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RNA Interference: Molecular mechanism and milestone therapeutic applications

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Abstract

In time, we have become gradually more aware of the fact that RNAs play a vital role in regulation of gene expression. The discovery of RNA interference (RNAi) also called post transcriptional gene silencing (PTGS), gave a booster shot to the world of RNA and molecular biology, a set of reactions involving the sequence-specific degradation of targeted mRNA by short double-stranded RNA molecules. Swift progress in our knowledge of RNAi-based mechanisms has made us enabled to use this powerful molecular process in studies of gene action as well as in biological and therapeutic applications for the treatment of disease. RNAi has become a valuable research tool, both in cell culture and in living organisms, because synthetic dsRNA introduced into cells can selectively and vigorously induce suppression of specific genes of interest. RNAi may be used for large-scale screens that can systematically silent the specific gene, which can help to identify the components necessary for a particular cellular process or an event such as cell division. The pathway is also used as a practical tool in biotechnology and medicine. Currently there are various clinical trials involving RNAi, and there is an expectation for the list of new applications to grow at an exceptional rate. This article focuses on our modern understanding about the mechanisms and therapeutic applications of RNAi.

Keywords: RNA interference, microRNA, tumor inhibition, infectious diseases.

Özet

RNA İnterferans: Moleküler mekanizma ve terapötik uygulamalardaki önemi

Zaman ilerledikçe, gen anlatımının düzenlenmesinde RNA'nın hayati rolünün arttığı gerçeğinin giderek daha fazla farkına varmaktayız. Transkripsiyon sonrası gen susturulması (PTGS) olarak da adlandırılan RNA interferansın (RNAi), kısa çift zincirli RNA molekülleri yoluyla hedeflenmiş mRNA'nın dizi-spesifik yıkımını kapsayan bir reaksiyon setinin keşfi, RNA ve moleküler biyoloji dünyasını güçlendirici bir aşı oldu. Bildiğimiz RNA tabanlı mekanizmalardaki hızlı ilerleme bizi, hastalıkların tedavisi için biyolojik ve terapötik uygulamalarda olduğu kadar gen aktivitesi çalışmalarında da bu güçlü moleküler süreci kullanmakta etkinleştirmektedir. RNAi, hücrelerin içine giren sentetik çift zincirli RNA'nın (dsRNA) kuvvetli ve seçici olarak ilgilenilen spesifik genin baskılanmasını tetikleyebilmesi nedeniyle hem hücre kültüründe hem de yaşayan organizmalarda değerli bir araştırma aracı haline gelmiştir. RNAi, hücre bölünmesi gibi belli bir hücresel bir işlemcinin bileşenlerinin tanımlanmasına yardımcı olmak üzere sistematik olarak spesifik bir geni susturabilen büyük ölçekli taramalarda kullanılabilir. Bu yolak aynı zamanda biyoteknoloji ve tıpta pratik bir araç olarak kullanılmaktadır. Bugünlerde RNAi'yi içeren çok çeşitli klinik denemeler ve olağanüstü bir hızla gelişen yeni uygulamaların listesi için beklentiler bulunmaktadır. Bu makale RNAi'nin terapötik uygulamaları ve mekanizması hakkındaki modern anlayışımıza odaklanmıştır.

Anahtar Kelimeler: RNA interferans, mikroRNA, tümör inhibisyonu, enfeksiyöz hastalıklar

