Cell. Mol. Life Sci. 64 (2007) 1785 – 1792
1420-682X/07/141785-8
DOI 10.1007/s00018-007-7057-3

Cellular and Molecular Life Sciences

Predicting siRNA efficiency

W. Li* **and L. Cha**

Center of Computational Biology, Beijing Institute of Basic Medical Sciences, P.O. Box 130(3), Beijing 100850 (China), Fax: +86 10 68213039, e-mail: liwj@nic.bmi.ac.cn

Online First 6 April 2007

Abstract. Since the identification of RNA-mediated interference (RNAi) in 1998, RNAi has become an effective tool to inhibit gene expression. The inhibition mechanism is triggered by introducing a short interference double-stranded RNA (siRNA,19~27 bp) into the cytoplasm, where the guide strand of siRNA (usually antisense strand) binds to its target messenger RNA and the expression of the target gene is blocked. RNAi has been widely applied in gene functional analysis, and as a potential therapeutic strategy in viral diseases, drug target discovery, and cancer therapy. Among the factors which may compromise inhibition efficiency, how to design siRNAs with high efficiency and high specificity to its target gene is critical. Although many algorithms have been developed for this purpose, it is still difficult to design such siRNAs. In this review, we will briefly discuss prediction methods for siRNA efficiency and the problems of present approaches.

Keywords. RNAi, siRNA, algorithm, prediction.

Introduction

RNA interference (RNAi) is an ancient mechanism for gene regulation which is mediated by short interfering doublestrand RNA (siRNA) [1–2]. The basic steps in knockdown expression of an siRNA target gene are as follows. First, long doublestrand RNA in the cytoplasm is processed into 21- to 23-nucleotide siRNAs with the characteristic 3′ overhangs of two nucleotides by Dicer, a ribonuclease III enzyme. Second, the siRNA is incorporated into an RNA-induced silencing complex (RISC). Third, the RISC complex uses the guide strand (usually the antisense strand) of the siRNA to bind to its target messenger RNA (mRNA). Finally, the target mRNA is cleaved, and the expression of the target gene is inhibited. This process of siRNA inhibition is shown schematically in Figure 1.

Because carefully designed siRNA can specifically inhibit the expression of its target gene without affecting other genes, RNAi has become an effective tool for gene functional analysis [3–10]. For example, using an RNAi library targeted to nearly 90 % of the 19,427 predicted genes of *Caenorhabditis elegans*, Simmer et al. investigated the relationship between the phenotype and related sets of genes [6]. In addition, Keating et al. explored the functions of 60 G protein-coupled receptors in *C. elegans* by RNAi on the whole-genome level [3]. In addition to gene functional analysis, RNAi has also been widely studied for genetic-based therapies. As such, RNAi is a potentially useful method to develop highly specific dsRNA-based gene-silencing therapeutics [11]. RNAi has been called 'one of the most exciting discoveries in biology in the last couple decades' [1]. However, designing siRNAs with high efficiency and high specificity is critical. Here we present a minireview about this topic.

We will briefly describe the potential applications of RNAi as an alternative therapeutic strategy, and provide a more detailed discussion of the main prediction methods for siRNA efficiency. Next, we review off-target effects analysis, and indicate some Web servers for siRNA design. Finally, we summarize the review and present possible directions for future research in developing new prediction methods for siRNA efficiency.

* Corresponding author.

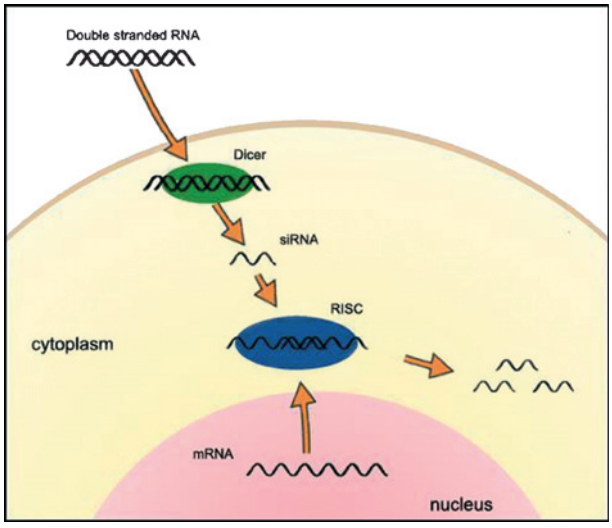

Figure 1. The main processes of short interfering RNA inhibiting expression of its target gene.

Potential applications of RNAi as an alternative therapeutic strategy

As an alternative therapeutic strategy, compared to conventional drugs, an RNAi-based potential drug has the following advantages [12]: easy to select target sites, high efficiency, ability to degrade cognate mRNA in cells of different species, no side effects, and stability. From a theoretical point of view, any gene whose expression contributes to disease is a potential target, from viral genes to oncogenes to genes responsible for heart disease and so on [1]. Through the design of specific siRNAs for the relevant genes, the expression of the target gene can be destroyed without affecting the expression of other genes. RNAi thus holds promise to become a therapeutic agent in the near future.

