Acta Physiologica Hungarica, Volume 98 (3), pp. 243–251 (2011)
DOI: 10.1556/APhysiol.98.2011.3.1

The huge world of small RNAs: Regulating networks of microRNAs (Review)

Zs Rácz, T Kaucsár, P Hamar

Institute of Pathophysiology, Faculty of Medicine, Semmelweis University, Budapest, Hungary

Received: December 10, 2010
Accepted after revision: March 21, 2011

MicroRNAs (miRNAs) are a recently discovered class of small, non-coding RNAs which do not code proteins. MiRNAs regulate gene expression by inhibiting protein translation from the messenger RNA. MiRNAs may function in networks, forming a complex relationship with diseases. Furthermore, specific miRNAs have significant correlation with diseases of divergent origin. After identification of disease-associated miRNAs, their tissue expression could be altered in a beneficial way by inhibiting or mimicking their effects. Thus, modifying the expression of miRNAs is a potential future gene-therapeutic tool to influence post-transcriptional regulation of multiple genes in a single therapy. In this review we introduce the biogenesis, mechanism of action and future aspects of miRNAs. Research on the post-transcriptional regulation of gene expression by miRNA may reshape our understanding of diseases and consequently may bring new diagnostic markers and therapeutic agents. Therapeutic use of miRNAs is already under clinical investigation in RNA interference trials.

Keywords: microRNA, post-transcriptional gene silencing, nucleic acid therapy

Since the first description of the genetic material in 1953 by James Watson and Francis Crick (65) three discoveries honoured by a Nobel Prize have reshaped our understanding of gene-expression regulation. The "central dogma" of unidirectional genetic information flow (DNA→RNA→protein) by Francis Crick, 1958 (7) has been broken first in 1970 by David Baltimore's discovery of reverse transcriptase (54), and than in 1982 by Stanley Prusiner's description of a proteinaceous infectious substance (prion) (47). In 1986 Ecker and Davis described that antisense RNA can inhibit gene expression in plants (10) providing a new role for RNAs. However, the relevance of this discovery was not given wide attention until 1998 when Craig C. Mello and Andrew Fire described RNA interference (RNAi) in eukaryotes *(C. elegans)* (14). This discovery of small RNAs not coding proteins opened a brand new field in biology: investigating regulatory roles of RNAs, previously thought to merely pass information from DNA to proteins.

Analysis of the human transcriptome revealed that it contains thousands of functioning RNAs that are not included in protein synthesis (non-coding RNAs) (41). Some of these non-coding RNAs are short microRNAs (miRNAs) with gene expression regulatory function first described in 1993 (38). Genes encoding miRNAs have been shown to make up to 1% of the

Corresponding author: Péter Hamar MD, PhD
Institute of Pathophysiology, Faculty of Medicine, Semmelweis University
Nagyvárad tér 4, H-1089 Budapest, Hungary
Phone/Fax: +36-1-210-0100; E-mail: hampet@net.sote.hu

genome (5). MiRNAs are short: 18–25 nucleotide (nt), double stranded RNAs (58), and their action is achieved through RNAi, following DNA → RNA transcription (36). Understanding the effects of miRNAs could dramatically change our schemes of gene regulation.

Controlling functions in our body form networks, in which miRNAs might play a crucial role (30). A single miRNA can alter the expression of many messenger RNAs (mRNA), furthermore, expression of one mRNA might be under the control of several miRNAs. The disease and stage specific miRNA expression profile can be detected by multiplex methods, such as microarray or microbead hybridization methods (Luminex /Biomedica Hungaria/, Bioplex /Biorad/). Together with these methods, real time quantitative polymerase chain reaction (qPCR) might give us more precise results on individual miRNA expressions leading to a better insight into the mechanism of miRNome network.

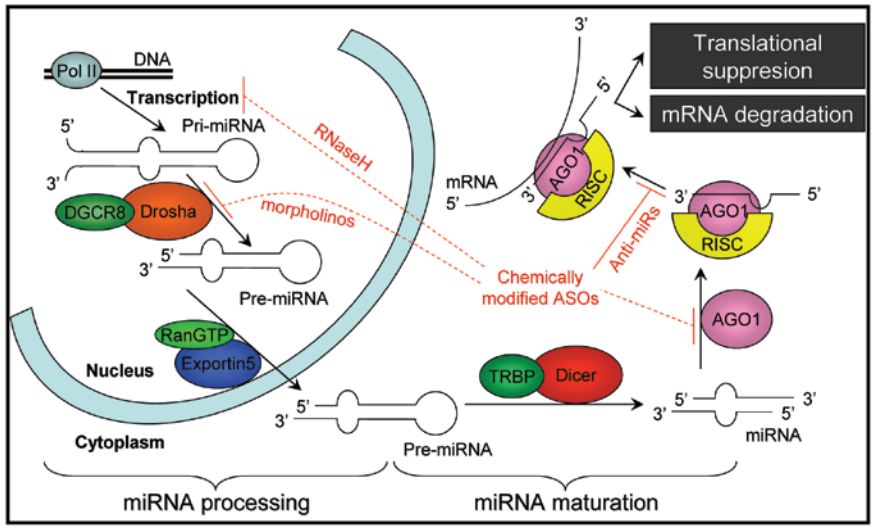

Fig. 1. MiRNA mechanism: biogenesis and function (9, 12, 20, 32, 44)

