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Commentary

Short interfering RNA-mediated gene silencing; towards successful application in human patients☆

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ABSTRACT

Gene silencing mechanisms that are mediated by small RNAs of 20-30 nucleotides(nt) are collectively called RNA silencing. The representative mechanism is RNA interference (RNAi), in which ~21-nt small RNAs (short interfering RNAs or siRNAs)efficiently trigger cleavage of target gene transcripts. As a result, proteins are no longer made from the targeted mRNAs. RNAi is tremendously specific and efficient with regard to recognizing target gene and disrupting their expression. The triggering molecules, double-stranded (ds) RNAs, are quite stable *in vivo* and are basically indistinguishable from natural endogenous RNAs; thus, RNAi intrinsically has great potential for therapeutic use. In fact, RNAi-based clinical trials for treating human diseases are currently ongoing worldwide. However, optimization of the protocols still requires substantial investigation; indeed, relevant studies have been undertaken internationally by many researchers. Given the growing anticipation of RNAi as a disease therapy, re-examining the RNAi mechanism and machinery from a biochemical perspective might provide a way to advance its successful application in the human body; this, therefore, is the focus of this Commentary.

1. Discovery of RNAi; a powerful tool for gene silencing

RNAi was discovered a decade ago through basic research in *C. elegans* as a model system [1]. It promptly attracted a large number of researchers worldwide because of its methodological potential, including great silencing efficiency and a high degree of target transcript specificity amidst the astonishing numbers of transcripts in cells. The RNAi system can be easily manipulated. For example, in *C. elegans*, feeding the animals dsRNAs or soaking them in a dsRNA solution easily induces RNAi. In addition, the fact that dsRNAs are much more effective than single-stranded (ss) RNAs in gene silencing is remarkable and should be widely appreciated.

Before the discovery of RNAi in nematodes, plant scientists had noticed a gene silencing phenomenon similar to RNAi in the plant kingdom, which was originally referred to as "co-suppression" [2,3]. Researchers in the fungal field termed the phenomenon "quelling" [3]. These observations lead us to anticipate that RNAi was not restricted to *C. elegans*, but highly conserved in divergent organisms. Indeed, many species, including flies, were shown to have RNAi [4–6]. Such strong conservation implied that RNAi was not only a powerful tool for silencing specific genes, but that it also has an intrinsically important physiological function in living creatures. Indeed, it has been shown that in some species, such as *Drosophila*, RNAi functions as an innate immune system for defending against infectious viruses and other invasive elements [7–9].

2. RNAi pathways and factors

Research on RNAi factors and their functions has been conducted worldwide. *Drosophila* was chosen as a model system because RNAi is inducible and effective in *Drosophila* cultured cells and embryos [4–6], and both biochemical and genetic approaches are applicable in this system. In parallel, cultured human cell lines were also employed because RNAi without undesirable side effects such as apoptosis could be induced when siRNAs, the minimum size of RNAi triggers, were utilized as the inducers [10,11].

After being introduced into living cells, dsRNAs are processed by an RNaseIII-domain containing nuclease, Dicer (in humans [12,13]; Dicer2 in flies [14,15]), into siRNA duplexes of ~21 nt (for review, see [16,17]). During this primary step, Dicer forms a complex with the dsRNA-binding domain containing protein, TRBP (TAR RNA-binding proteins, also known as TARBP2 [18,19]; R2D2 in flies [20])or PACT (also known as PRKRA [21]). In addition to its siRNA processing activity, the DICER complex also functions at the effector step in the RNAi process [22–24]; the loading of siRNA onto RISC (RNA-induced silencing complex) [25,26]. Within RISC, ss-siRNA (termed 'guide siRNA' [27]) is associated with Argonaute2 (AGO2in both humans and flies) [28–30], a member of the Argonaute family of proteins [17,31].

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AGO2 contains a PAZ domain, which is required for siRNA binding [16]. The PAZ domain is located around the center of the protein and is conserved in all members of Argonaute family in all species [15]. AGO2 also serves as the catalytic factor in RNAi (i.e. it is the enzyme responsible for cleaving target RNAs) [28–30]. The C-terminal PIWI domain of AGO2 is required for endonuclease activity (or Slicer activity) [16,32–34].This domain is also conserved in all Argonaute proteins [31].

