsequently, the binding competition assays are performed on lysed cell material. The choice of a mild lysis buffer allowed us to identify, not only CDK9, but also it's molecular partners in the p-Tefb complex (Cyclin T1, Cyclin T2 and Aff4) with similar concentration response behavior. The results for the kinases identified in the study were strikingly similar when also profiling the compound without the chemical modification using the kinobeads assay. In the CETSA experiments, where both lysed cells and intact cells were profiled, the lysate experiment most closely resembles that of the previous pull-downs. Here, only direct binders of Compound 1 show a thermal shift, for example several of the pulled down kinases but not the p-Tefb complex partners that were co-competed previously. In the intact cell version of CETSA, not only the direct binders of the compound show stability shifts, but also downstream events and other secondary modulatory effects leave thermal traces in the cell. For example, Compound 1 also binds to GSK3A/B, causing their melting temperature to increase. Inhibition of GSK3 further affects the phosphorylation state and cellular location of FOXK1, which in turn is identified as a destabilized protein. Finally, Limited Proteolysis was used to identify target protein and using the LiP-Quant approach their LiP scores were assigned. Further, out of the identified CDK targets, mapping of

peptide cleavage pattern was performed for the members of the CDK family for which structural data is published. The result identified the peptides to be directly adjacent to the ATP binding pocket of CDK9 or regions of high homology.

Novel Aspect

The use of complementary techniques, based on unique biological and biochemical processes, allow robust and confident characterization of protein inhibitors.