ACTA MEDICA LITUANICA. 2007. Vol. 14. No. 3. P. 165–169

RNA interference: from a research tool to a novel therapeutic agent

Jolanta Vidugirienė[1],

Said Goueli[1],

Kęstutis Sužiedėlis[2]

[1] *Department of Cell Analysis, Promega Corporation, USA*

[2] *Institute of Oncology, Vilnius University, Lithuania*

RNA interference (RNAi) is a powerful and highly specific gene-silencing approach that is triggered by the formation of double-stranded RNA helices and directs silencing of gene expression in a sequence specific manner. RNAi is an evolutionarily conserved natural mechanism that plays an important role in maintaining the genomic integrity of eukaryotic organisms, controlling gene expression, and guarding against exogenous viral infection. Over the past decade, this ancient cellular response has been exploited as an invaluable research tool for functional characterization of known genes, aided in identification of genes with unique functions, and has evolved as a novel approach for post-transcriptional gene silencing with potential clinical applications. In this review we will briefly highlight our current understanding of the RNAi process and discus how the use of RNAi technology has facilitated the advent of major discoveries in functional genomics and development of novel gene therapies.

Key words: RNA interference, gene-silencing, gene therapy

INTRODUCTION

Over the past decade, RNA interference has emerged as a natural mechanism for silencing gene expression (1, 2). The phenomenon was first noticed in plants, when attempts to enhance the violet color of petunias by introducting extra copies of pigment gene resulted in an unexpected decrease in gene expression and the appearance of several white flowers (3).

However, these and similar observations remained an enigma until it was reported by Fire and Mello (2006 recipients of the Nobel Prize in Physiology or Medicine) (4) that double-stranded RNA molecules injected into nematode worm *C. elegans* directed the degradation of messenger RNA in a sequence specific matter. This sequence-specific degradation of messenger RNA (mRNA) was coined RNA interference (RNAi) and since injection of small amounts of double-stranded RNA was efficacious, it has been proposed that RNA interference is a catalytic process.

The discovery of new enzymatic machinery in cells raised several fundamental questions to be addressed. What is the natural role of RNAi process? What is the cellular structure and function of the gene silencing machinery? And more importantly, does this mechanism exist in other organisms and can it be used for specific gene silencing?

During the next few years the molecular mechanism involved in handling dsRNA molecules has been extensively researched. In a very short time RNAi became a "powerful new tool" for functional genomic analysis, target validation and gene knockdown in cell and animal models. The main components of RNAi enzymatic machinery have been uncovered, and the important natural role of RNAi process has been elucidated.

Correspondence to: J. Vidugirienė, Department of Cell Analysis, Promega Corporation, 2800 Woods Hollow Road, Madison, WI 53711, USA. E-mail: jvidugiriene@promega.com

RNAI SILENCING MECHANISM

Depending on the organism, RNAi can be triggered by various sources of dsRNA molecules, including long dsRNAs, exogenously introduced small dsRNAs known as siRNAs or plasmid-based short hairpin RNAs (shRNAs) (Fig. 1). In cells, long dsRNAs and hairpin RNAs are recognized by an endonuclease Dicer and are converted into short-interfering RNAs (siRNAs) with 2-nt overhangs at the 3' ends and phosphate groups at the 5' ends (5, 6). Small interfering siRNAs generated by Dicer or introduced exogenously are loaded into RNA-induced silencing complexes (RISCs). RISC complex unwinds the double-stranded siRNA and preferentially utilizes one of the RNA strand as a guide to target complementary RNA molecules for degradation (7–9). The minimal defining features of RISC include the presence of an Argonaute family member and the guide strand of a small RNA. The guide-strand-containing RISC complex then binds to the corresponding mRNA and directs it for cleavage which is carried out by the catalytic domain of Argonaute 2 (10–12). The 5' end of the guide strand sets the location of the cleavage site for target-RNA and the cleavage which occurs between 10 and 11-nt upstream of the 5' end. The cleaved messenger RNA is targeted for degradation, while RISC complex is recycled and can undergo multiple rounds of mRNA cleavage (for more information see recent reviews 13–16).

