Antibiotic Lethality Is Impacted by Nutrient Availabilities: New Insights from Machine Learning

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In this issue of *Cell*, Yang, Wright et al. describe a machine learning approach that that can provide mechanistic insight from chemical screens. They use this approach to uncover how the nutritional availability for *Escherichia coli* impacts lethality toward three widely used antibiotics.

With the rise of antibiotic resistance, there is a need for novel antibiotics, but more important is the need for efficient methods of chemical screening to identify the mechanisms governing antibiotic lethality. Chemical and genetic screens are widely used for drug discovery, but they generally suffer from low hit rates. Furthermore, when new drug candidates are identified, it is often cumbersome to identify the mode of action. For treatment of infectious diseases, this is further complicated by complex interactions between the host and microbe; for example, the microbe may identify a niche environment that supports survival even in the presence of antibiotics.

In this issue of *Cell*, Yang, Wright et al. combine flux balance analysis with machine learning to get new insight into the epistatic factors underlying the lethality of three different antibiotics (Yang et al., 2019). *Escherichia coli* was grown in a wide range of different media, including many different carbon and nitrogen sources, with the objective of measuring the effect of metabolic supplementation on the lethality of three widely used antibiotics: ampicillin (AMP), ciprofloxacin (CIP), and gentamicin (GENT). For each case, the IC_{50}, i.e., the concentration of antibiotic needed to inhibit bacterial growth by 50%, was reduced in the presence of certain nitrogen sources, meaning that the bacteria had improved tolerance toward the antibiotics when the nutritional availability changed. In order to identify the causal effect of this observation, the authors performed flux balance analysis (FBA) (O'Brien et al., 2015) for each of the different applied media. By FBA, it is possible to calculate the flux through the different parts of the metabolic network in the cell, and the set of fluxes defines the phenotype at a detailed level (Nielsen, 2003) (Figure 1). For each condition, the calculated fluxes were used as inputs for machine learning to identify which flux changes may alter lethality of the three antibiotics, leading to the identification of 477 reactions that altered the antibiotic lethality. Next, the authors computed pathway scores for each pathway and antibiotic by performing least-squares regression on the changes in antibiotic IC_{50}, thereby identifying several pathways of the central carbon metabolism (i.e., glycolysis, tricarboxylic acid [TCA] cycle, and glyoxylate pathway) and amino acid biosynthesis. This is hardly surprising as flux through these metabolic pathways is strongly correlated with growth. More interestingly, the authors found that decreased flux through a cluster of pathways involved in purine metabolism resulted in decreased lethality of the antibiotics AMP and CIP but increased lethality of GENT. This was confirmed by deletion of key genes in purine metabolism or by inhibition of purine biosynthesis through addition of 6-mercaptopurine. The authors further showed that
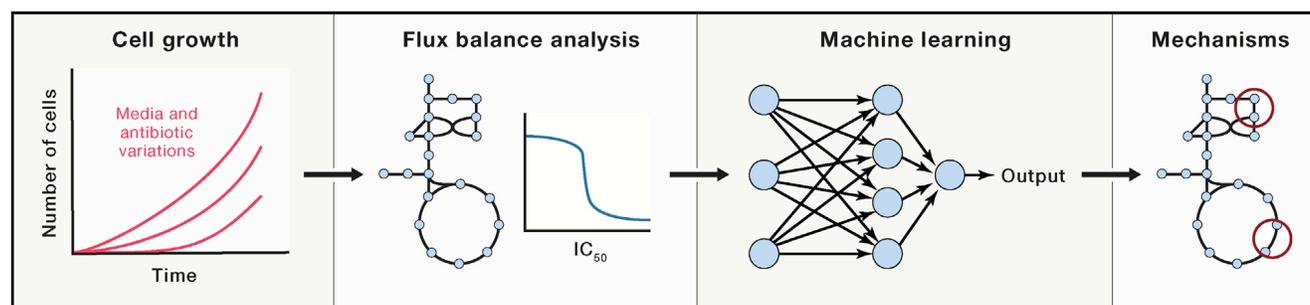

Figure 1. Quantitative Approach to Understand How Media Composition Impacts Drug IC_{50}
Flux balance analysis (FBA) can be used to compute fluxes through all the different metabolic pathways operating within the cell. By using the computed fluxes and the measured IC_{50} values in different media and drugs, it is then possible to identify correlations between fluxes' reduced antibiotic lethality, i.e., which flux changes enables the cells to better resist the antibiotics. Hereby, there is gained mechanistic insight into the causes of reduced antibiotic lethality, which can be used to design new therapies.

supplementation with adenine, but not guanine, decreased antibiotic lethality in wild-type cells.

The finding that there is a strong effect of adenine supplementation points to a key role of energy metabolism, and indeed, the authors found that there is increased respiratory metabolism in the presence of antibiotics, indicating an important role of respiratory metabolism for antibiotic lethality. Adenine supplementation results in decreased activity of purine biosynthesis, which further results in a decreased activity of energy metabolism, including respiration.

This approach of using computed fluxes to represent cellular phenotype showed that it is possible to identify how interactions between different pathways in the metabolic network determine antibiotic lethality. As the approach by Yang, Wright et al. could easily be translated for use with other cell types and other types of chemical screens, it is poised to significantly advance our capability to gain mechanistic insight into the mode of action of many different drugs beyond antibiotics.

The findings of the authors provide another important lesson: namely, that the activity of antibiotics is highly dependent on environmental conditions. Even potent antibiotics may lose their function if the environment provides conditions that allow the cells to grow at an altered growth rate. In this case, if adenine is pre-sent, the cells reduce the activity of energy metabolism, i.e., respiration, which may result in reduced growth and, hence, less sensitivity toward antibiotics.

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