

 CANCER

Identifying drug–genotype interactions

Mutations in oncogenes and tumour suppressor genes that are responsible for tumorigenesis represent potential therapeutic targets in oncology. Although current strategies to study cancer mutations in human cells have yielded positive results, in general these approaches do not accurately recapitulate the occurrence of cancer mutations in human tumours. Now, Di Nicolantonio and colleagues present a new approach to introduce cancer mutations endogenously into the genome of human cells that could provide a more accurate and patient-relevant pharmacogenomic platform for the design of targeted anti-cancer therapies.

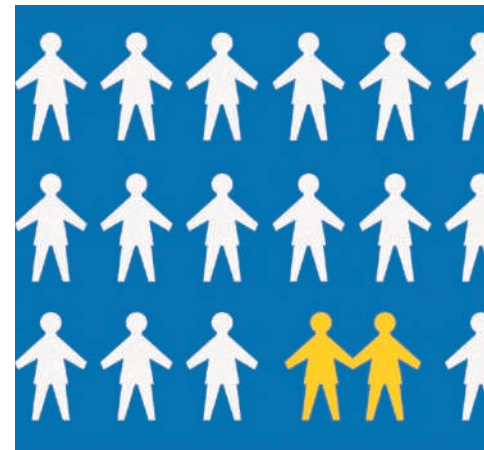
The authors used targeted homologous recombination to introduce (or ‘knock in’) a panel of cancer alleles into the genome of human cells by stable modification of the corresponding genomic locus. They focused on mutated alleles of *EGFR*, *KRAS*, *BRAF* and *PIK3CA* that are found in multiple cancer types. Mutant knock-in cells were then used in cell viability assays to produce genotype-specific pharmacological profiles using a panel of over 90 compounds, including Food and Drug Administration (FDA)-approved drugs, tyrosine kinase inhibitors and drugs currently in oncology clinical trials. The data were analysed using data-clustering algorithms to visualize mutation-specific phenotypes.

The vast majority of the drugs did not show selectivity towards

any specific genotype. However, the approach identified a number of drug interactions that were genotype-specific and that reflected the signalling pathways in which the corresponding oncogenic mutations are known to act: a distinct set of compounds, including the *EGFR* tyrosine kinase receptor inhibitors gefitinib and erlotinib, clustered according to their ability to selectively inhibit *EGFR*-mutated cells.

To assess whether the response of the knock-in cells to targeted drugs recapitulates that of naturally occurring cancer cells carrying equivalent cancer mutations, the authors performed an analysis of the effect of erlotinib on *EGFR*-knock-in cells in multiple cellular backgrounds. This drug preferentially inhibited the growth of cells with the *EGFR* mutant allele, with ten times the potency that it had in corresponding wild-type cells. Gefitinib showed a similar selectivity pattern, demonstrating that knock-in cells display drug responses resembling those of tumours carrying equivalent mutations. Further investigations into the mechanism responsible for the pronounced effect of erlotinib on the viability of *EGFR*-knock-in clones suggested that oncogenic addiction — the process by which cancer cells are dependent on the activity of specific oncogenes for maintenance of their malignant phenotype — was recapitulated in these clones.

Finally, the authors used the sequential introduction of two



mutations to model drug resistance. When double-knock-in clones carrying *EGFR* and *PIK3CA* mutations were treated with gefitinib and erlotinib, the presence of both mutations abrogated the sensitization seen with the *EGFR* knock-in alone. This showed the potential of the knock-in strategy to be used in the construction of tumour-progression models.

As knowledge of how oncogenic alleles affect selectivity and resistance to drugs is key to developing individualized therapies, this strategy could be exploited to identify new drugs or drug combinations that match the genetic profile of individual tumours.

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ORIGINAL RESEARCH PAPER Di Nicolantonio, F. *et al.* Replacement of normal with mutant alleles in the genome of normal human cells unveils mutation-specific drug responses. *Proc. Natl Acad. Sci. USA* **105**, 20864–20869 (2008)