Up to now, RNAi has been widely employed as a potential therapeutic strategy in viral infections [13–28], drug target discovery [29–33], cancers [34–38], and inherited genetic disorders [39, 40]. For example, in order to inhibit the infection of human immunodeficiency virus-1 (HIV-1), Novina et al. devised five siRNAs targeted to the HIV-1 cellular receptor CD4 and the viral structural Gag protein. They found that infection with HIV-1 can be efficiently inhibited in mammalian cells [41]. Song et al. studied the application of RNAi in fulminant hepatitis. By designing six siRNAs targeted to Fas, a gene related to apoptosis in a broad spectrum of liver diseases [42], the researches found that the longevity of 82% of experimental mice could be significantly prolonged. Based on conservation among the major HBV (hepatitis B virus) genotypes [15], seven siRNA sequences targeted to HBV mRNA were designed and used to inhibit HBV in mice by McCaffrey et al.. The results showed that six out of seven siRNAs have an antiviral effect. Two of them are very potent. In the antiviral application of RNAi, severe acute respiratory syndrome (SARS) coronavirus [26, 43], hepatitis C virus (HCV) [24], and herpes simple virus 2 (HSV-2) [28] have also been studied. For a recent review of siRNAs as the potential drugs, see [44]. For potential applications in cancer therapy, see [35, 45]. In addition, through the genome-wide RNAi screen in *Drosophila* cells infected with *Drosophila* C virus, Cherry et al. have found a related 66 specific ribosomal proteins which are required for RNA viruses with internal ribosome entry sites (IRESs) to be translated [33]. These host proteins may be potential therapeutic targets for curing the related RNA virus. For a review of its applications in drug target discovery, see [31].

From the point of view of systems biology, genes do not act independently. They interact with one another and form genetic networks. Mapping these networks plays an important role in understanding the state of biological systems, especially complex human disease [46]. In their recent paper [47], Lehner et al. developed a method for a high-throughput RNAi genetic interaction screen. In order to test the effects of the screen method, they chose the known pathways of EGF (epidermal growth factor), Wnt, Notch, SynMuuA, and SynMuv B. By considering all possible genetic interactions between ~31 genes in the above pathways and ~1750 library genes, they found 349 genetic interactions between 162 genes which function in the signaling and transcriptional networks. This is the first time RNAi technology has been used for systematic mapping of genetic interactions in *C. elegans* (animals). Through the experiments, the researches not only validated the known pathway components but also identified new pathway components. In addition, from the 349 genetic interactions, they also found six 'hub' genes, i.e., a class of highly connected genes. The hub genes are all chromatin-remodeling components. These genes can act as genetic buffers for a diverse set of genes, and therefore regulate many biological processes. Furthermore, Lehner et al. predicted that human orthologs of these genes are likely to be related to the human genetic diseases. This RNAi-based high-throughput screen method for genetic interaction may provide a way to predict complex human disease-related genes. From the above analysis, we conclude that RNAi is a powerful tool and has been applied in many aspects of molecular biology. Although there are many factors, such as target selection, delivery of siRNA, and so on, involved in the applications of RNAi, siRNA efficiency is one of the most important factors. We will discuss it in detail in following sections.

Predicting siRNA efficiency

Because of the wide application of RNAi, many prediction methods for siRNA efficiency have been developed in recent years [48–59]. These methods are mainly based on sequence characteristics of siRNAs or target mRNA secondary structures. For each kind of prediction method, the steps to construct a mathematical model are as follows. First, a certain number of siRNA sequences and related inhibiting efficiency vis-a-vis their target genes are collected. Second, the collected siRNA sequences are classified into two groups based on inhibition efficiency (high or low efficiency group). Third, the potential characteristics related to siRNA efficiency are extracted from siRNA sequences or target mRNA secondary structures. Finally, the significant characteristics between the two groups are identified, and these characteristics can be used to guide design of siRNAs with high efficiency for new target mRNA sequences.