After excising siRNAs, the Dicer-TRBP (or -PACT) heterodimer apparently releases the products and then binds the tiny dsRNA molecules once again to determine which strand of the duplex should be loaded onto RISC. Previous studies in *Drosophila* have indicated that R2D2 (the fly ortholog of TRBP or PACT) binds preferentially to the more thermodynamically stable end of the siRNA duplex [27,35,36]. As a result, Dicer2 is inevitably positioned close to the less stable end. Following this thermodynamically driven dsRNA end recognition, AGO2 supposedly receives the duplex from the Dicer2-R2D2 heterodimer. The mechanism for this transfer is not yet understood. Next, AGO2 cleaves one particular strand (termed the 'passenger strand' [27]),as if AGO2 cleaves target RNAs base-pairing with guide siRNAs [37–39]. It has been speculated that Dicer2 on the duplex would be displaced by AGO2 prior to this siRNA duplex receipt and passenger strand cleavage step. This is based on the observation that one strand of an siRNA duplex, whose 5′ end faces towards Dicer2, has the tendency to be loaded onto RISC.

3. RNAi in mammalian cells

Many researchers suspected that RNAi could not be used in mammalian cells or tissues because synthetic dsRNAs are notorious for inducing interferon responses, which further induce apoptotic responses [40]. However, papers published in 2001 showed that even in mammalian cells, RNAi can be activated without adverse side effects [10,11]. This finding had a tremendous impact on researchers in the RNA silencing field. The key was to utilize siRNA duplexes rather than long dsRNA precursors as RNAi inducers. Further investigation revealed that 27-nt dsRNAs might be more potent triggers than 21-nt siRNAs for mammalian RNAi [41]. 27 nt dsRNAs can serve as Dicer-nuclease substrates; the key to this solution might be that Dicer-nuclease activity from the precursors accelerates the effector step. The mechanism by which such excision accelerates the effector step remains unclear however.

4. RNAi machineries in mammalian cells

AGO2 has three distinct homologs in humans, AGO1, AGO3, and AGO4, all of which are known to be ubiquitous [17,31]. Strictly speaking, four additional Argonaute proteins (PIWIL1 to PIWIL4) can be detected in humans, but they are expressed exclusively in germline cells. To distinguish the two subgroups, ubiquitous Argonaute proteins are called AGO proteins, whereas germline-specific Argonautes are called PIWI proteins [17,31].

All four human AGO proteins (endogenously or exogenously expressed in cultured cells)can be loaded with siRNAs, but only AGO2 exhibits Slicer activity [28,29,42]. The amino acids necessary for Slicer activity, the D (Asp)-D (Asp)-H (His) triad residing in the PIWI domain, are conserved in all four AGO proteins [31].The cause of the functional differences between these proteins remains unclear. An obvious question now is whether or not AGO1, AGO3 and AGO4, which bind siRNAs but display no Slicer activity, act as "inhibitors" in the RNAi pathway.

All four AGO proteins can be loaded not only with siRNAs, but also with microRNAs (miRNAs) [28,29,42]. miRNAs are a subset of small RNAs that are thought to be endogenously expressed in all human cells [43]. Their size is very similar to that of siRNA (~21–23 nt), and they guide AGO proteins to their targets by directly associating with them, like siRNAs. AGO proteins associated with miRNAs appear to repress translation of target mRNAs by interfering with translation initiation, translation elongation or by some other means, but not by cleaving the transcripts [44]. In this sense, the functions of miRNAs are different from those of siRNAs. mRNAs targeted by miRNAs are sequestered and actively degraded in cytoplasmic foci (processing bodies or P-bodies) where various factors that function in mRNA degradation, such as decapping enzymes and nucleases, are concentrated [45]. This means that even mRNAs targeted by AGO proteins with no Slicer activity are eventually (actively) degraded in cells. In this regard, it can be noted that AGO proteins that do not have Slicer activity (namely AGO1, AGO3, and AGO4) are able to function in the siRNA-mediated gene silencing (RNAi) pathway. Indeed, it was previously shown that siRNAs can function as miRNAs [46]. The only concern would be that the turnover rates of AGO1 (or AGO3, or AGO4) and of AGO2 might be different. Speculatively, the turnover of AGO1-siRNA (or AGO3-siRNA, or AGO4-siRNA) complexes must be slower compared to that of AGO2-siRNA complexes because before entering the next round of silencing, anAGO2-siRNA complex can bypass the P-bodies (although AGO2 is detectable in P-bodies [42]) whereas the other AGO protein complexes have to be sequestered into P-bodies to release their target mRNAs.

