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Finding effective cancer therapies through loss of function genetic screens

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Intrinsic or acquired drug resistance often limits the success of cancer treatment. Loss of function genetic screens can help identify mechanisms of drug resistance and thereby deliver strategies to combat resistance. A further application of these genetic screens is the identification of drug targets whose inactivation is only effective in a specific context. This synthetic lethality approach enables the identification of drugs that act only in cancer cells having a cancer-specific mutation and the discovery of potent combination therapies. This review focuses on the question how functional genetic screens can help to improve the treatment of cancer.

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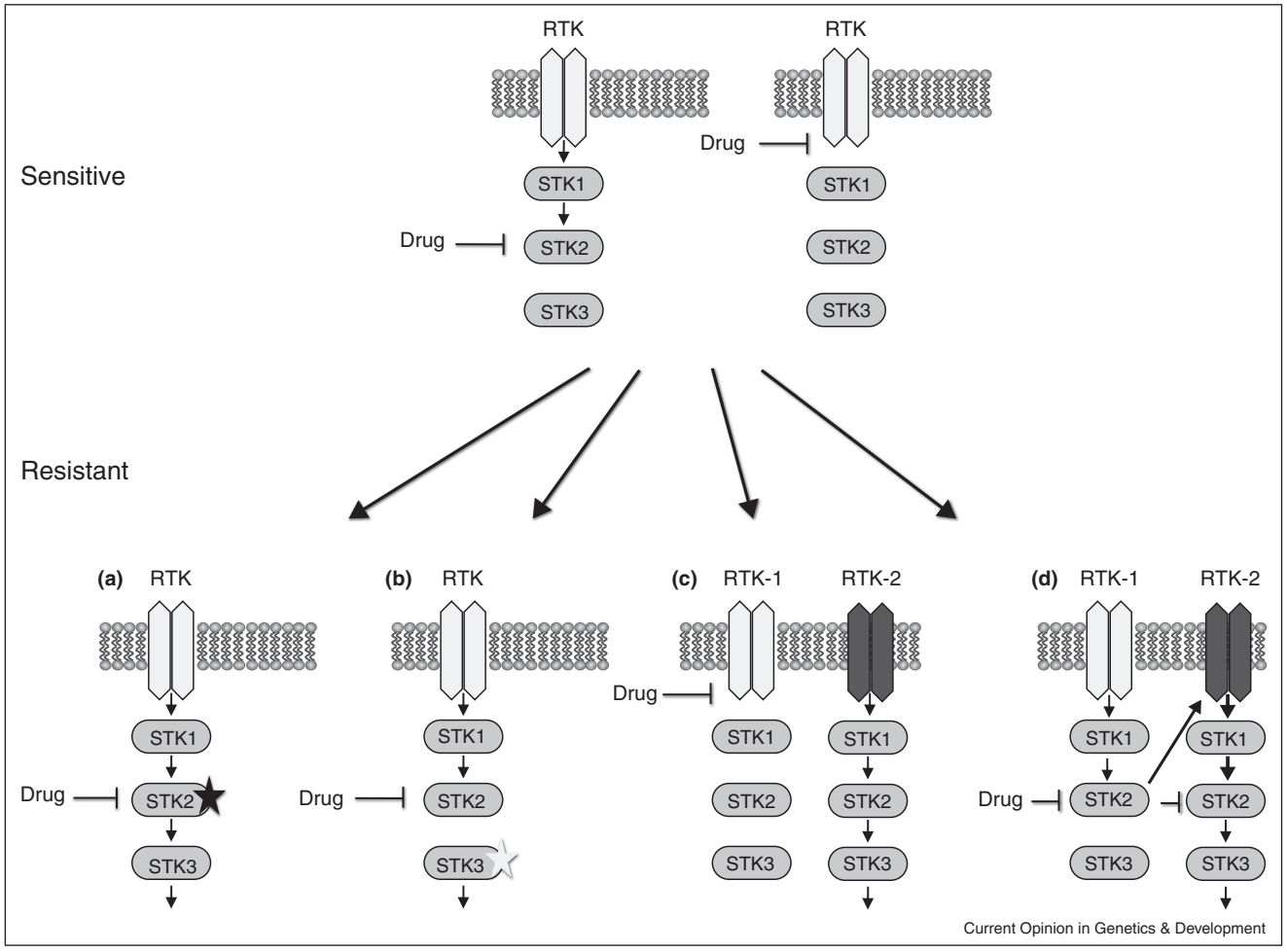
Predictable versus non-predictable drug resistance

