Functional genetic screens to identify cancer relevant genes

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Introduction

A general picture of the function of individual genes in both the healthy and diseased states of human tissues will suggest new therapeutic targets for the treatment of human disease. One of the ways in which such an overview can be obtained is through functional genetic approaches. The completion of the sequence of the human genome and the ability to silence gene expression by RNA interference (RNAi) in mammalian cells opens the possibility to perform systematic inactivation of large numbers of human genes.

Loss of function genetic screens

The most important feature of an ideal anti-cancer therapeutic is its ability to selectively target cancer cells while leaving normal non-malignant cells untouched. Many of the currently used cancer drugs are rather non-cancer-specific, acting indiscriminately on all rapidly dividing cells. Epigenetic and genetic alterations within cancer cells, while endowing these with increased abilities to survive and proliferate, may also generate specific weaknesses. Over the last 10 years we have gained insight in a number of signalling pathways that are frequently deregulated in human cancer. These are pathways that control cancer relevant processes such as cell proliferation, cell survival, cell differentiation and metastasis. Obviously, components of such signalling pathways receive broad attention of both cancer researchers and pharmaceutical companies and the search for new components of such signalling pathways is an important aspect of their research efforts. Nowadays, with the ability to silence thousands of human genes in cultured human cells in a single experiment by RNA interference, loss of function screens can be carried out to discover cancer cell vulnerabilities faster than ever.

RNA interference

In several organisms, introduction of double-stranded RNA has proven to be a powerful tool to suppress gene expression through a process known as RNA interference. In the recent past we have focused on the development and validation of tools that allow efficient loss-of-function genetic screens in mammalian cells [1]. This resulted in the generation of a vector system that uses a RNA-polymerase III promoter to direct the synthesis of short hairpin RNA (shRNA) molecules, which are processed intracellular into siRNA-like molecules and direct gene silencing through RNA interference [2]. The shRNAs contain a double-stranded stem of 19–29 base pairs, which is identical in sequence to the mRNA that is targeted for suppression, connected by a loop of 6–9 bases, which is efficiently removed *in vivo*. Expression of shRNAs has nowadays turned into a widely used technique for cell biologists to study gene function. Vector-produced shRNA molecules have the advantage that they can be used to study loss-of-function phenotypes that develop over longer periods of time, which can be important especially if one wants to study cancer relevant phenotypes. Furthermore, stable integration of these expression cassettes in the genome can be efficiently achieved through retroviral delivery [3]. We have generated and used collections of shRNA vectors (Nki shRNA library) to conduct genetic screens in human cells to study cancer relevant processes. For example we have studied the family of de-ubiquitinating enzymes resulting in the identification of the cylindromatosis tumour suppressor gene as a regulator of NF-κB activity [4] and USP1 as a negative regulator of the Fanconi Anaemia pathway [5]. Likewise we have identified several new regulators of the p53 pathway using a library that targets 8000 human genes [6]. Moreover we have developed a sophisticated approach to rapidly screen shRNA expression libraries, named shRNA bar code screens (described in more detail below) that will be useful for studying drug mode-of-action for cancer therapeutics.

shRNA bar code screens

To study the role of individual gene products in the response of tumour cells to therapeutics we have developed a technique that allows efficient loss-of-function genetic screens in mammalian cells named RNAi bar code screens. This technique is based on the notion that expression of short hairpin RNA (shRNA) molecules in mammalian cells by stably integrated vectors not only creates a gene-specific knockdown phenotype, but also introduces a gene-specific fingerprint (molecular barcode) in cells that express these siRNAs. In short, DNA microarrays are used to follow the relative abundance of each shRNA vector in a large population of shRNA vector library-infected cells under a particular stress condition. In the current approach we are able to use a mixed culture of 24,000 different RNAi knockdown genotypes that we expose to a signal of interest. Subsequently, genomic DNA is isolated and used to determine the relative abundance of all 24,000 knockdown genotypes in the population by array hybridisation of DNA barcodes. Such array hybridisations elucidate which knockdown genotypes are more sensitive or less sensitive to the treatment and thereby provide an overview of which cellular components are important for the response to the signal of interest.

Recently we have introduced 24,000 RNAi knockdown constructs into MCF-7 breast cancer cells and exposed this mixed culture to a drug that inhibits MDM2 (Nutlin-3). Nutlin-3 is a small molecule inhibitor of MDM2, which can activate the p53 pathway. Nutlin-3 displays strong anti-tumour effects in mice with surprisingly few side-effects on normal tissues. We performed a large-scale RNA interference screen to identify cellular factors that are required for the anti-cancer effects of Nutlin-3. Quantification of the knockdown genotypes after drug exposure revealed that not only p53 was required for the cytotoxic effects of Nutlin-3, but for instance also a cellular factor, named 53BP1 [7]. Interestingly, 53BP1 is part of a DNA damage-induced signalling network that is frequently activated in cancer, but not in healthy tissues [8–10]. These results suggest that Nutlin-3's tumour specificity results from its ability to turn a cancer cell-specific property (activated DNA damage signalling) into a weakness that can be exploited therapeutically. This study demonstrates the powerful application of RNAi bar code screens in elucidating mechanism of action of cancer drugs.

Conflict of interest statement

None declared.

References

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