DRUG DISCOVERY

Combating natural selection with 'antiviral Judo'

A method of screening for dominant inhibitors of viral growth could aid in combating RNA viruses by neutralizing their most powerful weapon—natural selection.

Poliovirus, West Nile, dengue fever virus… such RNA viruses are a major threat, and yet almost no drugs combat them effectively. This is a result of the high error rate of their replication that quickly produces heterogeneous viral populations including drug resistant variants. Judo, however, teaches that an apparently undefeatable foe can be defeated by neutralizing his strength. Karla Kirkegaard at Stanford has been thinking along these lines for quite some time. She says, "Since the major problem of targeting RNA viruses is drug resistance, and that is a genetic phenomenon that we can't avoid, then we have to use the genetics to understand how to get around it." She has now provided an intriguing solution (Crowder and Kirkegaard, 2005).

The basic tenet of natural selection requires that the advantage conferred by a mutated protein is transmitted to the organism's progeny via genetic inheritance. If the link between phenotype and genotype is broken and the progeny of an advantaged organism does not carry the advantageous gene, the selection of a stronger population cannot occur. A hint of such a process was reported under the name of 'phenotypic masking'. This term was coined to describe an observation that the frequency of virus resistance to neutralizing antibodies is lower than expected (Holland *et al.*, 1989). A possible explanation may be in the sharing of capsid proteins by viral variants that co-appear in a cell. If sharing of a capsid protein transfers its phenotype, then the phenotype becomes delinked from the organism possessing the gene. Owing to the oligomeric nature of the viral capsid, it is possible that mixing of mutated capsids with wild-type proteins will make the viral particle resistant to neutralization by antibodies. This will result in the formation of, on one hand, resistant viral particles that have a wild-type genome, and on the other hand, genomes that have the resistance mutation but are packaged into particles that are sensitive to neutralization—the link between genotype and phenotype is broken. Kirkegaard says, "We thought, 'sure that makes sense for capsids, but what about other components of the genome, and wouldn't they be the best drug targets?'"

How does one find such targets? The answer lies in an oddity of genetics: dominant negative effects. Typically, dominant negative mutants retain just enough function to get involved in the viral life cycle but then block it. Because of viral protein sharing, a dominant negative mutation can also block the function of variants that do not carry the protein. As the dominant negative effect is mediated by the same process as phenotypic masking, a screen for proteins that can function as dominant negatives should find drug targets for which resistant strains can't easily dominate a heterogeneous population.

What was needed was a screen for dominant inhibitors of viral growth. Scott Crowder developed a method in which he specifically mutated polioviruses, infected cells, and screened for mutants that produced almost no progeny. Once nonviable viral genomes were found, they were cotransfected into cells with wild-type genomes. To mimic a situation in which a drug-resistant, replication-competent virus emerges in a viral population inhibited by a drug, he used tenfold less wild type. Then it was a simple matter of looking for mutants that inhibited wild-type viral growth.

As predicted, many of these dominant inhibitor mutations were found to affect proteins that assemble as oligomers. Reassuringly, the authors also demonstrate that the one drug that has been somewhat efficient at combating these viruses seems to exploit this formation of chimeric capsids. Application of this 'antiviral Judo' should be able to guide drug discovery efforts to other suitable targets in these formidable foes.

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RESEARCH PAPERS

Crowder, S. & Kirkegaard, K. *Trans*-dominant inhibition of RNA viral replication can slow growth of drug-resistant viruses. *Nat. Genet.* **37**, 701–709 (2005).

Holland, J.J. *et al.* Virus mutation frequencies can be greatly underestimated by monoclonal antibody neutralization of virions. *J. Virol.* **63**, 5030–5036 (1989).

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