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PROTEOMICS

Insider information

Chemotherapy might be the gold-standard treatment for the majority of cancers but how much do we really know about what happens inside a cell once a drug has been added? "Not enough," argue Ariel Cohen and colleagues, who assert that a clearer understanding of protein dynamics in response to drug treatment might shed light not only on how drugs function, but also on why apparently identical cells respond differently following treatment. Guided by this philosophy, the team from Uri Alon's laboratory has recently set up an elegant system allowing high temporal resolution of individual proteins following drug treatment.

Using the H1299 human lung cancer cell line, the authors constructed a library of over 1,200 single cell clones, each expressing a different fluorescently tagged protein from its endogenous chromosomal location. Clones were expanded individually and incubated in the presence of camptothecin for 48 hours. Using fluorescence microscopy, each tagged protein could be monitored for changes in concentration (measured by the strength of the fluorescent signal) and localization in real time.

Satisfyingly, topoisomerase 1 — the molecular target for camptothecin — was one of the first proteins observed to respond, displaying both rapid degradation and a change in localization from the nucleolus to the cytoplasm, in agreement with previous reports. Overall, translocation events were relatively rare (~2% of the proteins relocated following camptothecin treatment), but the authors noted that these proteins displayed similar dynamics in response to the transcription inhibitor actinomycin D, leading them to propose that, in addition to causing DNA breaks, camptothecin might also function by inhibition of transcription.

The team also observed that the behaviour of many proteins, including several associated with cell death pathways, showed significant cell–cell variability, but was this biologically meaningful? To address this question, they asked whether any of these differences correlated with cell fate and found that decreases in the levels of the RNA helicase DDX5 and the replication factor RFC1 following camptothecin treatment were strongly associated with cell death, whereas cells which did not show these decreases tended to survive. Moreover, small interfering RNA-mediated downregulation of DDX5 activity increased camptothecin-induced cell death 2–3-fold. Taken together, these observations implicate both of these proteins in mediating cellular resistance to camptothecin, although further experimentation will be required to pinpoint their precise roles.

The authors argue that their strategy provides a valuable insight into the spatial and temporal effect of a drug at the level of the individual protein, which is essential for a complete appreciation of drug function and cellular resistance. Although further studies are undoubtedly needed to achieve this aim, their work is a step in the right direction.

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ORIGINAL RESEARCH PAPER Cohen, A. A. *et al.* Dynamic proteomics of individual cancer cells in response to a drug. *Science* 20 Nov 2008 (doi:10.1126/science.1160165)