Prediction methods of siRNA efficiency based on siRNA sequence characteristics

For prediction methods based on siRNA sequence characteristics, the basic assumption is that siRNA efficiency is mainly determined by the siRNA sequence itself. Currently, many sequence characteristics-based methods have been developed. For example, using the experimental data of 180 siRNAs targeted to two target genes, Reynolds et al. presented eight rules for designing high-efficiency siRNAs [48]. These eight rules are as follows: 1. The GC content of the sense strand of siRNA is between 30 and 50%. 2. There are more than three bases A or U in the region (15th–19th base) of the sense strand. 3. The stable hairpin structure of the sense (antisense) strand of siRNA is not permitted, and the melting temperature (Tm) should be less than 20°C. 4. The 19th base of the sense strand of the siRNA should be A. 5. The third base of the sense strand should be A. 6. The 10th base of the sense strand should be U. 7. The 19th base of the sense strand should not be G or C. 8. The 13th base of the sense strand should not be G. Furthermore, a simple algorithm was developed for high-efficiency siRNA design using the above eight rules. For each candidate siRNA sequence, a score S is assigned with initial value $S=0$. If a candidate siRNA satisfies conditions 1, 3, 4, 5, or 6, then S is added one ($S=S+1$). If a candidate siRNA can not meet the conditions 7 or 8, then1 is subtracted from S ($S=S-1$). For condition 2, for each base A or U in the region (15th–19th base) of the sense strand of siRNA, S is added one ($S=S+1$). Finally, for each candidate siRNA, there is a corresponding score S. The larger S is, the more likely the siRNA has high efficiency. In general cases, the score S is required to be more than 6 ($S\geq 6$). Through an independent test of 30 siRNAs targeted to 6 genes, the results indicated that there were 17 siRNAs with inhibiting efficiency 80%. The correct rate is 56.7%. The shortcoming of the method is that the above eight rules are treated equally. In fact, there is no reason to suppose that the efficiency contribution of the eight rules is equal in inhibiting target mRNA.

Recently, Jagla et al. used a larger experimental dataset of 601 siRNAs targeted to 4 genes to deduce the sequence characteristics for high-efficiency siRNA design [49]. From the whole dataset, four separate training datasets were extracted. For each training set, the decision-tree classification method was used to deduce criteria for siRNAs with high efficiency. There were four sets of criteria altogether. These four sets of criteria were merged, and the significant rules were found as follows: 1. The 10th and 19th bases of the sense strand of siRNA should be A or U. 2. The first base of the sense strand should be G or C. 3. There are more than 3 bases A or U in the region (13th–19th base) of the sense strand of siRNA. Obviously, rule 1 is basically the same as Reynolds rules 6 and 7. Rule 3 contains the information of Reynolds rule 2. Finally, the results indicated that prediction accuracy is about 30% when the inhibiting efficiency of 70% was taken as a threshold to distinguish a high- or low-efficiency group based on an independent test dataset.

Even through the above algorithms conclude that sequence characteristics are very important in determining the efficiency of siRNAs, Patzel et al. consider that the secondary structure of siRNA antisense strand plays an important role [50]. From the experimental data of nine siRNAs targeted to one gene, they found that the siRNA efficiency would be improved if there were more free bases at the 5' or 3' end of the antisense strand. Here free bases refers to the bases that form no base pairs with other bases. The correlation between the number of free bases and siRNA efficiency was as high as 0.94. Further analysis showed that siRNA efficiency was basically irrelevant to the accessibility of target mRNA, sequence characteristics, and the 5' end stability of the antisense strand. Although the correlation between the number of free bases and siRNA efficiency is high, the number of siRNAs is too few. The above conclusions may need further verification.

In order to improve the success rate of designing high-efficiency siRNAs, Hall et al. have constructed a neural network based on the experimental data of 2182 siRNAs targeted to 34 genes [51]. First, from 2182 siRNAs, they extracted the 200 siRNAs with the highest efficiency and the 200 siRNAs with the lowest efficiency as the training dataset. Second, the neural network was constructed using the characteristics of

siRNA sequences. Finally, an independent test dataset containing 249 siRNAs was used to test the neural network. The results indicated that the Pearson coefficient between the prediction results and experimental data was 0.66. Even so, as pointed out by Miyagishi et al. in a comment, the prediction accuracy will improve further if the secondary structures of the targets are considered. Therefore, for the improvement of prediction accuracy of siRNA efficiency, more factors based on the sequence characteristics should be taken into account.

Prediction methods of siRNA efficiency based on secondary structures of target mRNA

The typical prediction method based on secondary structures of the target mRNA is the H-bond method, introduced by Luo et al [53]. In order to deduce the relationship between the target secondary structures and siRNA efficiency, the H-bond index (H-b) was calculated for the siRNAs according to the following steps. First, a certain number of target secondary structures, whose free energy is close to the minimum free energy, were predicted using the Mfold Web server [60]. Second, the frequency for each base in the target region paired to other bases was calculated. For example, if the number of the calculated target secondary structures is 50, and there are 30 opportunities for the first base in the target region to be paired with other bases, the frequency is 0.6 (30/50). Furthermore, if the base in the target region is G or C, the corresponding frequency is multiplied by 3. If the base in the target region is A or U, the corresponding frequency is multiplied by 2. Finally, H-b is calculated by summing of all the weight frequencies in the target region.

Based on H-b, the quantitative relationship between siRNA efficiency and target secondary structure was observed using an experimental dataset of 14 siRNAs targeted to 3 genes. The results indicated that siRNAs with high efficiency had a low H-b. Finally, according to the above conclusions, four siRNAs targeted to a new target gene were designed. The experimental results further confirmed the conclusions. Obviously, if the target region is in the single-chain state, H-b will be very low. From this point of view, siRNAs with high efficiency should be single chains. This conclusion was also observed by Schramm et al [54]. In addition, Overhoff et al. confirmed that siRNA efficiency should be high if the target region was accessible in the target mRNA [55]. Recently, Yiu et al. presented a repelling loop method to evaluate siRNA efficiency based on five minimum or near-minimum free-energy target mRNA secondary structures [56].

