*Nature Reviews Cancer* | Published online 29 Apr 2016; doi:10.1038/nrc.2016.50

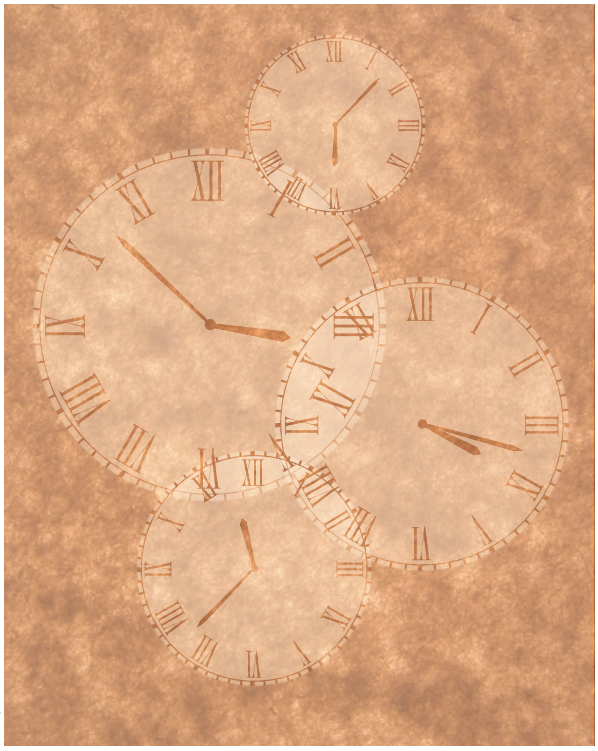
CHEMOTHERAPY

# Clocking up resistance

> “cells… gradually accumulate p53 at different rates after drug treatment”

Research using time-lapse imaging has revealed a mechanism underlying how cancer cells of the same or similar genotype can display different susceptibilities to chemotherapy. Paek *et al.* report that the level of p53 required to induce apoptosis in human colon cancer cells increases with time following treatment with DNA damage-inducing agents. This increase, moreover, appears to be dependent on the chemotherapy-induced increase in expression of inhibitor of apoptosis (IAP) proteins, which compete with p53 to promote survival.

Resistance to chemotherapy can be caused by fluctuations in protein levels that occur in response to treatment. As a result, isogenic populations of cancer cells can have heterogeneous responses to chemotherapeutic agents, undermining the effectiveness of these drugs. p53 is induced in response to DNA damage, and p53 levels have been linked to cell fate decisions, with low levels associated with cell-cycle arrest and higher levels associated with apoptosis. Here, the authors investigated whether differences in p53 levels over time influenced the ability of individual human colon cancer cells to survive treatment with DNA damage-inducing chemotherapeutic drugs.



Philip Patenall/Lara Crow/NPG

Fluorescently tagged p53 was used to track relative p53 levels at the single-cell level in a human colon cancer cell line. Cells were imaged over 72 hours in the presence of cisplatin, which induces DNA crosslinking. p53 dynamics were then correlated with if and when individual cells underwent apoptosis. Cells were found to gradually accumulate p53 at different rates after initiation of drug treatment, before either undergoing apoptosis or surviving to the end of the assay. Intriguingly, although apoptosis was linked to a particular p53 threshold, this threshold increased with time after initiation of drug treatment, such that cells that accumulated p53 more quickly required lower levels of the protein to trigger apoptosis than cells that were slower to accumulate p53. The same trends were observed with the DNA-damage inducing drugs camptothecin and etoposide.

Hypothesizing that increases in anti-apoptotic proteins in response to chemotherapy might be responsible for this time-dependent effect, the authors used previously published microarray data from colon cancer cells treated with cisplatin. Indeed, expression of three IAP genes — *BIRC2*, *BIRC3* and *BIRC7*, which encode cIAP1, cIAP2 and ML-IAP, respectively — increased significantly in response to cisplatin.

Next, overexpression of these IAP proteins, along with an additional IAP, XIAP, increased the resistance of colon cancer cells to cisplatin. Similarly, CRISPR-mediated knockout of *BIRC2* and *BIRC3*, and the broad inhibition of IAP proteins with the small molecule LCL-161 both significantly decreased cell viability in response to DNA-damaging agents in a p53-dependent manner. Moreover, this decrease in viability was associated with a lowering and flattening of the p53 threshold needed to trigger apoptosis, indicating that IAPs increase the level of p53 required to induce apoptosis.

These findings could have important implications for the design of combination chemotherapeutic regimens. Indeed, when the authors increased p53 levels in cisplatin-treated cells using the stabilizing molecule Nutlin-3, the greatest increase in cell death occurred when cells were treated early after chemotherapy application, indicating that timing of the use of different anticancer agents relative to pro-apoptotic and anti-apoptotic protein levels could be crucial to achieving cancer cell killing.

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**ORIGINAL ARTICLE** Paek, A. L. *et al.* Cell-to-cell variation in p53 dynamics leads to fractional killing. *Cell* http://dx.doi.org/10.1016/j.cell.2016.03.025 (2016)