Validating Upstream Kinase Predictions by Linking Activity to Drug Target Proximity

Dóra Schuller, Gitanjali Dharmadhikari, Monique Mommersteeg, Liesbeth Houkes, Simar Pal Singh, Rik de Wijn
PamGene International B.V., 's-Hertogenbosch, The Netherlands

Contact:
dschuller@pamgene.com

Abstract
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Introduction

Sustained cell signaling is one of the hallmarks of tumor growth. Deregulation of kinase signaling can be studied by methods such as peptide microarrays or phospho-proteomics. For this, the prediction of kinases from phosphorylation signatures is a critical and complex task[1,2]. The aim of this study is to validate the biological relevance of kinase predictions, which we evaluate by the integration of kinase activity profiles with sensitivity data from the Genomics of Drug Sensitivity in Cancer (GDSC)[3].

Methods

Serine-Threonine Kinase activity profiling of 11 B-cell lymphoma cell lines was performed on the KineomePro® platform.

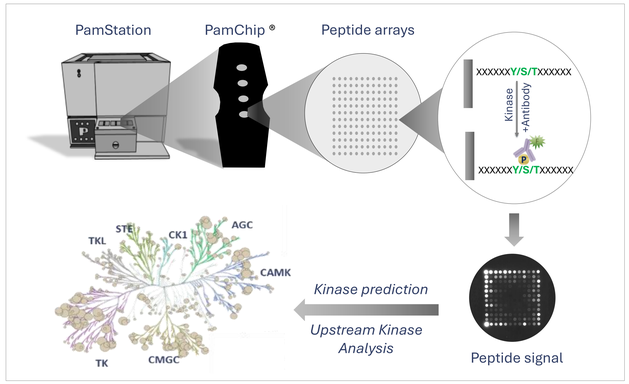

Fig.1. Kinase activity profiling on the KinomePro® platform. Kinases in the samples phosphorylate peptides which are printed on the PamChip. Phosphorylation is detected by fluorescent signal. Kinases are predicted based on the phosphorylation signature.

Kinases were predicted by Upstream Kinase Analysis[2] for 10 cell lines generally sensitive to multiple drugs (IC50 < 1 µM) compared to one relatively resistant control line (showing sensitivity to a smaller number of drugs).
Cell line-specific networks were generated from the top kinases and the target kinases of drugs the cells were most sensitive to, using the STRING[4] protein-protein interaction database and Prize-Collecting Steiner Forest[5] (PCSF) algorithm.

Network connectivity: scoring relevance of kinase predictions

The hypothesis: active kinases and drug targets interact in sensitive cells

A fundamental sensitivity mechanism involves repressing the activity of the drug's target kinase and its downstream survival pathways. Building on this mechanism, we can test the biological relevance kinase predictions, using the hypothesis that the signaling networks of active kinases and drug targets show high connectivity in sensitive cells. Connectivity serves as an indicator of the biological relevance of the kinase predictions.

Scoring biological relevance of kinase predictions

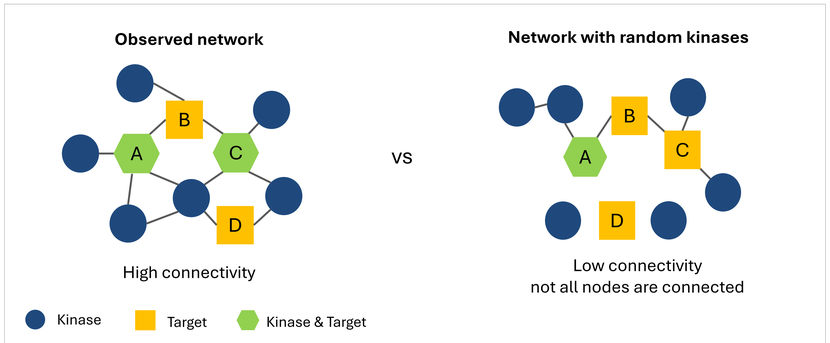

Fig.2. Testing networks for statistical significance against networks with randomized kinases.

To quantify signaling proximity of kinases and drug targets, we developed the network connectivity score. For each cell line-specific network, we calculated network connectivity using the median of the shortest paths between kinases and drug targets. This was then tested for statistical significance against a reference set of 50 random networks, generated using the original drug targets and randomized kinase data.

Networks of therapeutic vulnerability

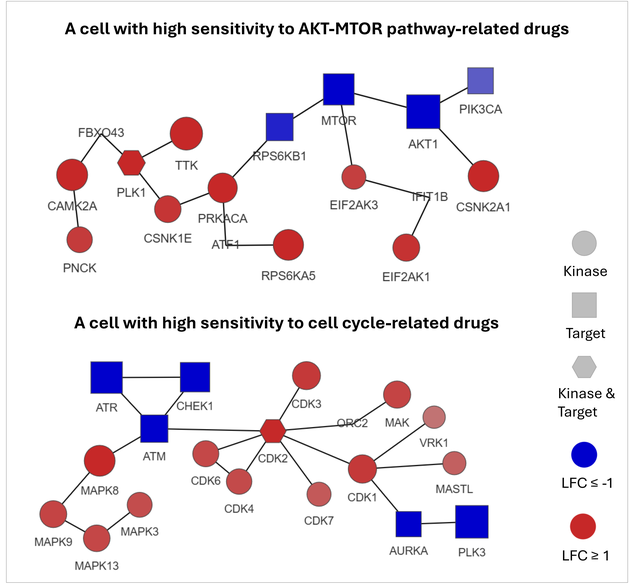

Fig.3. Parts of networks showing high connectivity of drug targets and kinases. *Top*: a cell sensitive to PIK3-AKT pathway-related drugs (WSUDCL2). *Bottom*: a cell sensitive to cell cycle-related drugs (NUDUL1). Blue squares indicate high drug sensitivity (low IC50) and red circles are kinases with higher activity compared to control (log fold change, LFC). Hexagonal nodes are kinases which are also targets of drugs the cells are sensitive to. Nodes without kinase or drug target are predicted by the PCSF algorithm.

Connectivity score is significant for B-cells

Kinase predictions for 7 out of 10 DLBCL cell lines resulted in significant network connectivity score or inverse network connectivity score ($p < 0.07$). The latter score is the inverse of the median of the shortest paths. This formulation emphasizes regions of higher network density.
The scores depend on the parameters used in the prediction algorithm. These results demonstrate the method's utility to identify optimal parameter settings for the prediction algorithm.

Conclusions

We have developed a method to validate the biological relevance of kinase predictions from phosphorylation signatures obtained in a cellular context.
Future work will focus on applying this method to improve studies of the role of kinases in signal transduction by evaluating and optimizing the performance of kinase prediction methods and their potential biases.

References:
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