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• REVIEW •

RNA interference: Antiviral weapon and beyond

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Abstract

RNA interference (RNAi) is a remarkable type of gene regulation based on sequence-specific targeting and degradation of RNA. The term encompasses related pathways found in a broad range of eukaryotic organisms, including fungi, plants, and animals. RNA interference is part of a sophisticated network of interconnected pathways for cellular defense, RNA surveillance, and development and it may become a powerful tool to manipulate gene expression experimentally. RNAi technology is currently being evaluated not only as an extremely powerful instrument for functional genomic analyses, but also as a potentially useful method to develop specific dsRNA based gene-silencing therapeutics. Several laboratories have been interested in using RNAi to control viral infection and many reports in *Nature* and in *Cell* show that short interfering (si) RNAs can inhibit infection by HIV-1, polio and hepatitis C viruses in a sequence-specific manner. RNA-based strategies for gene inhibition in mammalian cells have recently been described, which offer the promise of antiviral therapy.

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INTRODUCTION

RNA, long upstaged by its more glamorous sibling, DNA, is turning out to have star qualities of its own. *Science* hails the electrifying discoveries of RNA interference as 2002's breakthrough of the year. RNA interference, also named RNA silencing or post transcriptional gene silencing (PTGS), is a phenomenon in which small double-stranded RNA molecules induce sequence-specific degradation of homologous single-stranded RNA[1]. RNAi activity plays a role in host-cell protection from viruses and transposons in plants and insects. From a practical perspective, RNAi can therefore be used to target gene expression and has been proved to be a very powerful technique to knock down specific genes to evaluate their physiological roles in *Caenorhabditis elegans*, *Drosophila melanogaster*, and humans[2-5].

Previous reports have show that RNAi has following important characteristics[7-11]. (1) RNAi can be induced through transfection or microinjection of long double-stranded RNA. In plants and invertebrates, the double-stranded RNA is cleaved into 19- to 23-nt RNA fragments known as small interfering RNAs (siRNA). siRNAs are double-stranded RNA (dsRNA) molecules with characteristic 2-nucleotide over hanging 3' ends. They act as intermediates in the RNA interference (RNAi) pathway, which is thought to protect cells from harmful transposons and highly repetitive sequences by targeting their RNA transcripts for endonucleolytic cleavage and subsequent exonucleolytic degradation. siRNA-directed RNA degradation is central to the antiviral response in plants, where it represents a potent form of sequence-based immunity. Only RNA molecules <30 bases in length can be used to exclusively induce RNAi in mammalian cells because longer molecules also activate the nonspecific double-stranded RNA-dependent response. (2) RNAi has amplification activity and different durations. In plants and nematodes, RNAi activity is long-term and disseminates throughout the organism via an uncharacterized amplification mechanism. In mammalian cells, amplification activity seems absent, and interference activity is transient, lasting for only 3-5 days. More recently, DNA expression vectors have been developed to express hairpin or duplex siRNA, which employ the type III class of RNA polymerase promoters to drive the expression of siRNA molecules. In addition, stable cell lines containing siRNA expression plasmids have been produced to induce RNAi over longer durations. (3) RNAi can be induced locally and then spread throughout the organism in plants, and this aspect of the process likely reflects its role in viral defense.

The power of siRNAs springs from the cellular biochemistry of the RNAi pathway. Like antisense oligonucleotides, siRNAs use sequence complementarity to target an mRNA for destruction. Unlike the antisense pathway, the RNAi pathway couples the specificity of an RNA guide to the stability and efficiency of a multiple-turnover protein enzyme. The ability to manipulate RNA interference thus sets the stage for realizing a wide variety of practical applications of biotechnology ranging from molecular farming to possibly even gene therapy in animals. Gitlin *et al*[12] showed that RNAi drastically reduced polio infection in HeLa cells. While analysing the antiviral effects of siRNA over a course of viral infection, they found that siRNA-resistant viruses turned out to carry silent base-pair mismatches in the siRNA complementary sequences. The authors argued, therefore, that if RNAi was to be used for therapeutic reasons, siRNA needed to be designed against highly conserved parts of the viral genome. Adelman et *al*[13] demonstrated that dsRNA-mediated interference also could act as a viral defense mechanism in mosquito cells. These observations are consistent with RNA interference as the mechanism of resistance to DEN-2 in transformed mosquito cells. Kay *et al*[14] went beyond the *in vitro* systems and genetically engineered mice that expressed siRNA against hepatitis C RNA to show that this technique also worked well *in vivo* to prevent viral replication. After this bumper crop of promising results, it remains to be seen how close we are to RNAi-mediated antiviral therapy. Because siRNA taps into natural gene-silencing pathways, a new form of intracellular immunization against viral infection might be just around the corner.