There are three challenges in analyzing the relationship between siRNA efficiency and target mRNA secondary structures. First, the most popular method for RNA secondary structure prediction at present is Zuker′s Mfold Web server. The prediction accuracy is low for long target mRNA sequences. However, the target mRNA usually is long. For example, from the siRNA database siRecord [61] we obtained 1354 target mRNA sequences without missing values. Among 1354 targets, the shortest and longest sequence lengths are 51 and 2,314,078 bases, respectively. The average target sequence length is 5877 bases, which makes it difficult to get accurate target mRNA secondary structures. Therefore, reliable results of analysis based on target secondary structures cannot be guaranteed. Second, for long target mRNA sequences, the computation time is long and the demands on the computer are high. Sometimes, current PC′s struggle to meet the requirements of computation. Third, although Luo et al. formulated the H-b method to analyze the relationship between siRNA efficiency and secondary structures of target mRNA quantitatively [53], we still do not know how many target secondary structures need to be calculated. Furthermore, the number of samples used in Luo's analysis is very small, which makes it difficult to guarantee the reliability of H-b.

Analysis of off-target effects

For particular target mRNAs, successful design of siRNAs reguires not only high efficiency to silence the target gene but also high specificity. Here, specificity means that the designed siRNAs inhibit only the expression of the target gene, i.e., the designed siRNAs should not have off-target effects. Analysis of off-target effects plays an important role in applications of RNAi. For example, siRNAs may have potential as drugs for treatment of viral diseases. If the designed siRNAs have off-target effects on the human genes, side-effects will result.

In order to avoid off-target effects, many methods and programs have been developed [62–67]. However, the key issue is what criteria should be used to analyze these effects. At first, people found that silence effects can be abolished by only a single central mismatch between the siRNA and corresponding target mRNA [68]. Therefore, the blast program was used to check specificity [69]. The process is very similar to the probe specificity check for oligonucleotide microarrays [70]. First, related parameters such as word length and E value for the blast program are set up. In order to avoid potential other targets of the designed siRNAs, word length is often assigned as the minimum value. Second, the related transcriptome database is selected for the siRNAs. For example, if the target mRNA is a human gene, then the human transcriptome database should be selected. Better siRNAs are

Table 1. Selected Web servers for siRNA design.

Author(s)	Web page	Ref.
Schramm et al.	http://www.mwg-biotech.com	54
Naito et al.	http://design.rnai.jp/	75
Henschel et al.	http://cluster-1.mpi-cbg.de/Deqor/deqor.html	76
Cui et al.	http://bioit.dbi.udel.edu/rnai/	77
Dudek et al.	http://www.cellbio.unige.ch/RNAi.html	78
Arziman et al.	http://www.dkfz.de/signaling2/e-rnai/	79
Yuan et al.	http://jura.wi.mit.edu/bioc/siRNAext/	80
Wang et al.	http://www.genscript.com/sirna_ca.html#design	81
Levenkova, et al.	http://bioinfo.wistar.upenn.edu/siRNA/siRNA.htm	82
Santoyo et al.	http://side.bioinfo.cnio.es	83

found based on analysis of the blast results. In view of their fast running speed, blast programs are still used in the design of siRNAs.

However, people also found that the siRNAs could tolerate several mismatches between the siRNAs and the target. Findings show that blast programs may be not suitable for specificity analysis of siRNAs because blast programs can miss some potential targets, which leads to off-target effects. For example, in an investigation of 359 published siRNA sequences, Snove et al found that about 75 % of them had a risk of eliciting non-specific effects [71]. New programs have been developed based on these findings.

In Order to deduce the criteria for specificity analysis of siRNAs, gene expression profiling regulated by RNAi was used. Jackson et al. concluded that the similarity between siRNA and target mRNA is 79 % (15/19) and perhaps as few as 11 contiguous nucleotides between them are sufficient to direct silencing of nontargeted transcripts [72]. These results are very similar to Kane's criteria for probe design for oligonucleotide microarrays [73].

Recently, the criteria for off-target gene silencing have been studied in detail using gene expression profiling of human cells transfected with siRNAs [74]. From the expression profile regulated by 12 siRNAs, Birmingham et al. identified 347 off-target genes as the experimental off-targets set. The Smith-Waterman algorithm was used to calculate the similarity between the siRNAs and human genes, and the *in silico* off-targets set was obtained with similarity more than 79 %. Comparison of the experimental off-targets set and the *in silico* off-targets set showed that the false positive rate and false negative rate were more than 99 % and 93 %, respectively. In the meantime, the researches found that off-target effects were associated with the presence of one or more perfect matches (6–7 bases) between the antisense strand of the siRNA and the 3' untranslated region of the off-targets. These results showed that off-target effects were mainly caused by 3' UTR seed matches rather than the overall identity between siRNAs and non-target mRNAs.