Introduction

In the 1990s, a number of posttranscriptional gene silencing phenomena were discovered in different life forms like plants, fungi, animals and ciliates (Baulcombe, 2000; Matzke *et al*. 2001). The story of gene silencing at post-transcriptional level started in 1990 and observed in plants for first time when *Jorgensen laboratory* tried to up-regulate the activity of a gene for chalcone synthase, an enzyme involved in the production of specific pigments, by introducing exogenous transgenes into *petunias* (Agrawa *et al*., 2003; Napoli *et al*., 1990). Surprisingly, flower pigmentation did not get deeper, but rather showed variegation with complete loss of pigments in some cases. This revealed that that the added small DNA sequences influenced the expression of the endogenous loci (Hannon, 2002). This phenomenon was referred to as "cosuppression" (Napoli *et al*., 1990; Campbell, 2005). latter on Fire and coworkers (1998) coined term RNA interference (RNAi) to illustrate the inhibition of gene expression by injecting double-stranded RNAs (dsRNAs) into *Caenorhabditis elegans*. Guo and Kemphues (1995) found that *sense RNA* was as effective as *antisense RNA* for suppressing gene expression in worms, on the basis of this knowledge, Fire *et al.* (1998) investigate single-stranded antisense RNA and double stranded RNA in their experiments. Surprisingly they found that dsRNA was more effective for interference than any of individual strand. It was found that single-stranded antisense RNA had a modest effect in diminishing specific gene expression when injected into *C. elegans*, whereas double-stranded RNA induced powerful and specific interference (Fire *et al.,* 1998). RNAi is an *in vivo* multistep process which involves the production of small interfering RNAs (siRNAs) through the action of the 'Dicer' enzyme (RNase III endonuclease), resulting in 20-25 nucleotide siRNAs which mediate degradation of their complementary RNA (Shi, 2003). The following lines will review the fundamental mechanisms of RNAi and the usefulness of this approach in biology as to amend gene expression in mammalian cells.

Mechanism of RNA interference

Two types of small RNA molecules – microRNA (miRNA) and small interfering RNA (siRNA) are fundamental to RNA interference. (Hammond *et al*., 2000; Bernstein *et al*., 2001; Stevenson, 2004). These are generated via processing of longer dsRNA and stem loop precursors (Bernstein *et al*., 2001; Stevenson, 2004). RNAi is RNA-reliant gene silencing process that is controlled by the RISC (RNA-induced silencing complex) and is started when short double-stranded RNA molecules in a cell's cytoplasm interact with the argonaute (catalytic component of RISC) (Hamilton *et al*., 1999; Mette *et al*., 2000). The dsRNA which has to be processed for gene silencing, can be exogenous (from infection by a virus with an RNA genome or Lab. manipulations) or endogenous (originating in the cell). When the dsRNA is exogenous the RNA is brought directly into the cytoplasm and broken down to short fragments by Dicer (Elbashir *et al*., 2001). When it is endogenous as in pre-microRNAs, it is expressed from RNA-coding genes in the genome. The primary transcripts from such genes are first processed to form the characteristic stem-loop structure of pre-miRNA in the nucleus, and then exported to the cytoplasm to be cleaved by Dicer. Thus, the both pathways, exogenous and endogenous, converge at the RISC complex (Hammond *et al*., 2001).

Cleavage of dsRNA

Endogenous dsRNA starts RNAi by activating the ribonuclease protein Dicer, (Bernstein *et al.,* 2001) which binds and cleaves dsRNAs to produce double-stranded fragments of 20 pb with a 2-nucleotide overhang at the 3' end (Siomi *et al.,* 2009; Zamore *et al.,* 2000; Vermeulen *et al.,* 2005; Castanotto *et al.,* 2009) resulted small double-stranded fragments are called siRNAs. These then separated into single strands and integrated into an active RISC complex where they base-pair to their target mRNA and cleavage the mRNA thereby forbidding it from being used as atranslation template (Ahlquist *et al.,* 2002)

An effector protein known as RDE-4 in *C. elegans* and R2D2 in *Drosophila* induces dicer activity, by detecting an exogenous dsRNA and binding to it. This protein only binds long dsRNAs and then facilitates the transfer of cleaved siRNAs to the RISC complex (Parker *et al.,* 2006; liu *et al.,* 2003)

MicroRNA and RISC action

A pri-miRNA is transcribed from a much longer RNA-coding gene which is processed in the cell nucleus, to a 70-nucleotide stem-loop structure by the microprocessor complex, this complex consists of an RNase III enzyme called Drosha and a protein

DGCR8 which binds to dsRNA. *A pri-miRNA* must first undergo extensive post-transcriptional modification to become final mature miRNA. Mature miRNAs are structurally similar to siRNAs produced from exogenous dsRNA and can be integrated into the RISC complex; thus, miRNA and siRNA share the same cellular machinery downstream of their initial processing (Gregory *et al.,* 2006)

