RESISTANCE

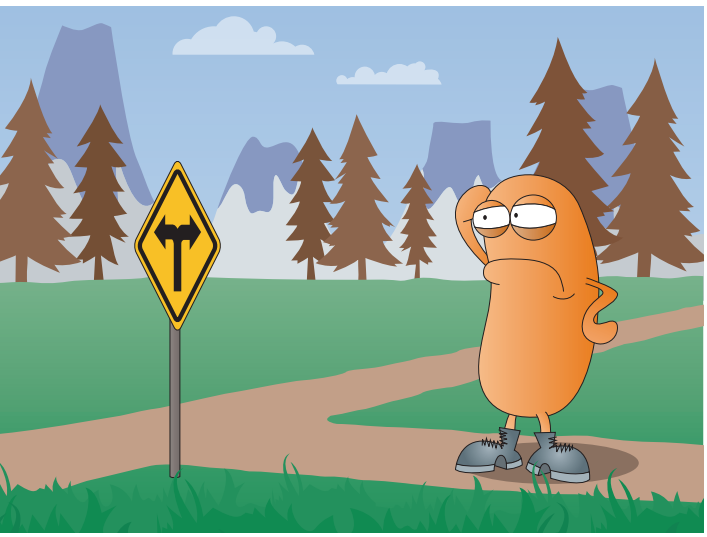
Following different paths

> mutations that promote drug resistance can be both pre-existing and acquired *de novo*

Two general pathways of resistance to targeted therapies have been proposed: the selection of clones carrying a pre-existing resistance mutation, or the eventual *de novo* development of a resistance mutation in cells that survive initial therapy (for example, through epigenetic adaptation or microenvironmental stimuli). Although evidence has been reported to support the first model, direct evidence of the second is lacking. Hata, Niederst *et al.* present data suggesting that genetic resistance to the epidermal growth factor receptor (EGFR) inhibitor gefitinib can develop through both paths.

The authors previously developed gefitinib-resistant cell lines from *EGFR*-mutant non-small-cell lung cancer (NSCLC) PC9 cells, and observed differences in the time required for the acquisition of the $EGFR^{T790M}$ gatekeeper mutation; for example, PC9-GR2 cells developed resistance in 6 weeks whereas PC9-GR3 cells required 24 weeks. To examine this further, they cultured >1,200 small pools of parental PC9 cells in the presence of gefitinib for 2 weeks. This resulted in two classes of surviving cells: rapidly growing 'early-resistant' colonies and small 'intermediate-resistant' colonies of drug-tolerant cells. Early-resistant colonies all carried the $EGFR^{T790M}$ mutation; these were derived from rare pre-existing $EGFR^{T790M}$ cells in parental PC9 cells that were selected by gefitinib treatment.

The drug-tolerant intermediate-resistant cells did not carry the $EGFR^{T790M}$ mutation. However, further long-term (10–30 weeks) culture in gefitinib led to the development of 'late-resistant' cells, several clones of which had acquired $EGFR^{T790M}$, suggesting that this mutation can arise *de novo* following prolonged exposure to gefitinib. Other resistant clones remained $EGFR^{T790M}$-negative, and the authors identified various mutations in other oncogenes (for example, *KRAS*, *BRAF* and *RET*) that might account for this resistance. Further experiments using PC9 cell subclones derived from single cells (to eliminate any pre-existing $EGFR^{T790M}$ cells) confirmed that resistant cells carrying $EGFR^{T790M}$ can arise from drug-tolerant cells that do not initially carry this mutation. Similar results were observed in another patient-derived NSCLC cell line that did not carry an initial $EGFR^{T790M}$ mutation. Although these results seemed surprising, given the timescales and initial cell population sizes, mathematical modelling predicted that the $EGFR^{T790M}$ mutation could indeed arise over a time course of several months.



Philip Patenall/NPG

Late-resistant cells had transcriptional profiles similar to those of intermediate-resistant drug-tolerant cells. Furthermore, intermediate- and late-resistant cells had decreased sensitivity to apoptosis induced by the third-generation EGFR inhibitor WZ4002 compared with early-resistant cells, suggesting that cells in which resistance arises later are less reliant on EGFR signalling for survival. The authors then examined several other $EGFR^{T790M}$-positive NSCLC cell lines derived from patients who had developed EGFR inhibitor resistance; these had differing sensitivities to WZ4002 *in vitro* and in xenograft models, suggesting that these different paths of resistance might be clinically relevant. Interestingly, the cells that were least sensitive to WZ4002 were derived from patients who had a long duration of response to initial EGFR inhibitor therapy, indicating that perhaps these cells arose from drug-tolerant cells *in vivo*. In addition, the combination of WZ4002 and navitoclax, which inhibits anti-apoptotic BCL-2 family proteins, could induce apoptosis of late-resistant cells and regression of xenograft tumours derived from these cells, suggesting a possible therapeutic strategy.

Overall, these data support the idea that mutations that promote drug resistance can be both pre-existing and acquired *de novo*. Furthermore, as genetic resistance might arise from small populations of drug-tolerant cells in patients, this argues that it is important to develop therapeutic strategies to eliminate these cells before resistance develops.

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