5. Possible new strategy for successful therapeutic application of RNAi

In any single cell, multiple physiological pathways are coordinated with other pathways and such coordination is maintained at a steady state, even when some pathways are constantly turned on and off. To successfully apply RNAi therapeutically, small exogenous RNAs must be introduced and activate RNA silencing machineries without disturbing the well-coordinated cellular environment. The question of how to achieve this, however, remains. From a biochemical perspective, one strategy would be to drastically minimize the dsRNA triggers (in amount) to be introduced into the cells. In this strategy, the optimization of siRNA loading onto AGO proteins would be crucial. Excess siRNAs, or their precursors, would cause severe unexpected problems in the cells. Indeed, artificially overexpressed siRNA precursors, which structurally mimicked miRNA precursors in mice, have previously been shown to be toxic to the animals [47]. The precursors occupied a large proportion of Exportin 5 [43], the factor necessary for exporting pre-miRNAs from the nucleus to the cytoplasm and thus severely disturbed endogenous miRNA activities. In the worst cases, the transgenic mice died [47]. To avoid such a serious problem, siRNAs could be directly introduced in mice as in an siRNA duplex form. However, even in this case, we can predict that excess siRNAs would mostly occupy the Dicer-TRBP (or -PACT) complexes and might block the miRNA activities.

One molecule of AGO protein binds one guide siRNA molecule [16]. This means that exogenous siRNA that has been successfully introduced into living cells may not find an AGO recipient, if the AGO proteins were mostly occupied by endogenous small RNAs (miRNAs and endogenous siRNAs (endo-siRNAs); the latter was recently discovered in mouse oocytes) [48,49]. In this case, the exogenous siRNAs would be degraded without exerting their functions. In this regard, we can conclude that the RNAi efficacy in cells would largely depend on the availability of AGO proteins for the siRNAs of interest. The levels of AGO proteins in any given cell or tissue could be examined, for example, by western blotting utilizing specific antibodies against them. Currently, however, there is no means to assess what proportion of cellular AGO proteins are bound or unbound to endogenous small RNAs and under what circumstances the ratio of the bound form over the unbound form is altered in cells. We do not even have a clear answer for the basic questions of how much AGO2, or other AGO proteins, are expressed in any cell or tissue in the human body or how their expression changes throughout development.

Hopefully, the answers to these important questions will be determined experimentally.

What if it was discovered that 'empty' AGO proteins were largely unavailable in the cellsor tissues where you were considering applying exogenous RNAi? A possible strategy would be to raise the cells' expression rates to increase the supply of 'empty' AGO proteins available for exogenous siRNAs. Indeed, it has been shown that overexpression of AGO2 enhances RNAi effects in mammalian cells [50,51]. However, currently it remains unknown how the expression of AGO proteins is regulated *in vivo* or how the expression can be manipulated. Clearly, further research on RNAi machineries will be needed to answer this type of question.

6. Final remarks

Clinical trials using 'RNAi' to treat human diseases were first launched in 2004. Several trials are currently ongoing worldwide (for review, see [52]). However, the various protocols involved have not yet been optimized. Current issues that need to be addressed so that therapeutic strategies can be optimized include; how to improve the systems that deliver synthetic siRNAs or their expression vectors to the targeted cells (this theme is focused on by Y. Takakura and other authors in the reviews); how to modify siRNAs for longer durability; and how to minimize off-target effects to eliminate side effects. A wide range of studies have demonstrated that certain kinds of RNA modifications, such as a 2′-*O*-methyl in the 2′ ribose at some nucleotides in siRNAs, convey an increased resistance to ribonucleases [53]. Another modification was effective for reducing off-target effects. Conjugation of cholesterol to siRNAs, or siRNA capsulation by a lipid layer or polycation showed an increased uptake by living cells [54–56]. These non-viral siRNA delivery and viral delivery issues (the latter is for siRNA-expression vectors) remain 'hot' topics, and continuous, intensive investigation will certainly be needed to optimize them (refer to the reviews in this issue). As mentioned above, even newer strategies, such as manipulating AGO expression *in vivo* and assembling RISC with minimal exogenous siRNA levels, will also be helpful. Clearly, further investigations, from both basic and applied science perspectives, need to be pursued to achieve the successful application of RNAi in disease therapy.