RISC comprises of a protein (with ribonuclease activity) and RNA component, first time discovered in *Drosophila* by Hammond *et al.* (2000). In addition to the ribonuclease activity RISC also contains a PAZ domain. Extra components of RISC consist of two RNA binding proteins i.e. *Vasa* intronic gene and *dFMR* proteins (Agrawal *et al.* 2003; Arenz and Schepers 2003; Kuznetsov 2003). There are still other elements of RISC yet to be recognized. For example, it remains unreadable that how the siRNAs become integrated and oprated in RISC, and how the siRNA works within the complex. The general agreement among scientists is that there is protein-protein interaction through the PAZ domain, between Dicer and RISC which enable small single stranded RNA fragments (less than 30 nucleotides) to enter the RNAi pathway. This trend arises two questions: 1) Does *RISC* recruit small single stranded fragment of RNA into the complex or does *Dicer* first pick up the small single stranded fragment of RNA and 2) How does the siRNA functions inside the complex? Investigator discovered only the answer to the second question. They got fond evidence to propose that RISC degrades the sense strand and uses the antisense strand only of the siRNA. RISC uses the siRNA and looks for the corresponding base sequence of the targeted mRNA. The degradation process is started once, by the siRNA-RISC complex, successful locating and cutting of the complementary mRNA happens, thus exposing the newly cut mRNA to exonucleases.

The Therapeutic Prospective of RNAi

Although it is difficult to insert long dsRNA strands into mammalian cells due to the interferon response, even then it may be promising to utilize RNAi in therapy. The use of short interfering RNA has been more successful rather than dsRNA (Paddison *et al.,* 2002; Whitehead KA *et al.,* 2011). The application of RNAi to clinical medicine has the ability to decode molecular advances directly into therapy, the eventual realization of rational remedies. Optimistic developments in animal models of cancer, infectious disease, and Genetic disorders propose that RNAi may be the most advanced track to cure the life threatening diseases in the near future.

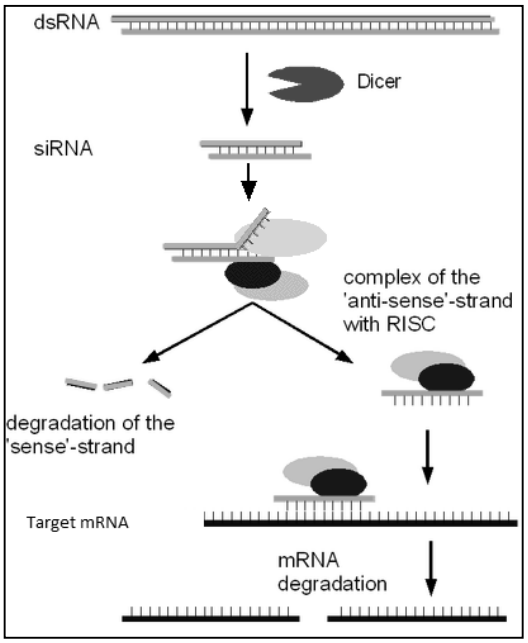

Figure 1*:* Schematic picture of RNAi mechanism.

Role of RNAi in Tumor Inhibition

In metastatic skin cancer, an oligonucleotide targeting the anti apoptotic gene *Bcl2* has revealed promising results in the clinical trials (Buchele, 2003). RNAi used for the treatment of chronic *myelogenous leukemia* (CML) for the first time, a disorder in which active form of the *Abl tyrosine kinase* produced by a chromosomal translocation. siRNAs can silence the oncogenic fusion protein by targeting the fusion transcript while conserving expression of its wild-type form (Wohlbold *et al.,* 2003; Scherr *et al.,* 2003). Point mutations in *Ras* oncogenes are associated with Pancreatic and colon carcinomas. Retroviral vectors express shRNAs, which target the mutant transcripts and selectively silence the *K-RASV12* mutant, and restrict tumor growth (Brummelkamp *et al.,* 2002). Rapid data collection on the types of mutations linked with various types of cancer will furnish an extended list of targets for this type of RNAi-based approach. Tumors often start expression of MDR1, a multidrug transporter that cells can use to expel chemotherapeutic drugs when they become resistant to standard pharmacological treatments over time. Researchers have transfected tumor cells with siRNAs targeting MDR1 to demonstrate that RNAi can be used to maximize the benefit of

existing chemotherapies for the treatment of cancer (Nieth *et al.*, 2003).