In future, to determine the criteria for siRNA specificity checks, we may need further experiments. Also, the secondary structures of nontarget mRNAs and the potential target regions of nontarget mRNAs should be considered.

Software for higly efficient siRNA design

The point of predicting siRNA efficiency and analysis of off-target effects is to design siRNAs with high efficiency and high specificity for further experiments. Therefore, it is very important to develop software for automatic design of siRNAs. Many programs have been developed. Some of them are listed in Table 1 [75–83]. In view of the long running time needed to predict the secondary structures of target mRNA, the programs are basically based on the methods of siRNA sequence characteristics.

Conclusions

From above analysis, we conclude that there are many factors involved in siRNA efficiency. These factors include secondary structures of the sense and antisense strands of siRNAs, secondary structures of target mRNAs, sequence characteristics of siRNAs, and the specificity of siRNA to its target. The analysis of siRNA efficiency, poses three problems at present. The first is the relationship between siRNA efficiency and the secondary structures of target mRNAs. Even though some point out that there is a strong relationship between them, others think that the siRNA efficiency is mainly determined by siRNA itself. The second problem is that current analysis of

siRNA efficiency is mainly based on factors such as the secondary structure of siRNA antisense strands rather than comprehensive analysis of all factors. The third problem is the off-target analysis of siRNA. Even though some criteria have been formulated, there is no standard for siRNA specificity. In order to design siRNAs with high efficiency and high specificity, all these problems should be considered comprehensively, and new algorithms should be developed. To meet this objectives, both supervised learning methods and optimal feature selection procedures may be needed.

Acknowledgements. This work is supported by grant no. 5042021 from the Beijing Natural Science Foundation and grant nos. 30470411 and 90608004 from the National Natural Science Foundation of China.