INTERFERING HCV

HCV genome is a single-stranded RNA that functions as both a messenger RNA and a replication template, making it an

attractive target for the study of RNA interference[15-21]. Previous results from Izumi RE' s laboratory[22] identified a small (60 nt) RNA from the yeast S. cerevisiae that specifically inhibited internal ribosome entry site (IRES)-mediated translation programmed by poliovirus (PV) and hepatitis C virus (HCV) 5' -untranslated region (5' UTR). The yeast inhibitor RNA (called RNAi) was found to efficiently compete with viral 5' UTR for binding to several cellular polypeptides that presumably play important roles in IRES-mediated translation. RNA interference offers further hope that a novel approach to silencing troublesome genes will become a valuable disease-fighting tool. But the therapy must leap many hurdles before it can be safely applied to humans. The power of small RNAs to shut down specific gene activities has now been brought to bear on an animal model of hepatitis. Mice infused with a siRNA against a cell death receptor recovered their liver function after experimentally induced injury. The work of Song *et al*[23] suggests that one type of entirely natural nucleic acid, small interfering RNAs (siRNAs), may hold promise as a therapeutic agent even without further engineering. These investigators provided the first *in vivo* evidence that infusion of siRNAs into an animal could alleviate disease, in this case hepatitis. Assembly of a siRNA strand into an RISC seemed to protect it from rapid degradation, the normal fate of small single-stranded RNA in cells. With this durability in mind, Song *et al*. set out to test whether direct infusion of siRNAs into mice might protect them from fulminant hepatitis. Both mice and humans with this disease suffered severe hepatic failure complicated with consequent encephalopathy, cerebral edema, metabolic imbalance and organ collapse. Infusing a solution of siRNA into a mouse' s tail, in massive amount, equivalent to half the animal' s blood volume protected it against hepatitis. And in animals that were already ailing, RNAi shut down the inflammation enough to allow the liver to recover. Despite the traumatic delivery method, the mice didn' t appear to suffer side effects. They gave mice injections of siRNA designed to shut down a gene called Fas, when over-activated during inflammatory response, it induced liver cells to self-destruct. The next day, the animals were given an antibody that sent Fas into hyperdrive. Control mice died of acute liver failure within a few days, but 82 % of the siRNA-treated mice remained free from serious disease and survived. About 80 % and 90 % of their liver cells incorporated the siRNA. Furthermore, the RNA molecules functioned for 10 days before fading completely after 3 weeks, lasting roughly three times longer than in previous studies. Another set of animals faced a different challenge: injections of cells with ConA, which compelled the immune system to attack the liver and produced the scarring seen in viral hepatitis. Animals infused with siRNA developed no liver damage. Silencing Fas expression with RNAi holds some therapeutic promise to prevent liver injury by protecting hepatocytes from cytotoxicity. In addition, biologists have agreeed that the best strategy would be to aim siRNA directly at hepatitis B or C viruses, but that would require a different siRNA than the one used by Song' s team. Evidences from several laboratories suggest that, in petri dishes, siRNA can stop hepatitis C from replicating. McCaffrey *et al*[24] showed that transgene expression could be suppressed in adult mice by synthetic small interfering RNAs and small-hairpin RNAs transcribed *in vivo* from DNA templates. They also showed the therapeutic potential of this technique by demonstrating effective targeting of a sequence from hepatitis C virus by RNA interference *in vivo*. Wilson *et al*[25] found RNA interference blocked gene expression and RNA synthesis from hepatitis C replicons propagated in human liver cells. Double-stranded small interfering RNA (siRNA) molecules designed to target the HCV genome were introduced through electroporation into a human hepatoma cell line (Huh-7) that contained an HCV subgenomic replicon. Two siRNA dramatically reduced virus-specific protein expression and RNA synthesis to levels that were 90 % less than those seen in cells treated with negative control siRNA. These same siRNA protected naive Huh-7 cells from challenge with HCV replicon RNA. Treatment of cells with synthetic siRNA was effective for more than 72 h, but the duration of RNA interference could be extended beyond 3 weeks through stable expression of complementary strands of the interfering RNA by using a bicistronic expression vector. These results suggest that a gene-therapeutic approach with siRNA can ultimately be used to treat HCV.