Role of RNAi Against Infection

The supreme role of RNAi in clinical medicine may be for the cure of infectious disease. To date, hepatic viral infection received the prime attention, because nucleic acid can be easily delivered to the liver of mice hydrodynamic shock and tail injection (Lewis *et al.*, 2002; McCaffrey *et al.*, 2002). Acute liver failure, cirrhosis, and liver cancer may result of chronic viral hepatitis infection. There is no available vaccine for hepatitis C virus (HCV) and limited options for its treatment (Davis, 1997), while in the case of hepatitis B virus (HBV), vaccine is available but once infected there is no treatment (Poland and Jacobson, 2004). McCaffrey *et al.* (2002, 2003) demonstrated that RNAi has ability to selectively repress viral protein expression in vivo. To achieve this finding, they performed two studies using tail vein injection of shRNAs targeting the HBV and HCV replicons. These results suggest new hope for hepatitis and other viral infection with reduced prognoses. One drawback of this strategy is that viruses like HIV that can evolve and change their genome rapidly; they modify their genomes to avoid sequence dependent silencing. A substitute approach is to use RNA interference to either target signaling pathways that commence the inflammatory response or host proteins necessary for pathogen intrusion. Another thrilling study was performed to relieve hepatitis in an animal model of acute liver failure by using shRNAs which target a host protein (Song *et al.*, 2003). In this in vivo research, siRNAs targeting the cell death receptor *Fas*, reached the liver, entered hepatocytes, and blocked *Fas* expression, limiting the severity of disease and expand the survival of treated animals.

HIV was the initial viral agent targeted by RNAi (Hannon and Rossi 2004). Exogenous engineered siRNAs and expressed shRNAs have been used to target RNAs encoded by Human Immune Virus in cell lines and in primary haematopoietic cells including the *tat* element (Lee *et al*. 2002), *gag* (Novina *et al*. 2002; Park *et al*. 2002), *env* (Park *et al*. 2002), *TAR* (Jacque *et al*. 2002), *nef* (Jacque *et al*. 2002), rev (Lee *et al*. 2002; Coburn and Cullen 2002), reverse transcriptase (Surabhi and Gaynor 2002; Hannon and Rossi 2004) and *vif* (Jacque *et al*. 2002). Unfortunately, because of the risk of high viral mutation rate by which viral genome can escape being targeted, RNA of HIV cannot be targeted directly by RNAi (Boden *et al*. 2003). An attractive and alternative approach is to target down regulation of the cellular cofactors required for HIV infection by RNAi. Cellular cofactors which can be down regulated, are the HIV receptor CD4 (Novina *et al*. 2002), the co-receptors CXCR4, CCR5 (Martinez *et al*. 2002b) and NF-κB (Surabhi and Gaynor 2002). It is exclusively because of the RNAi that all above mentioned cellular cofectors have been effectively downregulated resulting in the inhibition of HIV replication in several human cell lines and in primary cells like haematopoietic stem-cell-derived macrophages and T lymphocytes (Capodici *et al*. 2002; Coburn and Cullen 2002; Jacque *et al*. 2002; Martinez *et al*. 2002b; Surabhi and Gaynor 2002; Novina *et al*. 2002; Banerjea *et al*. 2003; Li *et al*. 2003; Hannon and Rossi 2004).

RNAi in Genetic and Developmental Diseases

Mutant transcripts can be targeted selectively by the engineered dsRNA, while leaving the role of wildtype alleles unchanged. The most apparent approach is to create dsRNA targeting the disease mutation itself. This strategy has revealed some promise in *amyotrophic lateral sclerosis (ALS)* cellular model (Ding *et al.*, 2003). Similarly, designing of siRNAs to target single nucleotide polymorphisms (SNPs) in mutant alleles is a substitute strategy. Efficient analysis of siRNA composition has revealed that single-nucleotide mismatches, when present in centre of siRNA, are the most compromising (Miller *et al.*, 2003). Researchers demonstrated that siRNA which structured in this way to target SNPs in mutant alleles, can decrease disease pathology in vitro by blocking expression of mutant alleles (Miller *et al.*, 2003,2004). Recently a research conducted on mouse model of spinocerebellar ataxia type I revealed that intracerebral delivery of adenoviral vectors encoding shRNAs targeting a mutant allele can lessen intranuclear inclusions and re-establish motor (Xia *et al.*, 2004).