References

[1] A. Fire, M.K. Montgomery, S.A. Kostas, S.E. Driver, C.C. Mello, Potent and specific genetic interference by double-stranded RNA in *C. elegans*, Nature 391 (1998) 806–811.
[2] R.A. Jorgensen, Sense cosuppression in plants: past, present and future, RNAi: a guide to gene silencing, 2003, pp. 5–21, Chapter 1.
[3] M.M. Matzke, M.F. Mette, T. Kanno, W. Aufsatz, A.J.M. Matzke, Regulation of the genome by double-stranded RNA, RNAi: a guide to gene silencing, 2003, pp. 43–63, Chapter 3.
[4] J.R. Kennerdell, R.W. Carthew, Use of dsRNA-mediated genetic interference to demonstrate that frizzled and frizzled2 act in the wingless pathway, Cell 95 (1998) 1017–1026.
[5] J.C. Clemens, C.A. Worby, N. Siomonson-Leff, M. Muda, T. Maehama, B.A. Hemmings, J.E. Dixon, Use of double-stranded RNA interference in *Drosophila* cell line to dissect signal transduction pathways, Proc. Natl. Acad. Sci. USA 97 (2000) 6499–6503.
[6] S.M. Hammond, E. Bernstein, D. Beach, G.J. Hannon, An RNA-directed nuclease mediates post-transcriptional gene silencing in *Drosophila* cells, Nature 404 (2000) 293–296.
[7] S.W. Ding, O. Voinnet, Antiviral immunity directed by small RNAs, Cell 130 (2007) 413–426.
[8] A. Girard, G.J. Hannon, Conserved themes in small RNA-mediated transposon control, Trends Cell Biol. 18 (2008) 136–148.
[9] G. Stefani, F.J. Slack, Small non-coding RNAs in animal development, Nat. Rev., Mol. Cell Biol. 9 (2008) 219–230.
[10] S.M. Elbashir, W. Lendeckel, T. Tuschl, RNA interference is mediated by 21- and 22-nucleotide RNAs, Genes Dev. 15 (2001) 188–200.
[11] S.M. Elbashir, J. Haborth, W. Lendeckel, A. Yalcin, K. Weber, T. Tuschl, Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells, Nature 411 (2001) 494–498.
[12] P. Provost, D. Dishart, J. Doucet, D. Frendewey, B. Samuelsson, O. Radmark, Ribonuclease activity and RNA binding of recombinant human Dicer, EMBO J. 21 (2002) 5864–5874.
[13] H. Zhang, F.A. Kolb, L. Jaskiewicz, E. Westhof, W. Filipowicz, Silencing processing center models for human Dicer and bacterial RNase III, Cell 118 (2004) 57–68.
[14] E. Bernstein, A.A. Caudy, S.M. Hammond, G.J. Hannon, Role for a bidentate ribonucluease in the initiation step of RNA interference, Nature 409 (2001) 363–366.
[15] Y.S. Lee, K. Nakahara, J.W. Pham, K. Kim, Z. He, E.J. Sontheimer, R.W. Carthew, Distinct roles for *Drosophila* Dicer-1 and Dicer-2 in the siRNA/miRNA silencing pathways, Cell 117 (2004) 69–81.
[16] M. Jinek, J.A. Doudna, A three-dimensional view of the molecular machinery of RNA interference, Nature 457 (2009) 405–412.
[17] H. Siomi, M.C. Siomi, On the road to reading the RNA-interference code, Nature 457 (2009) 396–404.
[18] T.P. Chendrimada, R.I. Gregory, E. Kumaraswamy, J. Norman, N. Cooch, K. Nichikura, R. Shiekhatter, TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing, Nature 436 (2005) 740–744.
[19] A.D. Haase, L. Jaskiewicz, H. Zhang, S. Laine, R. Sack, A. Gatignol, W. Filipowicz, TRBP, a regulator of cellular PKR and HIV-1 virus expression, EMBO Rep. 6 (2005) 961–967.
[20] Q. Liu, T.A. Rand, S. Kalidas, F. Du, H.E. Kim, D.P. Smith, X. Wang, R2D2, a bridge between the initiation and effector steps of the *Drosophila* RNAi pathway, Science 301 (2003) 1921–1925.
[21] Y. Lee, I. Hur, S.Y. Park, Y.K. Kim, M.R. Suh, V.N. Kim, The role of PACT in the RNA silencing pathway, EMBO J. 25 (2006) 522–532.
[22] R.I. Gregory, T.P. Chendrimada, N. Cooch, R. Shiekhatter, Human RISC couples microRNA biogenesis and posttranscriptional gene silencing, Cell 123 (2005) 631–640.
[23] E. Maniataki, Z.A. Mourelatos, Human, ATP-dependent, RISC assembly machine fueled by pre-miRNA, Genes Dev. 19 (2005) 2979–2990.