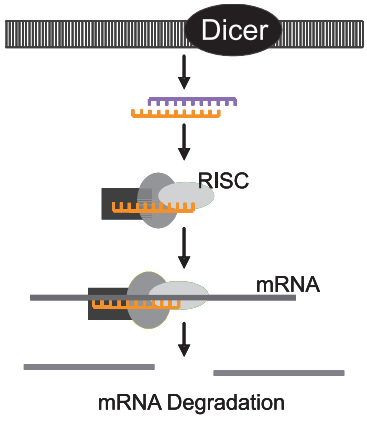

Fig. 1. Cellular mechanism of RNA interference

NATURAL FUNCTION OF RNAi MACHINERY

Once it was discovered that RNA interference is well conserved throughout eukaryotic organisms, it was important to find out what role RNAi plays in cellular processes. It was apparent that RNAi might have a more general role in gene regulations than once predicted. Short RNAs were suggested to be involved in the silencing of transposon elements, repetitive genes and viruses, processes that are crucial for maintaining the genome stability (17–19). While progress is being made in understanding RNAi, a very important class of short RNAs, named microRNAs, has been discovered (20, 21). MicroRNAs are abundant single-stranded RNAs (19–25-nt) encoded in the genome of all multicellular organisms and are processed from longer RNA precursors by components of RNAi machinery (22). MicroRNAs regulate the expression of distinct genes by base-pairing with partially complementary sequences in mRNAs and simply inhibiting their translation into proteins. Human microRNAs were identified only a short time ago but their involvement in widespread functions, including development, proliferation, heamatopoiesis and apoptosis, has been recognized (23–25). Therefore, although RNAi was first discovered as an experimental tool for sequence-specific gene silencing, it is clear now that RNAi is a well conserved natural mechanism found throughout eukaryotic organism and plays an important role in post-trancriptional gene regulation.

RNA INTERFERENCE AS A RESEARCH TOOL

Because it is very easy to carry out an RNAi study in a specific manner, the technology has emerged as a versatile tool with a wide range of applications from reverse genetics to high throughput screening of drug targets. Libraries of RNA interference molecules have been constructed, and gene function analysis on a genome-wide scale has been carried out (26–28).

In worms, fungi and plants, RNA interference can be achieved by simple introduction of long double stranded RNAs. Before RNAi interference became a powerful research tool in mammalian cells, several obstacles had to be overcome (Fig. 2). To begin with, introduction of long dsRNAs into mammalian cells induces a powerful set of antiviral responses, resulting in inhibition of all gene expression and rapid cell death. Fundamental insights into mammalian RNAi came after the discovery that all the multicellular organisms possess a conserved protein machinery that recognizes double-stranded RNA and that the molecules that lead to actual RNA interference and destruction of gene function are small double-stranded interfering RNAs (siRNAs) around ~19–31 nucleotide pairs in length (29). Since then, the field of RNAi-based technologies has grown at exponential rate.

There are two basic options for introducing the siRNA into mammalian cells. The first one deploys DNA-dependent expression vectors of short hairpin RNAs (shRNAs), which are subsequently processed by the cell to generate siRNAs. The second, short interfering RNAs targeting any gene of interest, can be made synthetically and delivered into the cells for specific inhibition of gene expression. The choice of method primarily depends on experimental design.

Synthetic siRNAs are easy to use and often produce reasonable results, however, their effects are transient and are restricted by rate of cell division (30). Since mammalian cells do not readily take up naked nucleic acid, double-stranded RNA molecules have to be specifically delivered into target cells. Synthetic siRNAs can be delivered to mammalian cells by electroporation