Theoretically, RNAi can be used to treat any disease linked with unsuitable or harmful gene expression i.e. in the case of Down's syndrome, caused by trisomy 21, are attributed to gene dosage imbalances (Antonarakis *et al.*, 2004). RNAi could be utilized to diminish the effect of those duplicated gene which are responsible for this phenotypic effect, as once identified by scientists.

In the case of *C. elegans*, it has been seen that miRNAs regulate the developmental timing and stage-specific processes (Lee *et al.*, 1993). If related mechanisms are preserved in humans, then

exogenous miRNAs might reactivate developmental processes to encourage repair. Additionally, researchers have also found that miRNA expressed in pancreatic islet cells can regulate insulin secretion, representing a new target for diabetes therapy (Poy *et al.,* 2004). Finally, RNAi has novel and powerful role in stem cell therapy .researchers have previously shown that miRNAs can influence the differentiation of hematopoietic stem cells (Chen *et al.,* 2004). Misexpressed miRNAs could be utilised to produce perfect populations of differentiated B-cells or other types of cell for ex vivo uses.

Limitations for RNAi as a therapy

Ensuring efficient delivery of siRNA and avoiding off-target effects are two main challenges for developing therapeutic RNAi approaches (Pasquinelli, 2002; Moss, 2002 Endogenous RNAi pathways could potentially be interfered and affected by siRNAs. Introducing siRNAs to target particular cellular or viral mRNA in essence hijacks the endogenous RNAi system (Hutvagner *et al.,* 2004). The prime attention is to be given to the basic research on off-target effects of siRNAs and on the design of effective siRNAs(Jackson *et al.,* 2003; Scacheri *et al.,* 2004; Saxena *et al.,* 2003). For oligonucleotide-based therapeutics, successful targeted delivery to particular cell or specific tissue types is still not a practical reality. For IV injection, either siRNAs are conjugated with cholesterol group or packed into liposomal particles. But these delivery approaches are effective for delivery to the liver and jejunum, but may not be suitable for delivery to other organs (Soutschek *et al.,* 2004). However the viral-vector-mediated delivery of therapeutic shRNA genes is an alternative approach. But still there are several safety concerns to this approach; one of them is a successful delivery of viral vector into targeted cell or tissue. As delivery related research is advancing, it remains to be answered that weather nuclease-resistant siRNAs will move to the clinic more quickly and efficiently than synthetic deoxyoligonucleotides.

Conclusion

In a surprisingly short time since its discovery in *C. elegans*, the RNAi metabolic pathway has came out as an influential tool for the study of gene function in mammals. As we improve our knowledge about the fundamental biology and biochemistry of this conserved gene-regulatory mechanism, so does our capability to utilize RNAi as an experimental tool. Despite substantial hurdles to conquer by using RNAi in whole animals, there is a hope that RNAi will discover a place alongside more formal approaches in the treatment of life threatening human diseases in near future.

References

Agrawal N, Dasaradhi PVN, Mohmmed A, Malhotra P, Bhatnagar RK, Mukherjee SK. RNA Interference: Biology, Mechanism, and Applications. *Microbiology and Molecular Biology Reviews* 67: 657-685. 2003.

Ahlquist P. RNA-dependent RNA polymerases, viruses, and RNA silencing. *Science.* 296: 1270–76, 2002.

Arenz C, Schepers U. RNA interference: from an ancient mechanism to a state of the art therapeutic application? *Naturwissenschaften* 90: 345-359, 2003.

Banerjea A, Li MJ, Bauer G, Remling L, Lee NS, Rossi J, Akkina R. Inhibition of HIV-1 by lentiviral vectortransduced siRNAs in T lymphocytes differentiated in SCIDhu mice and CD34+ progenitor cell-derived macrophages. *Mol Ther*. 8:62–71, 2003.

