

A cell-based screening technology reveals new potential targets of the tyrosine kinase inhibitor Purvalanol B

Application Note 1: Drug profiling

Summary

- The tyrosine kinase inhibitor Purvalanol B was linked to a methotrexate moiety
- The hybrid compound was screened against a normalized human universal expression cDNA library
- 192 potential interactors were analyzed
- Three new kinases and two kinase-associated proteins were identified as potential interactors of Purvalanol B
- The yeast three-hybrid technology is a valuable tool for identifying small molecule-protein interactions in an intact cellular environment
- The yeast three-hybrid system is a fast and robust method for identifying the unknown mechanism of action of a drug

Introduction

Mechanism of action studies play an important role in understanding the biological activity of small molecules identified in phenotype-based screenings. Finding the relationship between mode of action and mechanism of action allows more specific and mainly quicker development of drugs in different phases of the process. Here, we describe a particular profiling technology which is an adaptation of the yeast two-hybrid system allowing *in vivo* screening of small molecules against

a collection of expression cDNA libraries. Therefore, we are able to profile virtually any tissue, including diseased tissues. We used the tyrosine kinase inhibitor Purvalanol B as a bait and we identified, besides the known interactors CLK1 and CLK4, five novel interacting proteins. These results clearly show the potential of our profiling platform to get important information on the property of drug candidates.

Methods

Yeast three-hybrid

The DNA binding domain LexA is fused to a bait scaffold protein. The hybrid small molecule (consisting of the small molecule bound to the bait protein and the small molecule of interest) is captured by the bait scaffold. Upon interaction of a target with the small molecule the reporter genes in the yeast genome are activated (Figure 1).

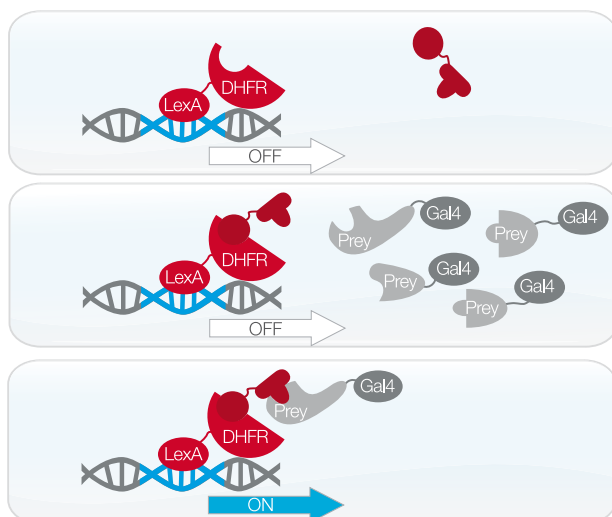


Figure 1: The yeast three-hybrid technology.

Yeast mating

The plasmid expressing the bait scaffold was transformed into an appropriate yeast strain. An overnight bait culture was mixed with a glycerol stock of normalized human universal cDNA library clones in a strain of the opposite mating type and left to mate overnight. After mating was complete, the diploid cells were plated on selective medium containing 50µM Purvalanol B conjugate (Figure 2).

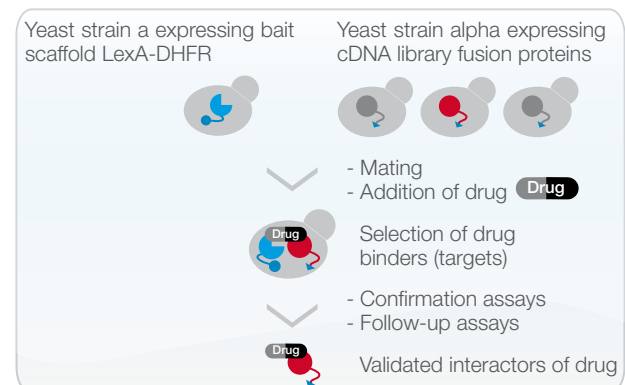


Figure 2: Schematic view of the screening procedure.