1 Robinson, R. (2004) RNAi therapeutics: how likely, how soon? PLoS Biol. 2, E28.
2 Fire, A., Xu, S., Montgomery, M. K., Kostas, S. A., Driver, S. E. and Mello, C. C. (1998) Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature 391, 8061 – 811.
3 Keating C. D., Kriek N., Daniels M., Ashcroft N. R., Hopper N. A., Siney E. J., Holden-Dye L. and Burke J. F. (2003) Whole-genome analysis of 60 G protein-coupled receptors in Caenorhabditis elegans by gene knockout with RNAi. Curr. Biol. 13, 1715 – 1720.
4 Barstead R. (2001) Genome-wide RNAi. Curr. Opin. Chem. Biol. 5, 63 – 66.
5 Agaisse H., Burrack L. S., Philips J. A., Rubin E. J., Perrimon N. and Higgins D. E. (2005) Genome-wide RNAi screen for host factors required for intracellular bacterial infection. Science 309, 1248 – 1251.
6 Simmer F., Moorman C., van der Linden A. M., Kuijk E., van den Berghe P. V., Kamath R. S., Fraser A. G., Ahringer J. and Plasterk R. H. (2003) Genome-wide RNAi of C. elegans using the hypersensitive rrf-3 strain reveals novel gene functions. PLoS Biol. 1, E12.
7 Sugimoto A. (2004) High-throughput RNAi in Caenorhabditis elegans: genome-wide screens and functional genomics. Differentiation 72, 81 – 91.
8 Kuttenkeuler D. and Boutros M. (2004) Genome-wide RNAi as a route to gene function in Drosophila. Brief Funct. Genomic. Proteomic. 3, 168 – 176.
9 Moffat J. and Sabatini D. M. (2006) Building mammalian signalling pathways with RNAi screens. Nat. Rev. Mol. Cell Biol. 7, 177 – 187.
10 Furlong E. E. (2005) A functional genomics approach to identify new regulators of Wnt signaling. Dev. Cell 8, 624 – 626.
11 Jana S., Chakraborty C., Nandi S. and Deb J. K. (2004) RNA interference: potential therapeutic targets. Appl. Microbiol. Biotechnol. 65, 649 – 657.
12 Tan F. L. and Yin J. Q. (2004) RNAi, a new therapeutic strategy against viral infection. Cell Res. 14, 460 – 466.
13 Silva J. M., Hammond, S. M. and Hannon G. J. (2002) RNA interference: a promising approach to antiviral therapy? Trends Mol. Med. 8, 505 – 508.
14 Bennink J. R. and Palmore T. N. (2004) The promise of siRNAs for the treatment of influenza. Trends Mol. Med. 10, 571 – 574.
15 McCaffrey A. P., Nakai H., Pandey K., Huang Z., Salazar F. H., Xu H., Wieland S. F., Marion P. L. and Kay M. A. (2003) Inhibition of hepatitis B virus in mice by RNA interference. Nat. Biotechnol. 21, 639 – 644.
16 Radhakrishnan S. K., Layden T. J. and Gartel A. L. (2004) RNA interference as a new strategy against viral hepatitis. Virology 323, 173 – 181.
17 Hu W. Y., Bushman F. D. and Siva A. C. (2004) RNA interference against retroviruses. Virus Res. 102, 59 – 64.
18 Lee N. S. and Rossi J. J. (2004) Control of HIV-1 replication by RNA interference. Virus Res. 102, 53 – 58.
19 Li X. P., Li G., Peng Y., Kung H. F. and Lin M. C. (2004) Suppression of Epstein-Barr virus-encoded latent membrane protein-1 by RNA interference inhibits the metastatic potential of nasopharyngeal carcinoma cells. Biochem. Biophys. Res. Commun. 315, 212 – 218.
20 Cristofaro P. and Ramratnam B. (2006) RNAi tackles a sexually transmitted disease. Nat. Biotechnol. 24, 48 – 49.
21 Sim A. C., Luhur A., Tan T. M., Chow V. T. and Poh C. L. (2005) RNA interference against enterovirus 71 infection. Virology 341, 72 – 79.
22 Stevenson M. (2003) Dissecting HIV-1 through RNA interference. Nat. Rev. Immunol. 3, 851 – 858.
23 Jacque J. M., Triques K. and Stevenson M. (2002) Modulation of HIV-1 replication by RNA interference. Nature 418, 435 – 438.
24 De Francesco R. and Migliaccio G. (2005) Challenges and successes in developing new therapies for hepatitis C. Nature 436, 953 – 960.
25 Bitko V., Musiyenko A., Shulyayeva O. and Barik S. (2005) Inhibition of respiratory viruses by nasally administered siRNA. Nat. Med. 11, 50 – 55.
26 Zhang Y., Li T., Fu L. Yu C., Li Y., Xu X., Wang Y., Ning H., Zhang S., Chen W. et al. (2004) Silencing SARS-CoV Spike protein expression in cultured cells by RNA interference. FEBS Lett. 560, 141 – 146.
27 Li Y., Wasser S., Lim S. G. and Tan T. M. (2004) Genome-wide expression profiling of RNA interference of hepatitis B virus gene expression and replication. Cell. Mol. Life Sci. 61, 2113 – 2124.
28 Palliser D., Chowdhury D., Wang Q. Y., Lee S. J., Bronson R. T., Knipe D. M. and Lieberman J. (2006) An siRNA-based microbicide protects mice from lethal herpes simplex virus 2 infection. Nature 439, 89 – 94.
29 Ito M., Kawano K., Miyagishi M. and Taira K. (2005) Genome-wide application of RNAi to the discovery of potential drug targets. FEBS Lett. 579, 5988 – 5995.
30 Hauber I., Bevec D., Heukeshoven J., Kratzer F., Horn F., Choidas A., Harrer T. and Hauber J. (2005) Identification of cellular deoxyhypusine synthase as a novel target for antiretroviral therapy. J. Clin. Invest. 115, 76 – 85.
31 Jones S. W., Souza P. M. and Lindsay M. A. (2004) siRNA for gene silencing: a route to drug target discovery. Curr. Opin. Pharmacol. 4, 522 – 527.
32 Lavery K. S. and King T. H. (2003) Antisense and RNAi: powerful tools in drug target discovery and validation. Curr. Opin. Drug Discov. Devel. 6, 561 – 569.
33 Cherry S., Doukas T., Armknecht S., Whelan S., Wang H., Sarnow P. and Perrimon N. (2005) Genome-wide RNAi screen reveals a specific sensitivity of IRES-containing RNA viruses to host translation inhibition. Genes Dev. 19, 445 – 452.
34 Ait-Si-Ali S., Guasconi V. and Harel-Bellan A. (2004) RNA interference and its possible use in cancer therapy. Bull. Cancer 91, 15 – 18.
35 Devi G. R. (2006) siRNA-based approaches in cancer therapy. Cancer Gene Ther. Cancer Gene Ther. 13, doi: 10.1038/sj.cgt.7700931
36 Sciamanna I., Landriscina M., Pittoggi C. , Quirino M., Mearelli C., Beraldi R., Mattei E., Serafino A., Cassano A., Sinibaldi-Vallebona P. et al. (2005) Inhibition of endogenous reverse transcriptase antagonizes human tumor growth. Oncogene 24, 3923 – 3931.
37 Pai S. I., Lin Y. Y., Macaes B., Meneshian A., Hung C. F. and Wu T. C. (2006) Prospects of RNA interference therapy for cancer. Gene Ther. 13, 464 – 477.

