A functional approach to questions about life, death, and phosphorylation

The success of the family of kinases as targets for small-molecule cancer therapeutics is probably best illustrated by the efficacy of the drug Gleevec. In spite of this, the function of many of the kinases in the mammalian genome remains unknown. In a recent paper, MacKeigan and colleagues report a functional genetic screen using RNA interference to identify kinases and phosphatases involved in programmed cell death (MacKeigan et al., 2005). Functional annotation is a prerequisite for selection of new drug targets. Such studies may therefore lay the foundation for the next generation of cancer drugs.

Phosphorylation of biomolecules is relevant for the control of nearly all cellular processes. Consequently, the enzymes that are primarily responsible for regulation of the phosphorylation state of biomolecules, the kinases and phosphatases, are attractive targets for drug development. In oncology, the remarkable success of Imatinib (also known as Gleevec, a small mole inhibitor of the BCR-ABL tyrosine kinase) and Trastuzumab (also known as Herceptin, an antibody targeting the ErbB2 growth factor receptor) has sparked an intense search for kinases involved in cancer-relevant pathways, such as cell cycle, signal transduction, and programmed cell death (apoptosis). Even though there are no blockbuster drugs targeting protein phosphatases on the market today, the interest of drug developers in this enzyme family has followed in the slipstream of the success of the kinases. In spite of this, the function of many of the over 500 kinases and 200 phosphatases in the human genome is understood poorly, leaving a large treasure trove of potential cancer drug targets untapped. The recent discovery that RNA interference can also be used to suppress gene expression in mammalian cells (see Brummelkamp and Bernards, 2003, for a review) has provided a tool for large-scale loss-of-function genetic screens in higher eukaryotes. Such genetic screens using RNA interference have already been used in mammalian cells to identify genes involved in a number of biological processes, including mitosis, proteasome function, and p53 function. In a new study, MacKeigan and colleagues (MacKeigan et al., 2005) use RNA interference to ask which kinases and phosphatases are involved in regulation of programmed cell death.

The group of Blenis generated two independent siRNAs for each of 650 known and putative kinases as well as 222 known and putative phophatases. In a first cell-based screen, kinases were identified whose suppression caused a greater than 2-fold increase in apoptosis in HeLa cells ("survival kinases"). Remarkably, 73 of the 650 kinases (11%!) scored as survival kinases in this assay, of which *CDK6*, *RPS6KL1*, *ROR1*, and *NLK* were the most potent (Figure 1). Since not all siRNAs used in the assay might be functional, this number may even be an underestimation. Add to that the fact that the effects of kinase suppression on apoptosis may be cell type dependent and one realizes that it is difficult to say exactly how many survival kinases the human genome harbors.

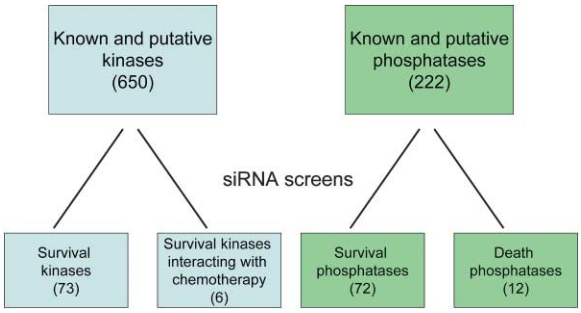

Figure 1. Functional annotation of kinases and phosphatases by RNA interference-based genetic screens in human cells

Using siRNA-based assays in human cells, a set of 650 known and putative kinases as well as 222 known and putative phosphatases were screened for their role in programmed cell death. A total of 73 kinases were identified whose suppression increased apoptosis ("survival kinases"). In a separate screen, a set of survival kinases was identified whose suppression sensitized cells to treatment with low-dose chemotherapy. Similar siRNA screens with a 222 member phosphatase knockdown library allowed the identification of 72 "survival phosphatases" and 12 "death phosphatases."