Baulcombe D. Unwinding RNA silencing. *Science.* 290: 1108-1109, 2009.

Bernstein E, Caudy A, Hammond S, Hannon G. Role for a bidentate ribonuclease in the initiation step of RNA interference. *Nature.* 409: 363–69, 2001.

Bernstein E, Caudy AA, Hammond SM, Hannon GJ. Role for a bidentate ribonuclease in the initiation step RNA interference. *Nature.* 409: 363–366, 2001.

Boden D, Pusch O, Lee F, Tucker L, Ramratnam B. Human immunodeficiency virus type-1 escape from RNA interference. *J Virol*. 77: 11531–11535, 2003.

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

Buchele T. Proapoptotic therapy with oblimersen (bcl-2 antisense oligonucleotide)- review of preclinical and clinical results. *Onkologie.* 26: 60–69, 2003.

Campbell TN, Choy FYM. RNA Interference: Past, Present and Future. *Curr Issues Mol. Biol.* 7: 1-6, 2005.

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

Castanotto, Daniela, Rossi, John J. The promises and pitfalls of RNA-interference based therapeutics. *Nature*. 457: 426–433, 2009.

Chen CZ, Li L, Lodish HF, Bartel DP. MicroRNAs modulate hematopoietic lineage differentiation. *Science.* 303: 83–86, 2004.

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

Davis GL. Treatment of acute and chronic hepatitis C. *Clin Liver Dis.* 1:615– 630, 1997.

Ding H, Schwarz DS, Keene A. Selective silencing by RNAi of a dominant allele that causes amyotrophic lateral sclerosis. *Aging Cell.* 2: 209–217, 2003.

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*. 20:6877-6888, 2001.

Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature (London)* 391: 806–811, 1998.

Gregory R, Chendrimada T, Shiekhattar R. MicroRNA biogenesis: isolation and characterization of the microprocessor complex. *Methods Mol Biol.* 342: 33–47, 2006.

Hammond S M, Boettcher S, Caudy A, Kobayashi R, and Hannon G. Argonaute2, a Link Between Genetic and Biochemical Analysis of RNAi. *Science*.293: 1146-1150, 2001.

Hannon G. J. and Rossi J. Unlocking the potential of the human genome with RNA interference. *Nature.* 431: 371–378, 2004.

Hannon GJ. Rossi J. Unlocking the potential of the human genome with RNA interference. *Nature.* 431: 371–378, 2004.

Hutvágner G, Simard MJ, Mello CC, Zamore PD. Sequence-specific inhibition of small RNA function. *PLoS Biol.* 2: E98, 2004.

Jackson AL. Expression profiling reveals off-target gene regulation by RNAi. *Nature Biotechnol.* 21: 635–637, 2003.

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

Kuznetsov VV. RNA Interference. An Approach to Produce Knockout Organism and Cell Lines. *Biochemistry (Moscow)* 68: 1301-1317, 2003.

Lee RC, Feinbaum RL, Ambros V. The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell*. 75: 843– 854, 1993.

Lewis DL, Hagstrom JE, Loomis AG. Efficient delivery of siRNA for inhibition of gene expression in postnatal mice. *Nat Genet.* 32:107–108, 2006.

Li MJ, Bauer G, Michienzi A, Yee JK, Lee NS, Kim J. Inhibition of HIV-1 infection by lentiviral vectors expressing Pol III-promoted anti-HIV RNAs. *Mol Ther*. 8: 196–206, 2003.

Liu Q, Rand T, Kalidas S, Du F, Kim H, Smith D, Wang X. R2D2, a bridge between the initiation and effector steps of the Drosophila RNAi pathway. *Science.* 301: 1921–5, 2003.

Martinez JM, Patkaniowskam A, Urlaubm H, Luhrmannm R, Tuschlm T. Single-stranded antisense siRNAs guide target RNA cleavage in RNAi. *Cell.* 110: 563–574, 2002.

Matzke M, Matzke A, Pruss G, Vance V. RNA-based silencing strategies in plants. *Curr. Opin. Genet. Dev.* 11: 221-227, 2001.