38 Bhattacharyya M. and Lemoine N. R. (2006) Gene therapy developments for pancreatic cancer. Best. Pract. Res. Clin. Gastroenterol. 20, 285 – 298.
39 Harper S. Q., Staber P. D., He X., Eliason S. L., Martins I. H., Mao Q., Yang L., Kotin R. M., Paulson H. L. and Davidson B. L. (2005) RNA interference improves motor and neuropathological abnormalities in a Huntington's disease mouse model. Proc. Natl. Acad. Sci. USA 102, 5820 – 5825.
40 Caplen N. J., Taylor J. P., Statham V. S., Tanaka F., Fire A. and Morgan R. A. (2002) Rescure of polyglutamine-mediated cytotoxicity by double-stranded RNA-mediated RNA interference. Hum. Mol. Genet. 11, 175 – 184.
41 Pomerantz R. J. (2002) RNA interference meets HIV-1: will silence be golden? Nat. Med. 8, 659 – 660.
42 Song E., Lee S., Wang J., Ince N., Ouyang N., Min J., Chen J., Shankar P. and Lieberman J. (2003) RNA interference targeting Fas protects mice from fulminant hepatitis. Nat. Med. 9, 347 – 351.
43 Li B., Tang Q., Cheng D., Qin C., Xie F. Y., Wei Q., Xu J., Liu Y., Zheng B. J., Woodle M. C. et al. (2005) Using siRNA in prophylactic and therapeutic regimens against SARS coronavirus in Rhesus macaque. Nat. Med. 11, 944 – 951.
44 Dykxhoorn D. M., Palliser D. and Lieberman J. (2006) The silent treatment: siRNAs as small molecule drugs. Gene Ther. 13, 541 – 552.
45 Tan F. L. and Yin J. Q. (2005) Application of RNAi to cancer research and therapy. Front. Biosci. 10, 1946 – 1960.
46 Bussey H., Andrews B. and Boone C. (2006) From worm genetic networks to complex human diseases. Nat. Genet. 38, 862 – 863.
47 Lehner B., Crombie C., Tischler J., Fortunato A. and Fraser A. G. (2006) Systematic mapping of genetic interactions in Caenorhabditis elegans identifies common modifiers of diverse signaling pathways. Nat. Genet. 38, 896 – 903.
48 Reynolds A., Leake D., Boese Q., Scaringe S., Marshall W. S. and Khvorova A. (2004) Rational siRNA design for RNA interference. Nat. Biotechnol. 22, 326 – 330.
49 Jagla B., Aulner N., Kelly P. D., Song D., Volchuk A., Zatorski A., Shum D., Mayer T., De Angelis D. A., Ouerfelli O. et al. (2005) Sequence characteristics of functional siRNAs. RNA 11, 864 – 872.
50 Patzel V., Rutz S., Dietrich I., Koberle C., Scheffold A. and Kaufmann S. H. (2005) Design of siRNAs producing unstructured guide-RNAs results in improved RNA interference efficiency. Nat. Biotechnol. 23, 1440 – 1444.
51 Huesken D., Lange J., Mickanin C., Weiler J., Asselbergs F., Warner J., Meloon B., Engel S., Rosenberg A., Cohen D. et al. (2005) Design of a genome-wide siRNA library using an artificial neural network. Nat. Biotechnol. 23, 995 – 1001.
52 Elbashir S. M., Harborth J., Weber K. and Tuschl T. (2002) Analysis of gene function in somatic mammalian cells using small interfering RNAs. Methods 26, 199 – 213.
53 Luo K. Q. and Chang D. C. (2004) The gene-silencing efficiency of siRNA is strongly dependent on the local structure of mRNA at the targeted region. Biochem. Biophys. Res. Commun. 318, 303 – 310.
54 Gerrit S. and Rebecca R. (2005) siRNA design including secondary structure target site prediction. Nat. Methods 2, DOI:10.1038/NMETH780.
55 Overhoff M., Alken M., Far R. K. , Lemaitre M., Lebleu B., Sczakiel G. and Robbins I. (2005) Local RNA target structure influences siRNA efficacy: a systematic global analysis. J. Mol. Biol. 348, 871 – 881.
56 Yiu S. M., Wong P. W., Lam T. W., Mui Y. C., Kung H. F., Lin M. and Cheung Y. T. (2005) Filtering of ineffective siRNAs and improved siRNA design tool. Bioinformatics 21, 144 – 151.
57 Schubert S., Grunweller A., Erdmann V. A. and Kurreck J. (2005) Local RNA target structure influences siRNA efficacy: systematic analysis of intentionally designed binding regions. J. Mol. Biol. 348, 883 – 893.
58 Pancoska P., Moravek Z. and Moll U. M. (2004) Efficient RNA interference depends on global context of the target sequence: quantitative analysis of silencing efficiency using Eulerian graph representation of siRNA. Nucleic Acids Res. 32, 1469 – 1479.
59 Heale B. S., Soifer H. S., Bowers C. and Rossi J. J. (2005) siRNA target site secondary structure predictions using local stable substructures. Nucleic Acids Res. 33, e30.