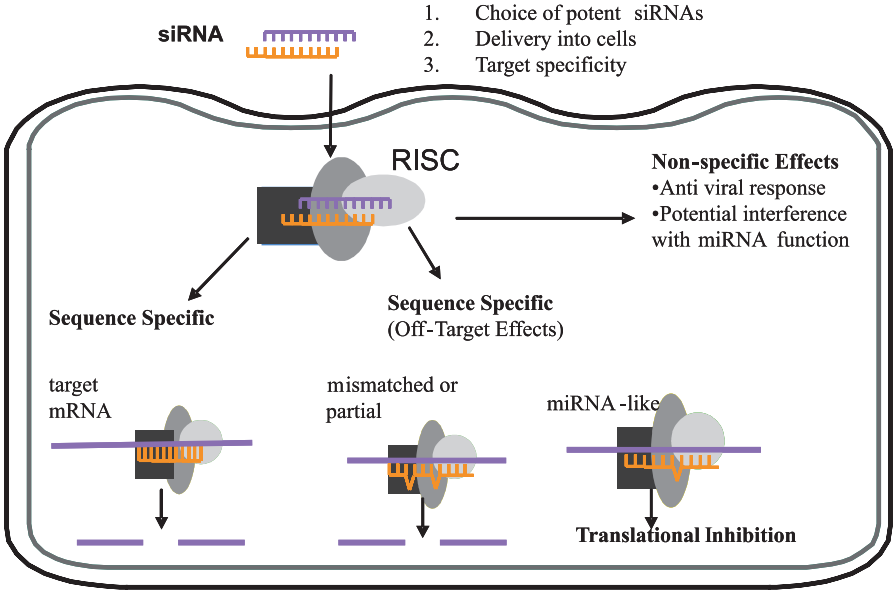

Fig. 2. Challenges for siRNA approaches

or by using lipophilic agents (31). Additionally, it has been demonstrated that ligands such as cholesterol, can be directly linked to the chemically stabilized siRNA molecule, facilitating cellular siRNA uptake through receptor-mediated endocytosis (32). More recently, with increased interest in cell-specific or tissue specific delivery, new strategies, such as coupling siRNAs to antibody fragments or packaging into nanoparticles coated with receptor-targeting ligands, have been explored (33, 34).

RNAi expressing vectors can either be transiently transfected into cells using standard DNA delivery approaches or are efficiently introduced and stabaly integrated into the host genome using viral systems (35, 36). Using viral approach shRNA-expressing cassettes can be delivered into both transformed and primary cells.

In general, using RNAi approach efficient (in many cases genes can be silenced by over 90%) and highly specific (in some cases, a single point mutation can abolish silencing effect) suppression of virtually any gene can be achieved. However, in practice different siRNAs often show a spectrum of potency with low or moderate efficacy (37, 38). Furthermore, siRNAs may nonspecifically target unrelated genes with only partial sequence complementarily and induce undesired "off-target effects" (39). Several commercial sources provide effective design algorithms online which are based on our current understanding of the function of RNAi machinery and are also aimed to minimize potential sequence-dependent "off-target effects". However, even with the well designed siRNAs and their efficient delivery into target cells, the proper controls and appropriate readout assays have to be selected for meaningful interpretation of RNAi experiments. For more comprehensive details on RNAi experimental design and applications see recent review (40).

RNA INTERFERENCE AS A THERAPEUTIC APPROACH

RNAi presents an attractive approach to treat pathological disorders that are linked to elevated expression of certain genes. Among the 30,000 genes of the human genome at least 1000 have already been linked to a genetic disease in humans and as such are potential targets for RNA interference. RNAi interference approach has already been exploited for the treatment of viral infection, ocular disease, disorders of the nervous system and cancer (for recent reviews, see 41–43). Clinical trials based on siRNAs targeting vascular endothelial growth factor (VEGF) or its receptor (VEGFR1) have been initiated for the treatment of 'wet' form of AMD (age-related macular degeneration) (Sirna Therapeutics). Intranasal delivery of siRNAs has been applied for inhibiting RSV infection (44). Studies on vaginal transmission of herpes simplex virus 2 (HSV-2) in mice showed that the infection can be blocked using a siRNA microbicide (45).

Other attractive targets for RNAi based therapies are neurological diseases and cancer. RNA interference has been demonstrated to be effective *in vivo* for reduction of pathological molecules in neurons, leading to robust efficacy in animal models of Alzheimer's disease, ALS, anxiety and depression (46). Different RNAi approaches have been successfully employed in a mouse model for effective inhibition of tumor growth (47).

However, a wide gap still exists between achieving such results in experimental models and its potential for therapeutic applications. Major challenges still remain that have to be addressed to be successfully considered as a platform for the development of thearpeutics. These include ensuring siRNA potency, specificity, delivery and, finally, cost.

OUTLOOK

Over the past few years the field of RNAi has matured and expanded. RNAi has become a standard experimental tool employed in various laboratories for sequence specific gene silencing. As we learn more about RNAi, improvements in siRNA stability, delivery and reduction of "off-target" effects will be made, and the high expectations of RNAi as an approach for development of potential therapeutics will be realized.