The utility of siRNA as a therapy against HCV infection will depend on the development of efficient delivery systems that induce long-lasting RNAi activity[26]. HCV is an attractive target for its localization in the liver, an organ that can be readily targeted by nucleic acid molecules and viral vectors. In future, chemically modified synthetic siRNA, with improved resistance to nucleases coupled with enhanced duration of RNAi, may become a possibility for therapeutic applications. On the other hand, gene therapy offers another possibility to express siRNA that targets HCV in a patient' s liver. Based on the above experiments, the use of siRNA as a treatment for HCV infections has great potential for use alone or in combination with conventional IFN/ribavirin therapy as means to decrease virus loads and eventually clear the persistent viruses from its host[27]. As therapeutic agents, siRNAs have enticing properties. Their actions appear to be short-lived in mammals, they are sequence specific and natural, cellular products and may therefore not produce toxic metabolites. Nonetheless, caveats for clinical use remain. Delivering siRNAs to the appropriate cells is a major challenge. siRNAs have thus far only been administered intravenously to mice by ' hydrodynamic transfection' , the rapid infusion of siRNA in a volume one-tenth the mass of the animal. Furthermore, the liver seems to be particularly receptive to exogenous RNA. Better delivery methods-such as formulation of siRNAs with compounds that promote transit across cell membranes-are clearly required before siRNAs can be used in therapy, especially to suppress gene expression in tissues other than in the liver. Nonetheless, the results of Song *et al*[23] have revealed the power of siRNAs in a disease model.

FACILITATING FUNCTIONAL GENOMICS

Until recently, RNA interference has been viewed primarily as a thorn in the side of plant molecular geneticists, limiting expression of transgenes and interfering with a number of applications that require consistent, high-level transgene expression. With our present understanding of the process, however, it is clear that RNA interference has enormous potential for engineering control of gene expression, as well as for a tool in functional genomics. It can be experimentally induced with high efficiency and targeted to a single specific gene or to a family of related genes. Likewise, dsRNA-induced TGS (transcriptional gene silencing) may have similar potential to control gene expression. Unwanted RNA interference, on the other hand, can be alleviated using viral suppressor technology or mutants impaired in silencing[28-30].

Genome-sequencing projects have provided tremendous amount of information about the genetic make-up of an organism. One way for finding out what genes do is to inactivate them, and to study the effects, in ' model' organisms. Kamath *et al*[4] used double-stranded RNAs to rapidly and transiently inactivate 16 757 of the worm' s predicted protein-coding genes. Meanwhile, Ashrafi and co-workers[31] have analysed these genes specifically to see if they had a role in regulating body fat. Together, their work has set a new standard

for systematic, genome-wide genetic studies. RNAi-based loss-of-function screens like these are tremendously powerful. Yet they have some disadvantages compared with classical genetic screening. For instance, some genes are more difficult to target by RNAi than others. And there are many non-coding RNAs, which are not translated into proteins. It remains to be seen if they are susceptible to RNAi. But there are still a vast number of protein-coding genes, from many different organisms, to study in detailed RNAi-based functional analyses, and this will keep the army of cell and molecular biologists busy for some time. Soon it will be possible to carry out RNAi-based screens in animal and human cells, using short synthetic double-stranded RNAs or plasmid- or virus-based DNA molecules that encode hairpin RNAs[32-34]. With the development of phenotypic read-outs based on cell biology, the hunt will begin.

