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Uncovering Scaling Laws to Infer Multidrug Response of Resistant Microbes and Cancer Cells

Kevin B. Wood,[1] Kris C. Wood,[2,3] Satoshi Nishida,[1] and Philippe Cluzel[1,*]
[1]FAS Center for Systems Biology, Department of Molecular and Cellular Biology, School of Engineering and Applied Sciences, Harvard University, 52 Oxford Street, Cambridge, MA 02138, USA
[2]Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, MA 02142, USA
[3]Present address: Department of Pharmacology and Cancer Biology, Duke University School of Medicine, Durham, NC 27710, USA
*Correspondence: cluzel@mcb.harvard.edu
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SUMMARY

Drug resistance in bacterial infections and cancers constitutes a major threat to human health. Treatments often include several interacting drugs, but even potent therapies can become ineffective in resistant mutants. Here, we simplify the picture of drug resistance by identifying scaling laws that unify the multidrug responses of drug-sensitive and -resistant cells. On the basis of these scaling relationships, we are able to infer the two-drug response of resistant mutants in previously unsampled regions of dosage space in clinically relevant microbes such as *E. coli*, *E. faecalis*, *S. aureus*, and *S. cerevisiae* as well as human non-small-cell lung cancer, melanoma, and breast cancer stem cells. Importantly, we find that scaling relations also apply across evolutionarily close strains. Finally, scaling allows one to rapidly identify new drug combinations and predict potent dosage regimes for targeting resistant mutants without any prior mechanistic knowledge about the specific resistance mechanism.

INTRODUCTION

Treatment strategies for infectious diseases and cancers often involve multiple drugs that must be combined, adapted, and refined to target evolving cell populations. Multidrug therapies can be difficult to design because drugs often interact, making their combined effects larger or smaller than expected from their individual effects (Bliss, 1956; Fitzgerald et al., 2006; Greco et al., 1995; Keith et al., 2005; Lehár et al., 2008; Loewe, 1953). Furthermore, well-developed multidrug treatments can be thwarted by the emergence of multidrug resistance, which arises in both bacterial infections and cancer, and represents a growing public health threat (Levy and Marshall, 2004). For example, potent drug regimens designed to target a particular cancer may be rendered ineffective by the rapid evolution of drug resistance (Garrett and Arteaga, 2011; Glickman and Sawyers, 2012; Poulikakos and Rosen, 2011). In addition, drugs may interact differently in each new resistant mutant, making the molecular characterization of resistance a time-consuming and at times untenable goal. Because of the rapidly increasing number of multidrug-resistant mutants, there is a significant need for new strategies to characterize and refine drug regimens in hopes of mitigating the effects of resistance.

Scaling laws can offer a complementary approach for simplifying the picture of multidrug-resistance without relying on highly time- and resource-consuming molecular studies. These laws, which can be surprisingly simple, are often based on symmetry arguments rather than system-specific microscopic details. Scaling is powerful because it offers a quantitative unifying framework for systems that appear, on the surface, to be very different. For example, allometric scaling laws (Shoval et al., 2012) connect anatomical and physiological features, such as body mass and metabolism, across a wide range of organisms. Similar relations have contributed to our understanding of phenotypic variability in populations of bacteria (Balaban et al., 2004) and eukaryotic immune cells (Feinerman et al., 2008), the fluctuation-response relationship in bacterial chemotaxis (Park et al., 2010), the structural properties of metabolic networks (Jeong et al., 2000), growth and gene expression in populations of *Escherichia coli* (Scott et al., 2010), and epistatic interactions between genes in yeast (Velenich and Gore, 2013). Motivated by the success of scaling laws across disciplines, we set out to identify similar principles that could unify the description of drug interactions in sensitive and resistant cells. The discovery of such scaling relations could provide an approach for systematically adapting multidrug treatments to effectively combat drug resistance, even before the molecular mechanisms have been fully elucidated.

RESULTS

Drug Interactions Can Change following Acquisition of Resistance

We first asked how acquired drug resistance affects the interactions between two drugs observed initially in wild-type (WT)