60 Zuker M. (2003) Mfold web server for nucleic acid folding and hybridization prediction. Nucleic Acids Res. 31, 3406 – 3415.
61 Ren Y., Gong W., Xu Q. , Zheng X., Lin D., Wang Y. and Li T. (2006) siRecords: an extensive database of mammalian siRNAs with efficacy ratings. Bioinformatics 22, 1027 – 1028.
62 Yamada T. and Morishita S. (2005) Accelerated off-target search algorithm for siRNA. Bioinformatics 21, 1316 – 1324.
63 Saxena S., Jonsson Z. O. and Dutta A. (2003) Small RNAs with imperfect match to endogenous mRNA repress translation: implications for off-target activity of small inhibitory RNA in mammalian cells. J. Biol. Chem. 278, 44312 – 44319.
64 Lin X., Ruan X., Anderson M. G., McDowell J. A., Kroeger P. E., Fesik S. W. and Shen Y. (2005) siRNA-mediated off-target gene silencing triggered by a 7 nt complementation. Nucleic Acids Res. 33, 4527 – 4535.
65 Qiu S., Adema C. M. and Lane T. (2005) A computational study of off-target effects of RNA interference. Nucleic Acids Res. 33, 1834 – 1847.
66 Naito Y., Yamada T., Matsumiya T., Ui-Tei K., Saigo K. and Morishita S. (2005) dsCheck: highly sensitive off-target search software for double-stranded RNA-mediated RNA interference. Nucleic Acids Res. 33, W589-W591.
67 Snove O., Jr. Nedland M., Fjeldstad S. H. , Humberset H., Birkeland O. R., Grunfeld T. and Saetrom P. (2004) Designing effective siRNAs with off-target control. Biochem. Biophys. Res. Commun. 325, 769 – 773.
68 Elbashir S. M., Martinez J., Patkaniowska A., Lendeckel W. and Tuschl T. (2001) Functional anatomy of siRNAs for mediating efficient RNAi in Drosophila melanogaster embryo lysate. EMBO J. 20, 6877 – 6888.
69 Altschul S. F., Madden T. L., Schaffer A. A. , Zhang J., Zhang Z., Miller W. and Lipman D. J. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389 – 3402.
70 Li W. and Ying X. (2006) MProbe 2.0: computer aided probe design for oligonucleotide microarrays. Appl. Bioinformatics 5, 181 – 186.
71 Snove O., Jr. and Holen T. (2004) Many commonly used siRNAs risk off-target activity. Biochem. Biophys. Res. Commun. 319, 256 – 263.
72 Jackson A. L., Bartz S. R., Schelter J. , Kobayashi S. V., Burchard J., Mao M., Li B., Cavet G. and Linsley P. S. (2003) Expression profiling reveals off-target gene regulation by RNAi. Nat. Biotechnol. 21, 635 – 637.
73 Kane M. D., Jatkoe T. A., Stumpf C. R., Lu J., Thomas J. D. and Madore S. J. (2000) Assessment of the sensitivity and specificity of oligonucleotide (50mer) microarrays. Nucleic Acids Res. 28, 4552 – 4557.
74 Birmingham A., Anderson E. M., Reynolds A., Ilsley-Tyree D., Leake D., Fedorov Y., Baskerville S., Maksimova E., Robinson K., Karpilow J. et al (2006) 3' UTR seed matches, but not overall identity, are associated with RNAi off-targets. Nat. Methods 3, 199 – 204.
75 Naito Y., Yamada T., Ui-Tei K., Morishita S. and Saigo K. (2004) siDirect: highly effective, target-specific siRNA design software for mammalian RNA interference. Nucleic Acids Res. 32, W124-W129.
76 Henschel A., Buchholz F. and Habermann B. (2004) DEQOR: a web-based tool for the design and quality control of siRNAs. Nucleic Acids Res. 32, W113-W120.
77 Cui W., Ning J., Naik U. P. and Duncan M. K. (2004) OptiRNAi, an RNAi design tool. Comput. Methods Programs Biomed. 75, 67 – 73.
78 Dudek P. and Picard D. (2004) TROD: T7 RNAi Oligo Designer. Nucleic Acids Res. 32, W121-W123.

79 Arziman Z., Horn T. and Boutros M. (2005) E-RNAi: a web application to design optimized RNAi constructs. Nucleic Acids Res. 33, W582-W588.
80 Yuan B., Latek R., Hossbach M., Tuschl T. and Lewitter F. (2004) siRNA Selection Server: an automated siRNA oligonucleotide prediction server. Nucleic Acids Res. 32, W130-W134.
81 Wang L. and Mu F. Y. (2004) A Web-based design center for vector-based siRNA and siRNA cassette. Bioinformatics 20, 1818 – 1820.
82 Levenkova N., Gu Q. and Rux J. J. (2004) Gene specific siRNA selector. Bioinformatics 20, 430 – 432.
83 Santoyo J., Vaquerizas J. M. and Dopazo J. (2005) Highly specific and accurate selection of siRNAs for high-throughput functional assays. Bioinformatics 21, 1376 – 1382.