[24] I.J. MacRae, E. Ma, M. Zhou, C.V. Robinson, J.A. Doudna, *In vitro* reconstitution of the human RISC-loading complex, Proc. Natl. Acad. Sci. USA 105 (2008) 512–517.
[25] G.J. Hannon, RNA interference, Nature 418 (2003) 244–251.
[26] J. Martinez, T. Tuschl, RISC is a 5′ phosphomonoester-producing RNA endonuclease, Genes Dev. 18 (2004) 975–980.
[27] Y. Tomari, C. Matranga, B. Haley, N. Martinez, P.D. Zamore, A protein sensor for siRNA asymmetry, Science 306 (2004) 1377–1380.
[28] J. Liu, M.A. Carmell, F.V. Rivas, C.G. Marsden, J.M. Thomson, J.J. Song, S.M. Hammond, L. Joshua-Tor, G.J. Hannon, Argonaute2 is the catalytic engine of mammalian RNAi, Science 305 (2004) 1437–1441.
[29] G. Meister, M. Landthaler, A. Patkaniowska, Y. Dorsett, G. Teng, T. Tuschl, Human Argonaute2 mediates RNA cleavage targeted by miRNAs and siRNAs, Mol. Cell 15 (2004) 185–197.
[30] J.J. Song, S.K. Smith, G.J. Hannon, L. Joshua-Tor, Crystal structure of Argonaute and its implications for RISC slicer activity, Science 305 (2004) 1434–1437.
[31] G. Hutvagner, M.J. Simard, Argonaute proteins: key players in RNA silencing, Nat. Rev., Mol. Cell Biol. 9 (2008) 22–32.
[32] J.J. Song, J. Liu, N.H. Tolia, J. Schneiderman, S.K. Smith, R.A. Martienssen, G.J. Hannon, L. Joshua-Tor, The crystal structure of the Argonaute2 PAZ domain reveals as RNA binding motif in RNAi effector complexes, Nat. Struct. Mol. Biol. 10 (2003) 1026–1032.
[33] J.B. Ma, Y.R. Yuan, G. Meister, Y. Pei, T. Tushl, D.J. Patel, Structural basis for 5′-end-specific recognition of guide RNA by the *A. fulgidus* Piwi protein, Nature 434 (2005) 666–670.
[34] M. Nowotny, S.A. Gaidanakov, R.J. Crouch, W. Yang, Crystal structure of RNaseH bound to an RNA/DNA hybrid: substrate specificity and metal-dependent catalysis, Cell 121 (2005) 1005–1016.
[35] A. Khvorova, A. Reynolds, S.D. Jayasena, Functional siRNAs and miRNAs exhibit strand bias, Cell 115 (2003) 209–216.
[36] D.S. Schwarz, Asymmetry in the assembly of the RNAi enzyme complex, Cell 115 (2003) 119–208.
[37] C. Matranga, Y. Tomari, C. Shin, D.P. Bartel, P.D. Zamore, Passenger-strand cleavage facilitates assembly of siRNA into Ago2-containing RNAi enzyme complexes, Cell 123 (2005) 607–620.
[38] K. Miyoshi, H. Tsukumo, T. Nagami, H. Siomi, M.C. Siomi, Slicer function of *Drosophila* Argonautes and its involvement in RISC formation, Genes Dev. 19 (2005) 2837–2848.
[39] T.A. Rand, S. Petersen, F. Du, X. Wang, Argonaute2 cleaves the anti-guide strand of siRNA during RISC activation, Cell 123 (2005) 621–629.
[40] M.P. Gantier, B.R. Williams, The response of mammalian cells to double-stranded RNA, Cytokine Growth Factor Rev. 18 (2007) 363–371.
[41] D.H. Kim, M.A. Behlke, S.D. Rose, M.S. Chang, S. Choi, J.J. Rossi, Synthetic dsRNA Dicer substrates enhance RNAi potency and efficacy, Nat. Biotechnol. 23 (2005) 222–226.
[42] A. Azuma-Mukai, H. Oguri, T. Mituyama, Z.R. Qian, K. Asai, H. Siomi, M.C. Siomi, Characterization of endogenous human Agronautes and their miRNA partners in RNA silencing, Proc. Natl. Acad. Sci. USA 105 (2008) 7964–7969.
[43] V.N. Kim, J. Han, M.C. Siomi, Biogenesis of small RNAs in animals, Nat. Rev., Mol. Cell Biol. 10 (2009) 126–139.
[44] P. Brodersen, O. Voinnet, Revisiting the principles of microRNA target recognition and mode of action, Nat. Rev., Mol. Cell Biol. 10 (2009) 141–147.
[45] A. Eulalio, I. Behm-Ansmant, E. Izzauralde, P bodies: at the crossroads of post-transcriptional pathways, Nat. Rev., Mol. Cell Biol. 8 (2007) 9–22.
[46] J.G. Doench, C.P. Petersen, P.A. Sharp, siRNAs can function as miRNAs, Genes Dev. 17 (2003) 438–442.