Ever since the first use of chemotherapy for the treatment of cancer in the 1960s, investigators have searched for the mechanisms underlying drug resistance with the ultimate goal of fighting drug resistance through an understanding of its nature. The overall success of these studies has been modest, most likely due to the myriad ways in which cancer cells can evade broadly toxic chemotherapies. With the advent of the new generation of targeted cancer drugs this situation has changed. Through the highly selective targeting of specific pathways that are activated in cancer, the repertoire of escape routes available to the cancer cell is also more limited and we begin to see recurring patterns of resistance (Figure 1). The most straightforward resistance to targeted cancer drugs involves mutation of the drug target itself. Thus, during drug treatment of BCR-ABL-positive Chronic Myelogenous Leukemia (CML) with imatinib, mutation of the T315 residue of the ABL kinase is frequently selected for. Similarly in lung cancer, targeting of the activated EGFR with small molecule drugs leads to the frequent emergence of the T790M mutant EGFR that is drug insensitive. These predictable resistance mechanisms have enabled the development of second-generation ABL and EGFR inhibitors that also target these mutant alleles, providing a secondary treatment option once the resistance manifests clinically. A second trick up the sleeve of cancer cells is to activate the signaling pathway that is blocked by a targeted cancer drug downstream of the blockade. Such a strategy effectively re-activates the signaling pathway that is inhibited by the cancer drug. Examples of this strategy are the appearance of *KRAS* mutant alleles in colon cancer upon treatment with EGFR inhibitor [1••,2••] and the appearance of gain of function mutations in the *MEK1* kinase in melanoma upon inhibition of the activated *BRAF(V600E)* oncogene [3]. Again, such predictable resistance mechanisms enable the design of effective second line therapies and more effective combination therapies aimed at preventing (or slowing down) the emergence of resistant variants. A third strategy employed by cancer cells is the activation of a parallel pathway to bypass the block imposed by a targeted agent. Thus, amplification of the *MET* receptor tyrosine kinase (RTK) has been seen in NSCLC patients treated with EGFR inhibitors [4]. Since EGFR and MET share downstream signaling pathways, these same pathways are now reactivated through MET signaling while EGFR signaling remains blocked by the drug treatment. A fourth approach that is seen repeatedly in drug resistant cancer is the re-activation of the inhibited signaling pathway upstream of the drug's effect in an attempt to evade pathway inhibition through hyperactivation of the inhibited pathway. Thus, inhibition of oncogenic BRAF signaling in melanoma can be countered by the upregulation of RTKs that signal through the RAS-RAF-MEK cascade [5].

RNAi to discover drug resistance mechanisms

RNA interference (RNAi) is a powerful tool to silence gene expression, whose effectiveness was first documented in somatic mammalian cells a dozen years ago [6]. Since then the technology has proven to be extremely powerful to uncover the molecular pathways that cancer cells use to develop drug resistance. The RNAi reagents can be either synthetic siRNAs that are produced *in vitro* or vector-encoded shRNAs that are cleaved intracellularly into siRNA-like molecules. When using siRNA reagents, individual siRNAs are introduced into the cancer cells through transfection in a multi-well format. SiRNA reagents only cause transient silencing of gene expression (lasting no more than on average 3–5 days). Not all cell

Figure 1



Mechanisms of resistance to targeted cancer drugs. Four recurrent mechanisms of resistance to targeted cancer drugs are schematically represented. Thickness of arrows represents strength of signal through a pathway. **(a)** Mutation of the drug target confers resistance to the drug and restores signaling through the pathway. RTK: receptor tyrosine kinase. STK: serine/threonine kinase. **(b)** Mutation of a component downstream of the drug target (black star) restores signaling through the pathway in the presence of drug. **(c)** *De novo* expression of a RTK circumvents a blockade of a RTK by a cancer drug and leads to re-activation of the same signaling pathway downstream of the RTK. **(d)** Inhibition of a downstream component of a RTK signaling pathway can lead to activation of a second RTK, which hyper-activates the signaling cascade inhibited by the cancer drug. As a consequence, the drug is now unable to fully inhibit the signaling through the pathway, allowing cell proliferation in the presence of drug. A variation on this theme is the activation of its own upstream RTK by the inhibition of a downstream STK.

types are equally efficient in taking up siRNAs following transfection, which somewhat limits the applications of this approach. On the other hand, viral delivery of shRNAs has the ability to deliver long-term gene silencing and, depending on the viral vector used, and allows infection of a wide range of cells, including primary cell cultures.

In the simplest iteration of an RNAi genetic screen, a collection of siRNA or shRNA reagents is introduced to silence a set of genes in a drug-sensitive cell line and drug resistant cells are identified (Figure 2). The most cost-effective way of performing such screens is through the use of pooled shRNA screening. In this method, thousands of different shRNAs are introduced into a drug-sensitive cancer cell line that is then exposed to the cytotoxic or cytostatic drug. The cancer cells that become drug-resistant by virtue of expression of a specific shRNA will continue to divide, while the majority of the population enters a growth arrested (or dies) due to drug treatment. As a result of the continued proliferation of the resistant cells, the shRNAs causing resistance will be enriched in the drug treated population. The relative abundance of the specific shRNAs can be determined by PCR amplification of the shRNA from genomic DNA followed by deep sequencing. Owing to the well-established off-target effects of RNAi, it is recommended that only those genes are studied for which multiple