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Mapping pathways to drug resistance with proteomics

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Emerging drug resistance thwarts progress in chemotherapy, resulting in increased morbidity, mortality and healthcare costs. Understanding the mechanisms by which drug resistance phenotypes emerge is important to prolong the useful life of existing drugs but may also highlight pathways that play a role in the acquisition of resistance and which may themselves present resistance-proof drug targets. Comparative proteomic approaches have demonstrated potential to link drug resistance phenotypes to molecular changes but will also prove powerful in the elucidation of the mechanisms by which drug resistance arises.

How can pathogens respond to drug exposure?

Pathogens encounter diverse physiological changes and environmental challenges as they transit through their lifecycles. For a pathogen, drug exposure is an environmental stress to which a response must be raised. At the molecular level, a drug perturbs some aspect of metabolism with deleterious consequences that may prove fatal depending on the level of drug exposure (time and concentration). However, for all drugs, there will be sublethal concentrations and there are many reasons why an effective drug concentration may not be achieved for a sufficient period of time. Furthermore, within a population of pathogens, some individuals may be less susceptible than others. If individuals are not killed by drug exposure, then drug challenge may be met by adaptive responses, which render the organism less susceptible to drug toxicity. These changes may enable a pathogen to survive transient drug exposure or to adopt a niche where drug concentrations are lower. The ability to survive drug exposure makes possible the selection of drug resistance, a process that is facilitated by the relatively small genome size and rapid generation time of microbes.

Pathways to drug resistance

Stable drug resistance is typically conferred through changes to a specific drug target, detoxifying enzyme or transport system, or by elaboration of alternate metabolic pathways [1]. Such phenomena can be mediated by single genes or operons, so there is often potential for advantageous drug resistance phenotypes to spread by horizontal gene transfer. However, the *de novo* evolution of resistance in a naïve population may be preceded by complex adaptive processes that enable individual organisms to survive drug challenge long enough for beneficial mutations to emerge. These adaptations may include lowering metabolic activity, thus reducing the consequences of target inhibition, or behavioral modifications, such as biofilm formation or the colonization of a privileged niche, thus reducing the encountered drug concentration. In a population, it appears that some individuals, known as persisters, have the capacity to survive drug exposure. Persisters are neither drug resistant nor are they a genetically distinct subpopulation, but they can enable an infection to recrudesce after drug pressure is removed [2]. Persistence seems to be triggered by environmental stresses such as nutrient deprivation and oxidative stress, and also by the stress of drug exposure [3]. How these adaptations are elicited by sublethal drug exposure is unclear, but it might be anticipated that common pathways could be modulated in response to

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stress resulting from different drugs. Furthermore, the induction of stress responses has been associated with increased rates of mutagenesis, potentially accelerating the emergence of drug resistance.

How do drugs kill cells?

A global analysis of the cellular response by *Bacillus subtilis* to 30 antimicrobial compounds was achieved through 2D gel analysis [4] and revealed overlapping profiles of protein modulation for drugs known to have related mechanisms of action, such as tetracycline and chloramphenicol. Moreover, the approach suggested mechanisms of action for novel compounds, based on similarity in the proteomic responses to drugs with known mechanisms of action. However, this study did not highlight a common stress response elicited by drugs with divergent mechanisms, perhaps because drug exposure was typically at a concentration of 5- to 10-times higher than the minimum inhibitory concentration, and the cells were thus likely on a pathway to death rather than adaptation.

It has been suggested that the pathways leading to cell death have common features regardless of the primary drug: target interaction. Van Bogelen *et al.* [5] found that antibiotics targeting the prokaryotic ribosome either elicit a heat-shock response in *Escherichia coli* or repress heat-shock proteins in favor of a cold-shock response. The magnitude of the response reflected the drug concentration employed, suggesting that the ribosome may have a role in responding to temperature shift and that pharmacological interactions can trigger this response without temperature change. This provides an example of how drug exposure can trigger preprogrammed physiological changes that might potentially modulate drug sensitivity. More recently, Kohanski *et al.* [6] found that exposure of *E. coli* to three different bactericidal antibiotic classes, represented by kanamycin, norfloxacin and ampicillin, led to upregulation of genes involved in NADH-coupled electron transport and triggered the production of hydroxyl radicals, which could result in cell death as a result of oxidative damage to DNA, lipids and proteins. The production of reactive oxygen species certainly does not explain all antibiotic activity as the same three antibiotics can also kill bacteria in the absence of oxygen [7,8], but a loss of redox control is often a consequence of metabolic perturbation, and thus, a likely consequence of drug exposure. Resistance to oxidative stress might thus buy time to allow for the selection of specific resistance mechanisms in drug-exposed organisms [9], particularly where pharmacodynamic reality results in exposure to sublethal concentrations.

For example, *Leishmania infantum* parasites, selected with gentamicin below the minimum inhibitory concentration, showed complex changes in components of the thiol redox control system [9]. The selected parasites were more susceptible to oxidative stress, suggesting an adaptation which, while it may contribute to drug tolerance, compromises parasite fitness.

Studies such as those described above report on the molecular changes that are triggered by lethal drug exposure or that accompany the acquisition of drug resistance. Such work could be extended to investigate the adaptive changes that are provoked in the initial response to sublethal drug exposure by sampling pathogens from cultures maintained in sublethal drug concentrations. Complex adaptations will best be approached through global, systems-level analysis [10] but, because they do not necessarily involve genetic changes, may not be evident in comparative genomic analyses. Adaptive changes may be highlighted by transcriptomic analyses, which have the major advantage of global coverage, but the important role of post-translational modifications in protein function makes proteomic or metabolomic analysis particularly attractive. The relatively simple proteomes and metabolomes that are elaborated by *in vitro* cultivated microbial pathogens should facilitate comprehensive coverage of key pathways [11], enabling characterization of the early adaptive events that are precursors to the selection of drug resistance. The application of systems biology approaches to understand the emergence of drug resistance holds the promise of revealing new drug targets and of strategies to limit the emergence and spread of drug resistance. The pathways that enable a population to survive drug exposure long enough to evolve drug resistance may themselves present resistance-proof drug targets or offer opportunities to avert the selection of resistance to more selective drugs.

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