[47] D. Grimm, K.L. Streetz, C.L. Jopling, T.A. Storm, K. Pandey, C.R. Davis, P. Marion, F. Salazaf, M.A. Key, Fatality in mice due to over saturation of cellular microRNA/short hairpin RNA pathways, Nature 441 (2006) 537–541.
[48] O.H. Tam, A.A. Aravin, P. Stein, A. Girard, E.P. Murchison, S. Chwloufi, E. Hodges, M. Anger, R. Sachidanandam, R.M. Schultz, G.J. Hannon, Pseudogene-derived small interfering RNAs regulates gene expression in mouse oocytes, Nature 453 (2008) 534–538.
[49] T. Watanabe, et al., Endogenous siRNAs from naturally formed dsRNAs regulate transcripts on mouse oocytes, Nature 453 (2008) 539–543.
[50] S. Diederichs, D.A. Haber, Dual role for argonatues in microRNA processing and posttranscriptional regulation of microRNA expression, Cell 131 (2007) 1097–1108.
[51] S. Diederichs, S. Jung, S.M. Rothenberg, G.A. Smolen, B.G. Mlody, D.A. Haber, Coexpression of Argonaute-2 enhances RNA interference toward perfect match binding sites, Proc. Natl. Acad. Sci. USA 105 (2008) 9284–9289.
[52] D. Castanotto, J. Rossi, The premises and pitfalls of RNA-interference-base therapeutics, Nature 457 (2009) 426–433.
[53] D.V. Morrissey, J.A. Lockridge, L. Shaw, K. Blanchard, K. Jensen, W. Breen, K. Hartsough, L. Machemer, S. Radka, V. Jadhav, N. Vaish, S. Zinnen, C. Vargeese, K. Bowman, C.S. Shaffer, J.B. Jeffs, A. Judge, I. MacLachlan, B. Polisky, Potent and persistent in vivo anti-HBV activity of chemically modified siRNAs, Nat. Biotechnol. 23 (2005) 1002–1007.
[54] C. Lorenz, P. Hadwiger, M. John, H.P. Vornlocher, C. Unverzagt, Steroid and lipid conjugates of siRNAs ti enhance cellular uptake and gene silencing in liver cells, Bioorg. Med. Chem. Lett. 14 (2004) 4975–4977.
[55] D.W. Bartless, H. Su, I.J. Hidebrandt, W.A. Weber, M.E. Davis, Impact of tumor-specific targeting on the biodistribution and efficacy of siRNA nanoparticle measured by multimodality in vivo imaging, Proc. Natl. Acad. Sci. USA 104 (2007) 15549–15554.
[56] J. Soutschek, et al., Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs, Nature 432 (2004) 173–178.