Received 02 July 2007
Accepted 01 August 2007

References

1. Mello CC, Conte DJr. Revealing the world of RNA interference. Nature 2004; 431: 338–42.
2. Meister G, Tuschl T. Mechanism by gene silencing by double-stranded RNA. Nature 2004; 431: 343–9.
3. Napoli C, Lemieux C, Jorgensen R. Introduction of a chimeric chalcone synthase gene into petunia results in reversible co-suppression of homologous genes in trans. Plant Cell 1990; 2: 279–89.
4. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, MelloCC. Potent and specific genetic interference by double stranded RNA in *Caenorthabditis elegans*. Nature 1998; 391: 806–11.
5. Hammond SM, Bernstein E, Beach D, Hannon G. J. An RNA-directed nuclease mediates post-transciptional gene silencing in Drosophila cells. Nature 2000; 404: 293–6.
6. Bernstein E, Caudy AA, Hammond SM, Hannon GJ. Role for a bidentate ribonuclease in the initiation step of RNA interference. Nature 2001; 409: 363–6.
7. Elbashir SM, Martinez J, Patkaniowska A, Lendeckel W, Tuschl T. Functional anatomy of siRNAs for mediating efficient RNAi in Drosophila melanogaster embryo lysate. EMBO J. 2001; 20: 6877–88.
8. Tuschl T, Zamore PD, Letmann R, Bartel DP, Sharp PA. Targeted mRNA degradation by double-standed RNA *in vitro*. Genes Dev 1999; 13: 3191–7.
9. Zamore PD, Tuschl T, Sharp PA, Bartel DP. RNAi: double-stranded RNA directs the ATP-dependent cleavage of mRNA at 21 to 23 nucleotide intervals. Cell 2000; 101: 25–33.
10. Chiu YL, Rana TM. RNAi in human cells: basic structural and functional features of small interfering RNA. Mol Cell 2002; 10: 549–61.
11. Haley B, Zamore PD. Kinetic analysis of the RNAi enzyme complex. Nature Struct Mol Biol 2004; 11: 599–606.
12. Miyoshi K, Tsukumo H, Nagami T, Siomi H, Siomi MC. Slicer function of *Drosophila* Argonautes and its involvement in RISC formation. Genes Dev 2005; 19: 2837–48.

13. Sontheimer EJ. Assembly and function of RNA silencing complexes. Nature Reviews / Molecular Cell Biology 2005; 6: 127–38.
14. Song JJ, Joshua-Tor L. Argonaute and RNA-getting into the groove. Curr Opin Struct Biol 2006; 16: 5–11.
15. Tolia NH, Joshua-Tor L. 2007 Slicer and the Argonautes. Nature Chemical Biology 2007; 3: 36–43.
16. Rana TM. Illuminating the silence: understanding the structure and function of small RNAs. Nature Reviews / Mol Cell Biol. 2007; 8: 23–34.
17. Sijen T, Pasterk RH. Transposon silencing in the Caenorhabditis elegans germ line by natural RNAi. Nature 2003; 426: 310–4.
18. Gitlin L, Karelsky S, Andino R. Short interfering RNA confers intracellular antiviral immunity in human cells. Nature 2002; 418: 430–4.
19. Lipman Z, Martienssen R. The role of RNA interference in heterochromatic silencing. Nature 2004; 431: 364–70.
20. Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochromic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 1993; 75: 843–54.
21. Reinhart BJ, Slack FJ, Basson M et al. The 21-nucleotide let-7 RNA regulates development timing in *Caenorhabditis elegans*. Nature 2000; 403: 901–6.
22. Hutvagner G, Zamore PD. A microRNA in a multiple-turnover RNAi enzyme complex. Science 2002; 297: 2056–60.
23. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281–97.
24. Farh KK-H, Grimson A, Jan C et al. The widespread impact of mammalian microRNAs on mRNA repression and evolution. Science 2005; 310: 1817–21.
25. Sage CI, Agami R. Immense promises for tiny molecules. Cell cycle 2006; 5: 1415–21.
26. Berdards R, Brummelkamp TR, Beijersbergen RI. shRNA libraries and their use in cancer genetics. Nature Methods 2006; 3: 701–6.
27. Fewell GD, Schmitt K. Vector based RNAi approach for stable, inducible and genome-wide screens. Drug Discovery Today 2006; 11: 975–82.
28. Root DE, Hacohen N, Hahn WC, Lander ES, Sabatini DM. Genome-scale loss-of-function screening with a lentiviral RNAi library. Nature Methods 2006; 3: 715–9.
29. Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Wieber K, Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature 2001; 411: 494–8.
30. Echeverri CJ, Perrimon, N. High-throughput RNAi screening in cultured cells: a user's guide. Nat rev genet 2006; 7: 373–84.
31. Elbashir SM, Hartborth J, Weber K, Tuschl T. Analysis of gene function in somatic mammalian cells using small interfering RNAs. Methods 2002; 26: 199–213.
32. Soutschek J, Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs. Nature 2004; 432: 173–8
33. Song E, Zhu P, Lee S-K et al. Antibody mediated in vivo delivery of small interfering RNAs via cell-surface receptors. Nature Biotechnol 2005; 23: 709–19.
34. Hu-Lieskovan S, Heidel JD, Bartlett DW, Davis ME, Triche TJ. Sequence-specific knockdown of EWS-FLI1 by targeted, nonviral delivery of small interfering RNA inhibits tumor growth in a murine model of metastatic Ewing's sarcoma. Cancer Ther 2005; 65: 8984–92.
35. Jr OS, Rossi JJ. Expressing short hairpin RNAs *in vivo*. Nature Methods 2006; 3: 689–95.
36. Wiznerowicz M, Szulc MJ, Trono D. Tuning silence: conditional system for RNA interference. Nature Methods 2006; 3: 682–8.
37. Khvorova A, Reynolds A, Jayasena SD. Functional siRNAs and miRNAs exhibit strand bias. Cell 2003; 115: 209–16.
38. Pei Y, Tuschl T. On the art of identifying effective and specific siRNAs. Nature Methods 2006. 3: 670–6.
39. Jackson A. Widespread siRNA 'off-target' transcript silencing mediated by seed region sequence complementarity. RNA 2006; 12: 1179–87.
40. Sachse C, Krausz E, Kronke A et al. High-Throughput RNA interference strategies for target discovery and validation by using synthetic short interfering RNAs: functional genomics investigations of biological pathways. Methods in Enzymology 2005; 392: 242–77.
41. Dykxhoorn DM, Palliser D, Lieberman J. The silent treatment:siRNAs as small molecule drugs. Gene Ther 2006; 13: 541–52.
42. Kim DH, Rossi JJ. Strategies for silencing human disease using RNA interference. Nature Reviews / Genetics 2007; 8:173–84.
43. de Fougerolles A, Vornlocher H-P, Maraganore J, Lieberman J. Interfering with disease: a progress report on siRNA-based therapeutics. Nature Reviews / Drug Discovery 2007; 6: 443–53.
44. Bitko V. Inhibition of respiratory viruses by nasally administered siRNA. Nature Med 2005; 11: 50–5.
45. Palliser D, Chowdhury D, Wang Q-Y et al. An siRNA based microbicide protects mice from lethal herpes simplex virus 2 infection. Nature 2006; 439: 89–94.
46. Sah DWY. Therapeutic potential of RNA interference for neurological disorders. Life Sciencies 2006; 79: 1773–80.
47. Takeshita F, Ochiya T. Therapeutic potential of RNA interference against cancer. Cancer Sci 2006; 97: 689–96.