McCaffrey AP, Meuse L, Pham TT. RNA interference in adult mice. *Nature.* 418:38–39, 2002.

Miller VM, Gouvion CM, Davidson BL, Paulson HL. Targeting Alzheimer's disease genes with RNA interference: an efficient strategy for silencing mutant alleles. *Nucleic Acids Res.* 32: 661–668, 2004.

Moss EG. MicroRNAs: hidden in the genome. *Curr. Biol.* 12: R138–R140, 2002.

Napoli C, Lemieux C, Jorgensen R. Introduction of chimeric chalcone synthase gene into *Petunia*

results in reversible cosuppression of homologous genes in trans. *J Plant Cell.* 2: 279-289, 1990.

Nieth C, Priebsch A, Stege A, Lage H. Modulation of the classical multidrug resistance (MDR) phenotype by RNAinterference (RNAi). *FEBS Lett.* 545: 144–150, 2003.

Novina CD, Murray MF, Dykxhoorn DM, Beresford PJ, Riess J, Lee SK. siRNA-directed inhibition of HIV- 1 infection. *Nature Med*. 8: 681–686, 2002.

Paddison P, Caudy A, Hannon G. Stable suppression of gene expression by RNAi in mammalian cells. *Proc. Natl. Acad. Sci. USA.* 99: 1443–8, 2002.

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*. 30: 4830–4835, 2002.

Parker G, Eckert D, Bass B. RDE-4 preferentially binds long dsRNA and its dimerization is necessary for cleavage of dsRNA to siRNA. *RNA.* 12: 807–18, 2006.

Pasquinelli AE. MicroRNAs: deviants no longer. *Trends Genet.* 18:171–173, 2002.

Poy MN, Eliasson L, Krutzfeldt J. A pancreatic islet-specific microRNA regulates insulin secretion. *Nature*. 432: 226–230, 2004.

Saxena S, Jonsson ZO, Dutta A. 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, 2003.

Scacheri PC. Short interfering RNAs can induce unexpected and divergent changes in the levels of untargeted proteins in mammalian cells. *Proc. Natl Acad. Sci. USA* 101: 1892–1897, 2004.

Scherr M, Battmer K, Winkler T. Specific inhibition of *bcr-abl* gene expression by small interfering RNA. *Blood.* 101: 1566–9, 2003.

Shi, Y. Mammalian RNAi for the masses. *Trends Genet.* 19, 9–12, 2003.

Siomi, Haruhiko, Siomi, Mikiko C. On the road to reading the RNA-interference code. *Nature*. 457:396–404, 2009.

Song E, Lee SK, Wang J. RNA interference targeting Fas protects mice from fulminant hepatitis. *Nat Med.* 9:347–351, 2003.

Soutschek JA, Akinc B, Bramlage K, Charisse R, Constien M, Donoghue S, Elbashir A, Geick. Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs. *Nature. 432*:173-178, 2004.

Stevenson M. Therapeutic Potential of RNA Interference. *The New England Journal of Medicine.* 351:1772-1777, 2004.

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

Vermeulen A, Behlen L, Reynolds A, Wolfson A, Marshall W, Karpilow J, Khvorova A . The contributions of dsRNA structure to Dicer specificity and efficiency. *RNA.*11: 674–82, 2005.

Whitehead KA, Dahlman JE, Langer RS, Anderson DG. Silencing or stimulation? siRNA delivery and the immune system. *Ann. Rev. Chem. and Biomol. Eng*. 2: 77-96, 2011.

Wohlbold L, van der Kuip H, Miething C. Inhibition of *bcr-abl* gene expression by small interfering RNA sensitizes for imatinib mesylate (STI571). *Blood*. 102: 2236–2239, 2003.

Xia H, Mao Q, Eliason SL. RNAi suppresses polyglutamine-induced neurodegeneration in a model of spinocerebellar ataxia. *Nat Med.* 10: 816–820, 2004.

Zamore P, Tuschl T, Sharp P, Bartel D. RNAi: double-stranded RNA directs the ATP-dependent cleavage of mRNA at 21 to 23 nucleotide intervals. *Cell.*101: 25–33, 2000.