In a related genetic screen, it was found that inhibition of 72 out of 222 phosphatases (32%!) caused a greater than 2-fold increase in apoptosis (named "survival phosphatases"). Conversely, a screen was performed to identify "cell death phosphatases": phosphatases that act to sensitize cells to apoptosis. Such phosphatases are potential tumor suppressors, as their inhibition would confer resistance to apoptosis. To identify these phosphatases, a screen was set up in which HeLa cells were induced to undergo apoptosis by treatment with different chemotherapeutic agents and the phosphatase knockdown library was screened for inhibition of apoptosis. Among the 12 cell death phosphatases identified in this screen are *MK-STYX*, *PPP3CB*, *ACP6*, *PPP4R1L*, *PTPRS*, and *PTPRD* (Figure 1).

Perhaps more interesting from a drug discovery perspective was a fourth screen carried out by MacKeigan et al., in which they searched for kinases whose inhibition synergizes with chemotherapeutic agents in induction of apoptosis. Inhibition of such kinases may sensitize cancer cells to chemotherapeutic agents, providing a potential basis for rational combination therapy. In this screen, HeLa cells were treated with low doses of an apoptosis-inducing chemotherapeutic agent (taxol, cisplatin, or etoposide), and the kinase knockdown library was screened for kinases whose inhibition increased the rate of apoptosis induced by the drug. Here one would anticipate the identification of DNA damage signaling kinases such as *ATM*, *ATR*, and *CHK* kinases, which confer resistance to low levels of DNA damage. Surprisingly, however, this was not the case, perhaps because the screen was performed in a checkpoint-defective tumor cell line. Instead, *SGK* (an *AKT*-related kinase), *mTOR*, *CDK6*, *CDK8*,

FER, and *PINK-1* were found to sensitize cells to taxol. Interestingly, *mTOR* can be inhibited by rapamycin, suggesting that combining rapamycin and taxol might be an effective strategy in cancer therapy. The authors also note that *PINK-1* maps to a genetic locus implemented in familial Parkinaon's disease, which is marked by the death of neurons.

The study by MacKeigan et al. shows that inhibition of certain kinases and phosphatases can render tumor cells more vulnerable to chemotherapeutic agents. Does this mean that the kinases and phosphatases identified in the present study are suitable targets for cancer drug discovery? A major question that remains unexplored in the present study concerns the selectivity of the identified targets for cancer cells. Most cancer drugs are administered systemically, reaching both cancer cells and normal cells. If inhibition of the identified targets also sensitizes normal cells to chemotherapy, not much is gained. In addition, most cancer cells have mutations that prevent the induction of apoptosis, which could adversely affect the way in which cancer cells respond to the inhibition of the targets identified here. One salient example of such an unexpected outcome may be the finding in the present study that inhibition of the tumor suppressor gene *PTEN* by siRNA resulted in potent induction of cell death in cancer cells. Selectivity for cancer cells may therefore be more readily obtained by identifying "genotype-specific" drug targets, i.e., targets whose inhibition is only toxic to cells carrying a defined (cancer-specific) genetic lesion. This concept of "synthetic lethality" in cancer drug development was first proposed as early as 1997 (Hartwell et al., 1997), but has unfortunately remained a subject about which more reviews have been written than data published. Nevertheless, given the frequent occurrence of synthetic lethal interactions in yeast (Tong et al., 2004), such cancer-relevant genetic interactions will sooner or later be found in mammalian cells using techniques similar to those employed by MacKeigan et al.

Functional annotation of mammalian kinases and phosphatases is far from complete today, and the present study shows how powerful RNA interference-based genetic screens can be to place (families of) genes in cancer-relevant pathways. An interesting issue is that many of the kinases and phoshatases identified in the screens described above are not overexpressed or mutated in cancer. This emphasizes a comment made by Stephen Friend at a discussion session during the recent 96th AACR meeting in Anaheim that drug developers have in the past focused too much on genes that are either amplified or mutated in cancer. Therefore, studies like the one by MacKeigan et al. will help reveal innovative classes of drug targets that were not obvious from more classical cancer genetic studies.

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Selected reading

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