Results

In this study the well known kinase inhibitor Purvalanol B was screened against a normalized human universal cDNA library in order to validate known targets and to find new targets in potentially novel indications for this drug. 192 potential interactors were analyzed and we identified previously known as well as novel targets, including kinases and kinase-associated proteins (Table 1).

10 targets were tested in a compound dependency test (Figure 2). Three kinases and two kinase-associated proteins were compound dependent. Here, we show that the yeast three-hybrid technology is a valuable tool for identifying small molecule-protein interactions.

Kinases identified	Function	Number of hits
CLK1	CDC-like kinase 1	4
CLK4	CDC-like kinase 4	4
MEKK 4	MEK kinase 4	4
STK33	Serine/threonine-protein kinase 33	5
EPHA4	Tyrosine-protein kinase receptor SEK	9
Kinase-associated	Function	Number of hits
Ik3-1	CDK5 & ABL1 enzyme substrate 1	20
hSRB11	Cyclin-C	11
Beclin-1	Coiled-coil myosin-like BCL2-interacting protein	1
Other proteins	Function	Number of hits
RIT2	Ras-like protein expressed in neurons	1
ATF-4	Cyclic AMP-dependent transcription factor	1

Table 1: Targets found by screening.

cDNA sequences were searched against NCBI database. Two known interactors (CLK1 and 4) and three new kinases were found. In addition, five proteins were identified as potential interactors, three of them were kinase-associated proteins.

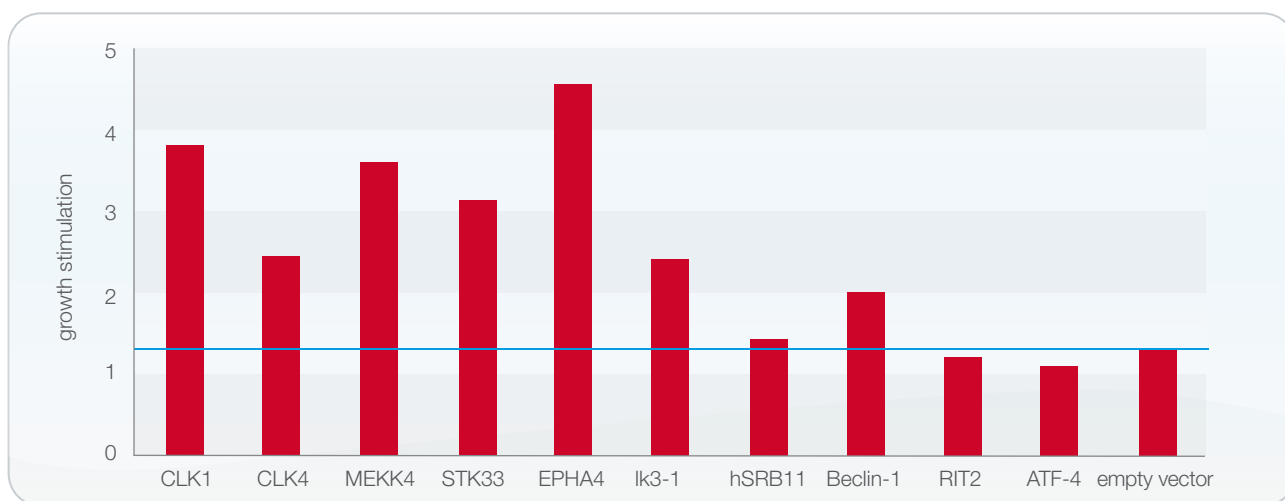


Figure 2: Compound-dependency assay.

The isolated prey plasmids were retransformed into the original yeast strain expressing the bait scaffold. The strains were grown *o/n*, ODs were adjusted and the strains were incubated in selective medium with or without 50µM Purvalanol B conjugate. OD's were measured over a time range of 6 days. Growth stimulation values ($OD + cpd / OD - cpd$) are shown.

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