Jolanta Vidugirienė, Said Goueli, Kęstutis Sužiedėlis

RNR INTERFERENCIJA (NEPRODUKTYVI SĄVEIKA): NUO TYRIMO METODO IKI NAUJO TERAPINIO VEIKSNIO

S a n t r a u k a

RNR interferencija (neproduktyvi sąveika, RNRi) yra efektyvus, labai atrankus genų raiškos slopinimo būdas, kurį lemia dvigrandės RNR komplekso susidarymas; dėl pastarojo genų veikla slopinama priklausomai nuo genų sekos. RNRi – natūralus, evoliucinės atrankos būdu atsiradęs konservatyvus biologinis reiškinys, veikiantis eukariotinių organizmų genomų vientisumą, genų raišką ir apsaugantis nuo virusinių infekcijų. Per pastarąjį dešimtmetį šis konservatyvus ląstelės atsako mechanizmas kartu tapo ir neįkainojamu tyrimo metodu, kuriuo funkciškai apibūdinami nustatyti genai, identifikuojami nauji genai, lemiantys savitas funkcijas ląstelėje. Metodas tapo nauju molekuliniu potransliacinio genų slopinimo įrankiu, potencialiai tinkamu klinikiniam taikymui. Šioje apžvalgoje naudojama tiek kitų autorių publikuota medžiaga, tiek šių autorių eksperimentinė patirtis siekiant supažindinti skaitytojus su esama RNRi būkle, kartu prisimenant, kaip RNR interferencijos technologija paskatino svarbiausius atradimus funkcinės genomikos srityje ir naujas kryptis genų terapijos srityje. Apžvalgoje taip pat pateikiamas kitų naujausių šios srities apžvalgų sąrašas, skirtas giliau susipažinti su RNRi fenomenu.

Raktažodžiai: RNR interferencija, genų slopinimas, genų terapija