SILENCING HIV-1

RNA interference represents an exciting new technology that may have therapeutic applications in the treatment of viral infections such as HCV and HIV. RNA interference is also found in HIV. Previous reports have shown that siRNA directed against the HIV genome can effectively inhibit virus production in cell-culture systems[35-38]. In addition, RNAi activity directed toward the major HIV receptor protein, CD4, led to decreased entry of HIV into cells[39-41]. However, replication of HIV occurred through an integrated DNA genome. Does it mean that RNAi is ineffective in clearing the virus? By targeting several regions of the HIV-1 genome, Jacque *et al*[42] showed that siRNA mediated viral genome degradation and caused downregulation of viral gene expression and they proved that RNAi worked even when the viral genome was contained within the nucleoprotein complex. They also showed that intracellularly made siRNA (transcribed from a plasmid) worked well, providing possible ways for delivering gene-therapy agents against HIV. To assess the effects of RNAi on HIV-1 infection, Novina *et al*[43] targeted both cellular and viral RNAs. The HeLa-derived cell line Magi-CCR5 (which expresses human CD4, and the chemokine receptors CCR5 and CXCR4) was transfected with short interfering RNA specific for the gene of interest and then infected with HIV-1. Cells transfected with siRNA specific for CD4 expressed CD4 mRNA at a level eight times lower than control cells, which led to a four-fold reduction in HIV-1 entry. Therefore, siRNA-directed silencing of CD4 specifically inhibited HIV entry and hence replication. Next, the viral structural protein Gag was targeted by transfecting cells with siRNA specific for the p24 component of this polyprotein. p24-siRNA-transfected cells showed a four-fold decrease in viral protein compared with controls, implying that viral amplification was inhibited by this approach. The authors also carried out transfection assays on human T cells, to assess the effect of RNAi on viral infectivity in a more physiological context. H9 cells were transfected with siRNA against green fluorescent protein (GFP) and were infected with an HIV-1 strain in which the *nef* gene was replaced with GFP. Again, silencing of viral gene expression occurred, resulting in reduced GFP and HIV-1 protein expression. These and other recent studies[44-46] show that siRNA can inhibit viral replication at several stages of infection, including very early stages, when viruses are most vulnerable. Infection can also be blocked by targeting either viral genes or host genes that are involved in the viral life cycle. It has been shown that siRNA directed against HIV-1 has the potential to be useful treatments. This study extended work by Lee *et al*[47,48] who used a vector-based RNAi strategy to silence an HIV-1 gene, and established that siRNA technology could be used to suppress multiple steps of the HIV-1 life cycle. They described a mammalian Pol III promoter system capable of expressing functional double-stranded siRNA following transfection into human cells. In the case of the 293 cells cotransfected with the HIV-1 pNL4-3 proviral DNA and the siRNA-producing constructs, they were able to achieve up to 4 logs of inhibition of expression from the HIV-1 DNA. Martinez *et al*[49] found that suppression of chemokine receptor expression by RNA interference could inhibit HIV-1 replication. Their results demonstrate that RNAi may be used to block HIV entry and replication through the blockade of cellular gene expression. Park *et al*[50] showed that HIV-1 replication was totally suppressed in a sequence-specific manner by six long dsRNAs containing the HIV-1 gag and env genes in HIV-1-infected cells. Especially, E2 dsRNA containing the major CD4-binding domain sequence of gp120, dramatically inhibited the expression of the HIV-1 p24 antigen in PBMCs for a relatively long time. Coburn *et al*[51] demonstrated that siRNA duplexes targeted against the essential Tat and Rev regulatory proteins encoded by HIV-1 could specifically block Tat and Rev expression and function. More importantly, they showed that these same siRNAs could effectively inhibit HIV-1 gene expression and replication in cell cultures, including those of human T-cell lines and primary lymphocytes. These results demonstrate the utility of RNAi for modulating the HIV replication cycle and provide the evidence that genomic HIV-1 RNA, existing within a nucleoprotein reverse-transcription complex, is amenable to siRNA-mediated degradation.

But does it actually work as an antiviral weapon? More recent studies have suggested that it does, at least in cells in culture dishes. Gitlin *et al*[12] reported that specific siRNA administered to human cells from the outside, like a drug, could enter them and protect them against infection by the rapidly multiplying poliovirus. Jacque *et al*[42], meanwhile, reported similar results in their studies with the AIDS virus HIV-1. These authors further demonstrated that if the siRNA were expressed from inside cells, rather than simply administered from the outside, the cells became largely immune to subsequent HIV-1 is infection. These results are exciting, and suggest that RNAi perfectly suitable to many antiviral applications. But one important factor is that not all viral RNA sequences are equally accessible to siRNA. Some sequences might be buried within secondary structures or within highly folded regions in target RNAs, whereas others might come form tight complexes with proteins that obscure their recognition. Optimal targets must be chosen by trial and error. Another issue is that viruses often produce mutated progeny molecules. Some of these naturally help the viruses escape immune surveillance or inhibition by drugs, but they might also prevent recognition by siRNA. To overcome this obstacle, one might need to target viral RNA sequences that are conserved and normally invariant between different strains, or to simultaneously target several viral sequences. Finally, the problem of how to deliver siRNA to cells needs to be addressed. They can certainly be delivered efficiently to cells in culture, but methods must be improved before RNAi can be used in animals, let alone patients.

FUTURE DIRECTIONS

The field of RNAi is moving at an impressive pace and generating exciting results that are clearly associated with RNA interference, transgene silencing and transposon mobilization[52-55]. Possible links to X-chromosome inactivation, imprinting and interferon response have also been suggested, but not yet firmly established. RNAi also has a considerable economic potential, especially in agriculture. A better understanding of PTGS should allow a more efficient response to viral infection and the development of transgene/host associations that can override silencing to allow the expression of interested proteins. Now that early mouse embryos are known to be susceptible to