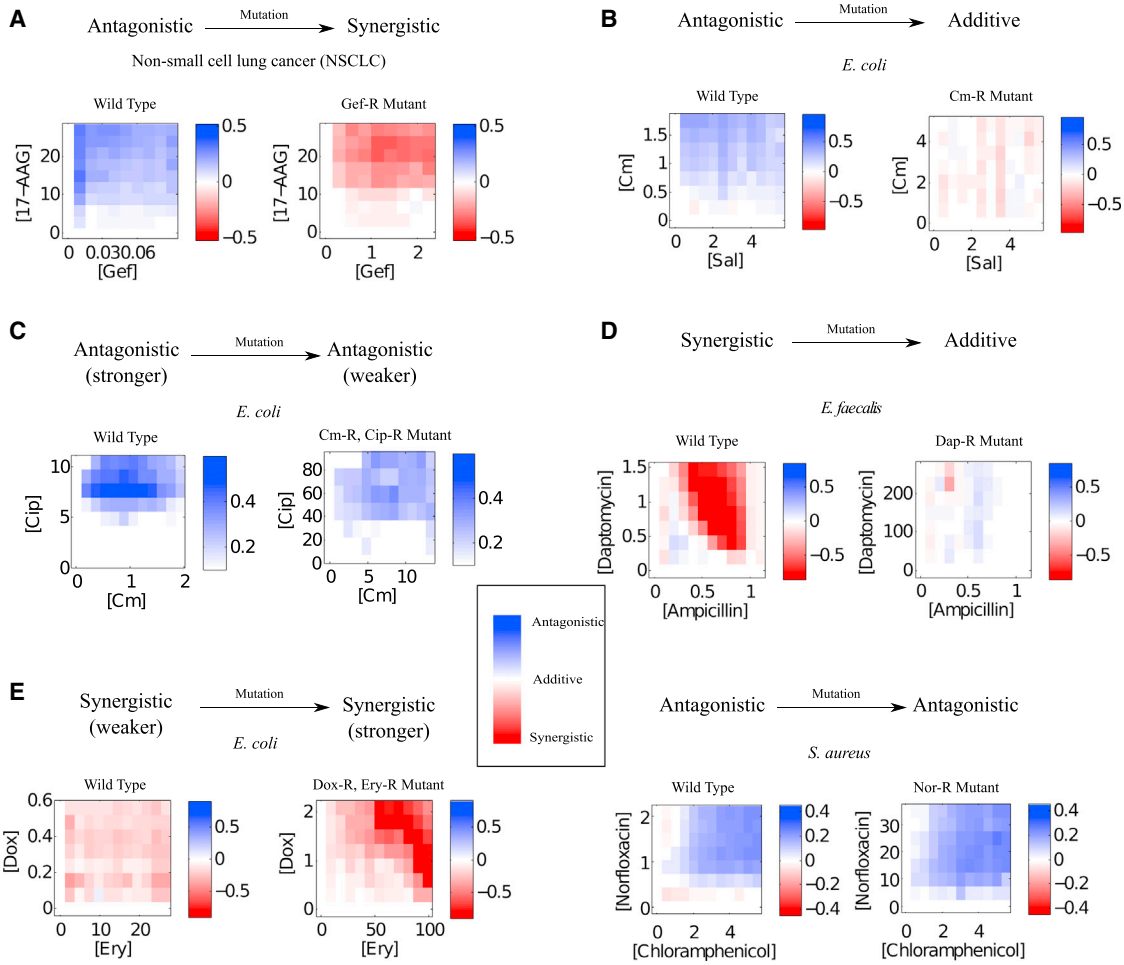

Figure 1. Resistance that Either Alters or Conserves Interactions between Drugs in Prokaryotic and Eukaryotic Cells

Heatmaps quantify the drug interaction and classify it as synergistic or antagonistic across a range of active concentrations for both WT and mutant cells. To quantify the drug interaction at each point on the response surface, we define the interaction parameter $I = \log_2(g_{12} - g_1\, g_2 + 1)$, which is positive (blue) for antagonistic, negative (red) for synergistic interactions (Bollenbach and Kishony, 2011), and zero when there is no interaction ($g_{12} = g_1\, g_2$, consistent with Bliss independence). In addition to modifying the resistance of cells to one or more drugs, resistance events can sometimes modify the interactions between drug pairs. See Figure S1 for an alternative quantification of drug interactions. We note that because the mutants in this study are resistant to at least one drug, we must use higher drug concentrations for the mutant cells to obtain growth reduction. However, we estimated the drug interactions over concentration ranges that yield approximately similar growth reductions in mutant and WT cells (Figure S1). Drug concentrations are given in units of μg/ml unless otherwise noted.

(A) Gefitinib (Gef) resistance in NSCLC cells changes the interaction between 17-AAG and gefitinib from strongly antagonistic (suppressive) to synergistic. [17-AAG] and [gefitinib] are in units of nM and μM, respectively.

(B) Chloramphenicol (Cm) resistance in *E. coli* changes the interaction between salicylate (Sal) and Cm from strongly antagonistic (suppressive) to additive/weakly synergistic.

(C) Cm and ciprofloxacin (Cip) resistance in *E. coli* weakens the strongly antagonistic (suppressive) interaction between Cm and Cip, but does not eliminate the antagonism. [Cip] is in units of ng/ml.

(D) Daptomycin (Dap) resistance in *E. faecalis* reduces the strongly synergistic interaction between ampicillin and Dap.

(E) Erythromycin (Ery) and doxycycline (Dox) resistance in *E. coli* increases the synergistic interaction between the two drugs.

(F) Norfloxacin (Nor) resistance in *S. aureus* does not change the interaction between Cm and Nor.

drug-sensitive cells. To answer this question, we measured the population growth of a wide range of organisms, including bacteria and human cancer cells, in response to drug pairs (Supplemental Experimental Procedures; Tables S1–S3). We then quantified the nature of the drug interaction, i.e., synergy or antagonism, in both WT and resistant cells using two standard pharmacology approaches (Figures 1 and S1). Interestingly, we find that resistance can alter not only the individual drug efficacies but also the interactions between drugs. That is, two drugs can interact quite differently depending on whether they are applied to drug-resistant mutants or drug-sensitive cells (Figures 1 and S1). For example, the combination of two anticancer agents, gefitinib and 17-AAG, is antagonistic for most dosages in *EGFR* mutant non-small-cell lung cancer (NSCLC) cells, making it an unlikely a priori choice for therapy (Figure 1A). However, the same drug pair becomes synergistic for most dosages (Xu et al.,