NEWS AND COMMENTARY

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RNA interference gene therapy
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A story of mice and men

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RNA interference (RNAi) has emerged as one of the hottest topics in molecular and organismal biology. A flurry of recent papers suggest that RNAi may be useful as a human therapeutic. Significantly, in a paper in September's *Nature Genetics*, David Lewis and his colleagues have shown that RNAi is functional in numerous tissues of therapeutic interest.[1]

RNAi is an evolutionarily conserved surveillance mechanism that silences RNAs having homology to a double-stranded RNA (dsRNA) trigger. It is an indispensable tool for targeted gene inhibition in plants, worms and flies. Recently, Elbashir *et al*[2] electrified the field by showing that RNAi functions in mammalian cells too. The new Lewis *et al* study provides additional evidence that RNAi is functional in adult mammals.

The excitement surrounding RNAi stems from the possibility that it could potentially be used to inactivate any pathogenic RNA. RNA from infectious agents, like viruses, as well as disease-causing host genes could be targeted for inactivation. The new *Nature Genetics* study extends our knowledge of the parameters that affect the gene silencing process *in vivo*.

From work in *Drosophila* extracts, plants, and *C. elegans*, a plausible model for RNAi gene silencing has emerged. The 'Dicer' enzyme cuts long double-stranded RNAs that the cell recognizes as pathogenic into much shorter sections (21–23 nucleotides).[3] These short dsRNAs, known as guide RNAs, are then incorporated into a large multi-subunit complex, the RNA induced silencing complex (RISC).[4] This complex subsequently identifies and cleaves RNAs homologous to the guide RNAs.

RNAi silencing was always going to be hard to detect in mammals. The RNA-dependent protein kinase response (PKR) causes global, non-specific repression of translation in response to dsRNAs longer than 30 nucleotides, thus masking any RNAi-based silencing. However with shorter dsRNAs (short interfering RNAs or siRNAs) Elbashir *et al*, in their groundbreaking study, were able to silence genes in mammalian cells without triggering the PKR response.[2] More recently small hairpins RNAs (shRNAs) have been used to silence genes in mammalian cells,[5] raising the prospect of therapeutic RNAi through the expression of hairpin RNAs from gene therapy vectors.

Recently, we described a method for inducing RNAi in adult mice.[6] We used hydrodynamic transfection[7,8] to co-deliver target genes with siRNAs or shRNAs. This strategy effectively silenced (up to 98%) luciferase RNAs, as well as RNAs derived from hepatitis C virus. It is clear, that all the tools necessary for RNAi gene therapy are now available.

The new study also uses hydrodynamic transfection to deliver siRNA to tissues in mice (this time without the use of RNase inhibitor) with similarly impressive results. Importantly, Lewis and his co-workers observed RNAi in liver, spleen, kidney and pancreas.[1] These results suggest a RNAi gene therapy strategy could be applied to a diverse array of tissues.

The new study also showed, for the first time in mice, that RNAi can suppress an endogenous chromosomal gene.[1] Lewis and his co-workers significantly reduced expression of the transgene in a large percentage of hepatocytes in an EGFP transgenic mouse. This is an impressive result, given hydrodynamic transfection of plasmids usually results in transfection of only 5–40% of hepatocytes. In this case, the small size of siRNAs may have led to greater hepatic transfection efficiency resulting in relatively successful *in vivo* RNAi.

The promising new data from Lewis *et al* appear as a number of other exciting RNAi studies are being published. For example, recent reports that describe inhibition of viral replication in cultured cells seem likely to lead to an entirely new class of therapeutic agents. However, the work of Gitlin *et al*[9] sounds a note of caution for the field. They found that polio escape mutants, with silent point mutations in the middle of the target sequence, emerged after treatment with RNAi.[9] Thus, it may be necessary to attack viruses with multiple RNAi sequences simultaneously to prevent the emergence of resistant strains.

This is clearly an exciting time for RNAi therapeutics. However, intracellular RNAi delivery in humans is a major obstacle that must be overcome. At least now we have evidence that any viral or non-viral vector system can deliver RNAi[6] and that it should be useful in many tissues.[1] As with current gene transfer technologies, the limitations of the available vectors may represent the rate-limiting step towards developing RNAi into a *bona fide* therapeutic. Nonetheless, the study of RNAi efficacy in pre-clinical models of disease will become a robust area of research. Over time, the true value of this new class of therapeutics will likely be realized.

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