RNAi, it will be critical to determine whether this technique can also be applied to tissue culture[56-60]. The possible repercussions of RNAi in mammals are potentially far-reaching in the fight against certain diseases such as cancer or virus/ parasite infection, as well as for the analysis of more fundamental problems in neurobiology and cell and developmental biology. In the next ten years, RNAi will probably be regarded as one of the major breakthroughs of the 2000s. A new report has shown that incubating a target mRNA with *Drosophila* embryonic extracts and the cognate dsRNA *in vitro* leads to its degradation in a process that recapitulates many of the features of RNA interference *in vivo* (including sequence specificity, length dependence and amplification). This important study has paved the way for a biochemical analysis of RNA interference. In relation to RNA interference in mammals, it is important to note that in contrast to the sequence-specific RNAi effect observed in mouse embryos, this new study has shown that incubation of an mRNA with rabbit reticulocyte lysates and dsRNA induces nonspecific mRNA degradation, one possible reason for this difference could be that the interferon response present in rabbit reticulocyte lysate is not functional in early mouse embryos[61-65].

Although antiviral RNAi technology has not yet been optimized, the phenomenon does appear to be both general and effective. In 1988, the concept of "intracellular immunization" was proposed, whereby one could express within cells inhibitory molecules (usually proteins) that could protect these cells from specific viral infections in the future[66-75]. The promise of intracellular immunization now appears to be closer to reality - although amazingly, through the use of small RNAs rather than peptides or proteins. The potential of using RNAi activity for the treatment of viral diseases and cancer has aroused a great deal of interests in the scientific community. Many laboratories have reported the use of RNAi activity in cultured cells infected with HIV, human papillomavirus, and polio or containing a variety of cancer genes. The clinical applications of RNA is just around the corner.

