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RNA interference: new drugs on the horizon

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Researchers at Sirna Therapeutics (San Francisco and Boulder, USA) and Protiva Biotherapeutics (Burnaby, Canada) have taken an important step towards developing therapeutically active short interfering RNAs (siRNAs) [1]. By wrapping chemically modified siRNA in a specialized liposome, Sirna's Senior Director of Biology, David Morrissey, and colleagues have greatly increased the *in vivo* potency and duration of action of a siRNA targeted against hepatitis B virus (HBV). 'Our main clinical target is HCV', explains Morrissey, 'but this work is an important proof-of-principle for demonstrating the potency of our siRNAs.'

The therapeutic promise of RNA interference

RNA interference (RNAi) is a highly conserved, endogenous gene-silencing mechanism. Discovered in 1998, RNAi involves double-stranded RNA-mediated sequence-specific degradation of mRNA. Because the development of many diseases requires the production of specific proteins (for example, the replicative enzymes involved in viral infections or the oncogenes that help to drive tumorigenesis), scientists quickly recognized the therapeutic potential of RNAi [2]. 'The fact that with siRNA-based drugs we would be tapping into an endogenous protein-driven mechanism,' explains Morrissey, 'makes the approach potentially very powerful.'

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But before siRNAs can be used therapeutically several challenges need to be addressed. For example, siRNAs are naturally degraded so their effect is only transient; consequently, ways must be found to prolong their gene-silencing activity. Furthermore, the delivery method must get the siRNAs to their target tissues efficiently and most importantly, once there, the cellular uptake of the siRNAs must be optimized and their cellular activity maximized.

Solving the delivery problems

Sirna scientists are working on these and other challenges and in June they reported that a daily intravenous dose of 30 mg/kg of a chemically optimized siRNA targeted to HBV RNA produced a 90% reduction of serum HBV DNA in a mouse model of HBV replication [3]. Recognizing that this is not a clinically viable dose, the Sirna scientists have now used lipid encapsulation to improve the pharmacology of their siRNA. In their current formulation, chemically modified siRNAs are covered with a lipid bilayer, designed to facilitate their cellular uptake and endosomal release, and then with a polyethylene glycol-lipid conjugate surface coating, to prevent rapid systemic clearance. This formulation produces a 95% reduction in HBV serum titers at doses 1–5 mg/kg per day (delivered in three intravenous doses). This reduction in HBV serum titers persists for seven days after the last dose and can be maintained by weekly doses of siRNA [1].

'Wrapping the modified siRNA in lipid did a remarkably good job at reducing the amounts of siRNA needed to see an effect *in vivo*', comments James Patton, Professor of Biological Sciences at Vanderbilt University, USA. 'However, it is relatively easy to target siRNAs to the liver so we are still left with questions about how to target siRNAs to other tissues.'

'Although getting siRNAs to the liver is relatively straight forward', comments Morrissey, 'achieving significant uptake by hepatocytes together with long duration of effect is an important breakthrough. In addition, we can change the parameters of our formulation to target it to other tissues or decorate our liposomes with targeting ligands to get specific delivery.' Sirna is now testing its current formulation in primate models of HCV and hopes to start Phase I clinical trials of an anti-HCV siRNA during 2006.

John Rossi, Chair of the Division of Molecular Biology at the Beckman Research Institute of the City of Hope and co-founder of Calando

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Pharmaceuticals (Duarte, USA), also describes the new data from Sirna as encouraging but notes that other recent studies also show *in vivo* effects of siRNAs. For example, he says, antibody-mediated *in vivo* delivery of siRNAs is looking very hopeful [4], and an siRNA designed to silence an oncogene expressed in Ewing sarcoma has inhibited human tumor growth in mice when packaged in a cyclodextrin polymer (designed by Calando) and targeted through the attachment of transferrin to the polymer (www.nci.nih.gov/NCICancerBulletin/NCI_Cancer_Bulletin_041905). 'Overall', concludes Rossi, 'with all the incremental improvements that are being made to siRNA delivery, I think we should have useful siRNA-based drugs in the very near future.'

References

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Reversing the evolution of antibiotic resistance

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Bacteria develop resistance to antibiotics through gene mutations. Conventional wisdom has long held that these mutations are inevitable, caused by imperfect polymerases replicating a large genome under pressure. However, increasingly evidence suggests that bacteria are active players in the process, inducing proteins responsible for generating various types of mutations. Now, researchers at the Scripps Research Institute in La Jolla, CA, USA, led by Floyd Romesberg, are showing that interfering with these mutation-inducing pathways can reverse antibiotic resistance and render bacteria completely susceptible to the once ineffective antibiotic. 'Inhibiting these mutation pathways from the outset', says Romesberg, 'could represent a novel approach to combating the evolution of antibiotic resistance' [1].

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Shutting down the SOS response

Resistance-conferring mutations arise from three recombination pathways involved in repairing DNA damage. The Scripps group examined the role of various genes encoding proteins involved in the bacterial SOS response to two antibiotics, ciprofloxacin and rifampicin (members of the important quinolone and rifamycin classes, respectively).

To test whether the SOS response is necessary for ciprofloxacin and rifampicin resistance to occur, they infected mice with pathogenic *Escherichia coli* containing either wild-type or mutant LexA (a protein induced during SOS repair of DNA damage). Autoproteolysis of LexA causes derepression of three polymerases (Pol II, Pol IV and Pol V) involved in generating mutations.

They found that interfering with LexA prevented its autoproteolysis and the derepression of the LexA-controlled polymerases. As a result, *E. coli* was unable to turn on its SOS genes and could not evolve resistance to either ciprofloxacin or rifampicin. By contrast, *E. coli* with wild-type LexA resulted in the generation of *E. coli* subsequently becoming resistant to these antibiotics. They concluded that the LexA-controlled polymerases are necessary for the evolution of ciprofloxacin and rifampicin resistance. *In vitro* studies that deleted or mutated various genes involved in the recombination pathways, including *lexA*, confirmed the conclusions made from the animal studies.

Resistance to ciprofloxacin and rifampicin is, therefore, a consequence of proteins being induced to increase mutation rates significantly and not simply a chance occurrence of errors during genome replication. Interfering with the genes involved in these recombination pathways prevented the bacteria from becoming resistant.

Small-molecule inhibitors

'The real motivation was for drug design and looking for novel targets', says Romesberg. 'Mutation plays a role and might be susceptible to this type of intervention. Inhibiting these pathways would have a significant impact on the evolution of resistance.'

He says they are currently trying to identify small molecules that could be administered in conjunction with antibiotics, preventing bacteria from acquiring resistance-conferring mutations in the first place.

'This is immensely important', says George Drusano, physician scientist at Albany Medical College and Ordway Research Institute in Albany, NY, USA. 'We can roll back the past in certain circumstances and prevent the erosion of the susceptibility profile of clinically important organisms to this class of agents.'

Furthermore, the phenomenon might not be unique to bacteria. 'The evolution of resistance in chemotherapy is a real problem in breast cancer', says Romesberg. 'In human cells, people just don't know what the pathways are'. He comments, 'one approach would be to do genome-wide screens in yeast to identify genes that, when deleted, would render the yeast susceptible to mutation'. For now, he and his colleagues are trying to determine whether this is a universal phenomenon in bacteria and other antibiotic classes, at the same time as devising a chemical approach to reversing resistance.

Reference

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