REFERENCES

1 **Zamore PD**. Ancient pathways programmed by small RNAs. *Science* 2002; **296**: 1265-1269
2 **Williams RW**, Rubin GM. ARGONAUTE1 is required for efficient RNA interference in Drosophila embryos. *Proc Natl Acad Sci U S A* 2002; **99**: 6889-6894
3 **Ge Q**, McManus MT, Nguyen T, Shen CH, Sharp PA, Eisen HN, Chen J. RNA interference of influenza virus production by directly targeting mRNA for degradation and indirectly inhibiting all viral RNA transcription. *Proc Natl Acad Sci U S A* 2003; **100**: 2718-2723
4 **Kamath RS**, Fraser AG, Dong Y, Poulin G, Durbin R, Gotta M, Kanapin A, Le Bot N, Moreno S, Sohrmann M, Welchman DP, Zipperlen P, Ahringer J. Systematic functional analysis of the Caenorhabditis elegans genome using RNAi. *Nature* 2003; **421**: 231-237
5 **Schwarz DS**, Hutvagner G, Haley B, Zamore PD. Evidence that siRNAs function as guides, not primers, in the Drosophila and human RNAi pathways. *Mol Cell* 2002; **10**: 537-548
6 **Pantaleo V,** Rubino L, Russo M. Replication of Carnation Italian ringspot virus defective interfering RNA in Saccharomyces cerevisiae. *J Virol* 2003; **77**: 2116-2123
7 **Dennis C**. Small RNAs: the genome's guiding hand? *Nature* 2002; **420**: 732
8 **Jia Q,** Sun R. Inhibition of gammaherpesvirus replication by RNA interference. *J Virol* 2003; **77**: 3301-3306
9 **Van Wezel R**, Liu H, Wu Z, Stanley J, Hong Y. Contribution of the zinc finger to zinc and DNA binding by a suppressor of post-transcriptional gene silencing. *J Virol* 2003; **77**: 696-700
10 **Qu F**, Ren T, Morris TJ. The coat protein of turnip crinkle virus suppresses posttranscriptional gene silencing at an early initiation step. *J Virol* 2003; **77**: 511-522
11 **Ray D**, White KA. An internally located RNA hairpin enhances replication of Tomato bushy stunt virus RNAs. *J Virol* 2003; **77**: 245-257
12 **Gitlin L**, Karelsky S, Andino R. Short interfering RNA confers intracellular antiviral immunity in human cells. *Nature* 2002; **418**: 430-434
13 **Adelman ZN**, Sanchez-Vargas I, Travanty EA, Carlson JO, Beaty BJ, Blair CD, Olson KE. RNA sicencing of dengue virus type 2 replication in transformed C6/36 mosquito cells transcribing an inverted-repeat RNA derived from the virus genome. *J Virol* 2002; **76**: 12925-12933
14 **McCaffrey AP**, Kay MA. A story of mice and men. *Gene Ther* 2002; **9**: 1563
15 **Gong GZ**, Lai LY, Jiang YF, He Y, Su XS. HCV replication in PBMC and its influence on interferon therapy. *World J Gastroenterol* 2003; **9**: 291-294
16 **Yan FM**, Chen AS, Hao F, Zhao XP, Gu CH, Zhao LB, Yang DL, Hao LJ. Hepatitis C virus may infect extrahepatic tissues in patients with hepatitis C. *World J Gastroenterol* 2000; **6**: 805-811
17 **Yu YC**, Mao Q, Gu CH, Li QF, Wang YM. Activity of HDV ribozymes to trans-cleave HCV RNA. *World J Gastroenterol* 2002; **8**: 694-698
18 **Song ZQ**, Hao F, Min F, Ma QY, Liu GD. Hepatitis C virus infection of human hepatoma cell line 7721 *in vitro*. *World J Gastroenterol* 2001; **7**: 685-689
19 **Dai YM,** Shou ZP, Ni CR, Wang NJ, Zhang SP. Localization of HCV RNA and capsid protein in human hepatocellular carcinoma. *World J Gastroenterol* 2000; **6**: 136-137
20 **Cheng JL**, Liu BL, Zhang Y, Tong WB, Yan Z, Feng BF. Hepatitis C virus in human B lymphocytes transformed by Epstein-Barr virus in vitro by in situ reverse transcriptase-polymerase chain reaction. *World J Gastroenterol* 2001; **7**: 370-375
21 **Meier V**, Mihm S, Wietzke Braun P, Ramadori G. HCV-RNA positivity in peripheral blood mononuclear cells of patients with chronic HCV infection: does it really mean viral replication? *World J Gastroenterol* 2001; **7**: 228-234
22 **Izumi RE**, Valdez B, Banerjee R, Srivastava M, Dasgupta A. Nucleolin stimulates viral internal ribosome entry site-mediated translation. *Virus Res* 2001; **76**: 17-29
23 **Song E,** Lee SK, Wang J, Ince N, Ouyang N, Min J, Chen J, Shankar P, Lieberman J. RNA interference targeting Fas protects mice from fulminant hepatitis. *Nat Med* 2003; **9**: 347-351
24 **McCaffrey AP,** Meuse L, Pham TT, Conklin DS, Hannon GJ, Kay MA. RNA interference in adult mice. *Nature* 2002; **418**: 38-39
25 **Wilson JA**, Jayasena S, Khvorova A, Sabatinos S, Rodrigue-Gervais IG, Arya S, Sarangi F, Harris-Brandts M, Beaulieu S, Richardson CD. RNA interference blocks gene expression and RNA synthesis from hepatitis C replicons propagated in human liver cells. *Proc Natl Acad Sci U S A* 2003; **100**: 2783-2788
26 **Randall G**, Grakoui A, Rice CM. Clearance of replicating hepatitis C virus replicon RNAs in cell culture by small interfering RNAs. *Proc Natl Acad Sci U S A* 2003; **100**: 235-240
27 **Zamore PD**, Aronin N. siRNAs knock down hepatitis. *Nat Med* 2003; **9**: 266-267
28 **Pomerantz RJ.** RNA interference meets HIV-1: will silence be golden? *Nat Med* 2002; **8**: 659-660
29 **Mallory AC**, Parks G, Endres MW, Baulcombe D, Bowman LH, Pruss GJ, Vance VB. The amplicon-plus system for high-level expression of transgenes in plants. *Nat Biotechnol* 2002; **20**: 622-625
30 **Caplen NJ,** Zheng Z, Falgout B, Morgan RA. Inhibition of viral gene expression and replication in mosquito cells by dsRNA-triggered RNA interference. *Mol Ther* 2002; **6**: 243-251
31 **Ashrafi K,** Chang FY, Watts JL, Fraser AG, Kamath RS, Ahringer J, Ruvkun G. Genome-wide RNAi analysis of Caenorhabditis elegans fat regulatory genes. *Nature* 2003; **421**: 268-272
32 **Miyagishi M**, Taira K. U6 promoter-driven siRNA with four uridine 3' overhangs efficiently suppress targeted gene expression in mammalian cells. *Nat Biotechnol* 2002; **20**: 497-500
33 **Nicholson RH**, Nicholson AW. Molecular characterization of a mouse cDNA encoding Dicer, a ribonuclease III ortholog involved in RNA interference. *Mamm Genome* 2002; **13**: 67-73
34 **Adelman ZN**, Blair CD, Carlson JO, Beaty BJ, Olson KE. Sindbis virus-induced silencing of dengue viruses in mosquitoes. *Insect Mol Biol* 2001; **10**: 265-273

35 **Hu WY,** Myers CP, Kilzer JM, Pfaff SL, Bushman FD. Inhibition of retroviral pathogenesis by RNA interference. *Curr Biol* 2002; **12**: 1301-1311

36 **Dector MA**, Romero P, Lopez S, Arias CF. Rotavirus gene silencing by small interfering RNAs. *EMBO Rep* 2002; **3**: 1175-1180

37 **Surabhi RM**, Gaynor RB. RNA interference directed against viral and cellular targets inhibits human immunodeficiency Virus Type 1 replication. *J Virol* 2002; **76**: 12963-12973

38 **Martinez de Alba AE**, Flores R, Hernandez C. Two chloroplastic viroids induce the accumulation of small RNAs associated with posttranscriptional gene silencing. *J Virol* 2002; 76: 13094-13096

39 **Park WS**, Miyano-Kurosaki N, Hayafune M, Nakajima E, Matsuzaki T, Shimada F, Takaku H. Prevention of HIV-1 infection in human peripheral blood mononuclear cells by specific RNA interference. *Nucleic Acids Res* 2002; **30**: 4830-4835

40 **Kitabwalla M**, Ruprecht RM. RNA interference-a new weapon against HIV and beyond. *N Engl J Med* 2002; **347**: 1364-1367

41 **Capodici J**, Kariko K, Weissman D. Inhibition of HIV-1 infection by small interfering RNA-mediated RNAinterference. *J Immunol* 2002; **169**: 5196-5201

42 **Jacque JM,** Triques K, Stevenson M. Modulation of HIV-1 replication by RNA interference. *Nature* 2002; **418**: 435-438

43 **Novina CD**, Murray MF, Dykxhoorn DM, Beresford PJ, Riess J, Lee SK, Collman RG, Lieberman J, Shankar P, Sharp PA. siRNA-directed inhibition of HIV-1 infection.*Nat Med* 2002; **8**: 681-686

44 **Wojtkowiak A**, Siek A, Alejska M, Jarmolowski A, Szweykowska-Kulinska Z, Figlerowicz M. RNAi and viral vectors as useful tools in the functional genomics of plants. Construction of BMV-based vectors for RNA delivery into plant cells. *Cell Mol Biol Lett* 2002; **7**: 511-522

45 **Xia H,** Mao Q, Paulson HL, Davidson BL. siRNA-mediated gene silencing *in vitro* and *in vivo*. *Nat Biotechnol* 2002; **20**: 1006-1010

46 **Rhodes A**, James W. Inhibition of heterologous strains of HIV by antisense RNA. *AIDS* 1991; **5**: 145-151

47 **Lee NS**, Dohjima T, Bauer G, Li H, Li MJ, Ehsani A, Salvaterra P, Rossi J. Expression of small interfering RNAs targeted against HIV-1 rev transcripts in human cells. *Nat Biotecrnol* 2002; **20**: 500-505

48 **Song E**, Lee SK, Wang J, Ince N, Ouyang N, Min J, Chen J, Shankar P, Lieberman J. RNA interference targeting Fas protects mice from fulminant hepatitis. *Nat Med* 2003; **9**: 347-351

49 **Martinez MA**, Clotet B, Este JA. RNA interference of HIV replication. *Trends Immunol* 2002; **23**: 559-561

50 **Park WS**, Miyano-Kurosaki N, Hayafune M, Nakajima E, Matsuzaki T, Shimada F, Takaku H. Prevention of HIV-1 infection in human peripheral blood mononuclear cells by specific RNA interference. *Nucleic Acids Res* 2002; **30**: 4830-4835

51 **Coburn GA**, Cullen BR. Potent and specific inhibition of human immunodeficiency virus type 1 replication by RNA interference. *J Virol* 2002; **76**: 9225-9231

52 **Couzin J**. RNA interference. Mini RNA molecules shield mouse liver from hepatitis. *Science* 2003; **299**: 995

53 **Jia Q,** Sun R. Inhibition of gammaherpesvirus replication by RNA interference. *J Virol* 2003; **77**: 3301-3306

54 **Agami R**. RNAi and related mechanisms and their potential use for therapy. *Curr Opin Chem Biol* 2002; **6**: 829-834

55 **Lin SL**, Ying SY. D-RNAi (messenger RNA-antisense DNA interference) as a novel defense system against cancer and viral infections. *Curr Cancer Drug Targets* 2001; **1**: 241-247

56 **Di Serio F**, Schob H, Iglesias A, Tarina C, Bouldoires E, Meins F Jr. Sense- and antisense-mediated gene silencing in tobacco is inhibited by the same viral suppressors and is associated with accumulation of small RNAs. *Proc Natl Acad Sci U S A* 2001; **98**: 6506-6510

57 **Vaucheret H**, Fagard M. Transcriptional gene silencing in plants: targets, inducers and regulators. *Trends Genet* 2001; **17**: 29-35

58 **Blair CD**, Adelman ZN, Olson KE. Molecular strategies for interrupting arthropod-borne virus transmission by mosquitoes. *Clin Microbiol Rev* 2000; **13**: 651-661

59 **Morel JB,** Vaucheret H. Post-transcriptional gene silencing mutants. *Plant Mol Biol* 2000; **43**: 275-284

60 **Hammond SM**, Bernstein E, Beach D, Hannon GJ. An RNA-directed nuclease mediates post-transcriptional gene silencing in Drosophila cells. *Nature* 2000; **404**: 293-296

61 **Yamamoto T**, Omoto S, Mizuguchi M, Mizukami H, Okuyama H, Okada N, Saksena NK, Brisibe EA, Otake K, Fuji YR. Double-stranded nef RNA interferes with human immunodeficiency virus type 1 replication. *Microbiol Immunol* 2002; **46**: 809-817

62 **Couzin J**. Breakthrough of the year. Small RNAs make big splash. *Science* 2002; **298**: 2296-2297

63 **Brummelkamp TR**, Bernards R, Agami R. Stable suppression of tumorigenicity by virus-mediated RNA interference. *Cancer Cell* 2002; **2**: 243-247

64 **Olson KE,** Adelman ZN, Travanty EA, Sanchez-Vargas I, Beaty BJ, Blair CD. Developing arbovirus resistance in mosquitoes. *Insect Biochem Mol Biol* 2002; **32**: 1333-1343

65 **Jiang M**, Milner J. Selective silencing of viral gene expression in HPV-positive human cervical carcinoma cells treated with siRNA, a primer of RNA interference. *Oncogene* 2002; **21**: 6041-6048

66 **Hao CQ**, Feng ZH, Zhou YX, Nie QH, Li JG, Jia ZS, Liang XS, Xie YM, Cao YZ, Kang WZ. Construction,package and identification of replication-deficient recombinant adenovirus expression vector of HCV C. *Shijie Huaren Xiaohua Zazhi* 2003; **11**: 144-147

67 **Jia ZS**, Chen L, Hao CQ, Feng ZH, Li JG, Wang JP, Cao YZ, Zhou YX. Intracellular immunization by hammerhead ribozyme against HCV. *Shijie Huaren Xiaohua Zazhi* 2003; **11**: 148-150

68 **Cheng YQ**, Nie QH, Zhou YX, Huang XF, Luo H, Yang HG. Ultrastructure characteristics of HCV infected human trophoblast cells in culture. *Shijie Huaren Xiaohua Zazhi* 2003; **11**: 151-156

69 **Liang XS**, Lian JQ, Zhou YX, Nie QH, Hao CQ. Inhibitory effect of IRES specific inhibitor RNA on HCV IRES mediated protein translation. *Shijie Huaren Xiaohua Zazhi* 2003; **11**: 157-160

70 **Sun L**, Zhou YX, Hao CQ, Feng ZH, Zhao J, Hu PZ, Fu Y, Ma FC, Chang JQ, Wang JP, Nie QH. Effect of DNA vaccine on anti-HCV infection in mice with subcutaneous inoculating tumor. *Shijie Huaren Xiaohua Zazhi* 2003; **11**: 165-168

71 **Sun Y**, Cheng RX, Feng DY, Ouyang XM, Zheng H. Effect of HCV NS3 on proliferation and phosphorylation of MAPK in human hepatocytes. *Shijie Huaren Xiaohua Zazhi* 2003; **11**: 173-177

72 **Zhang L**, Zhao GZ, Li Y, Shi LL. Dynamic changes of HVR1 quasispecies in chronic hepatitis C after IFN therapy. *Shijie Huaren Xiaohua Zazhi* 2003; **11**: 182-184

73 **Li L**, Cheng J, Li F, Wang JJ, Zhang J, Wu Q, Han P, Chen GF, Ji D, Li K. Clinical and pathological characteristics of steatohepatitis in patients with chronic hepatitis C virus infection. *Shijie Huaren Xiaohua Zazhi* 2002; **10**: 1009-1013

74 **Zhang J**, Cheng J, Li L, Liu AB, Wu Q, Li K, Dong J, Wang L, Lu YY. Apolipoprotein A I and A II in the patients infected with hepatitis C virus. *Shijie Huaren Xiaohua Zazhi* 2002; **10**: 1014-1017

75 **Cheng J**, Ren JY, Li L, Lu ZM, Li K, Hong Y, Lu YY, Wang G, Liu Y, Zhang LX, Chen JM. Liver steatosis of transgenic mice expressing hepatitis C virus structural proteins. *Shijie Huaren Xiaohua Zazhi* 2002; **10**: 1022-1026

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