Micro RNAs function by post-transcriptional gene silencing, meaning silencing the messenger RNA also called RNA interference (RNAi). Micro RNAs bind to and inhibit the translation of the complementary sequence messenger RNAs. Molecular mechanisms of RNAi were first described in detail for short interfering RNAs (siRNAs). For further information on siRNAs we refer to our recent reviews (52, 53). Double-stranded (ds)RNA can also activate gene expression (RNAa) by targeting gene promoters thus, inducing transcriptional activation (37).

MiRNAs are generated from endogenously transcribed, hairpin structures (5). MiRNA coding genes are transcribed by RNA polymerase II (pol II) and the transcript is named primary miRNA (pri-miRNA) (39), from which nucleotides are cleaved by Drosha (RNase enzyme) inside the nucleus. This second product is called pre-miRNAs and is recognized by nuclear export factor Exportin-5 (Exp5/Xpo5), which traffics pre-miRNAs into the cytoplasm (40). The following steps are mediated by Dicer (RNase III enzyme): it cleaves the hairpin structure and forms the miRNA-specific ends. The outcome of this processing is a double-stranded, 18–25 nt long dsRNA, named miRNA (28). The so-called guide (antisense) strand keeps its strong contact with the RNA-induced silencing complex (RISC), while the other strand is degraded (31). The guide strand binds the complementary messenger RNA (mRNA),

and is followed by the degradation of the complementary mRNA, a mechanism, that requires full match complementarity and predominantly occurs in plants (5). The mechanisms of post-transcriptional gene silencing mediated by miRNAs are summarized in Table I (27):

Table I. Possible gene silencing mechanisms of miRNAs

Inhibiting the translation: Blocking the initiation of translation (on eukaryotic initiation factor, eIF) Blocking the elongation
Degradation of newly synthesized proteins
Sequestration of miRNAs (i.e. in the P-bodies) (27)

Further silencing mechanisms of miRs

While miRNAs in plants direct cleavage of the targeted mRNA as described above, in animals there is usually a lack of extensive complementarity required for cleavage by Argonaute proteins (29), revealing prominent regulation at the protein level by ribosome drop off during elongation of translation (50). Furthermore, mRNA decreases are associated with poly(A)-tail shortening, leading to mRNA de-adenylation, de-capping and consequent rapid degradation (67). This indicates that microRNAs act as rheostats to make fine-scale adjustments of protein level (4). Further experiments suggest that miRNAs repress translation initiation by preventing 60S ribosome subunit joining to miRNA-targeted mRNAs (63). As to Guo and colleagues lowered mRNA levels account for most (≥84%) of the decreased protein production, indicating that mRNA destabilization plays a key role in reduced protein translation (18). Thus, miRNA-mediated silencing manifests at both protein and mRNA levels. Although there are abundant data on the proposed model of influencing translation initiation by miRNAs (8), further experiments are needed to reveal the mechanism of action of miRNAs in eucariots.

Nomenclature of miRNAs

The correct naming of miRNAs necessitated the establishment of a universal nomenclature of miRNAs (Table II). There are many online data bases, which help scientists to explore the sequence of different miRNAs or the structure of their precursor pri-miRNA molecule. Other online tools may be useful in identifying possible miRNA–mRNA interactions (Table III) (23–25). The new identification number is activated when the article describing the new miRNA is published (2, 17).

Table II. Short summary of miRNA nomenclature

Abbreviation	Description
miR	Mature miRNA
Mir	Precursor micro RNA
Identifying number	Is given in the order of description
Prefix made up by 3–4 letters (hsa-miR, mmu-miR)	Host organism (Homo Sapiens, Mus Musculus)
Number in the suffix (hsa-miR-194-1, hsa-miR-194-2)	Chromosomal localization (of same miRNAs)
Small letter in the suffix (hsa-miR-200a, hsa-miR-200b, hsa-miR-200c)	Paralogous miRNA sequences (differing only in a few nucleotides from each other)

Table III. Online microRNA databases and target prediction tools

Database name	Website	Description
miRBase	http://www.mirbase.org	The miRBase database is a searchable database of published miRNA sequences and annotations. The miRBase Registry provides miRNA gene hunters with unique names for novel miRNA genes prior to publication of results.
microRNA.org (miRanda)	http://www.microrna.org/microrna/home.do	Contains predicted microRNA targets and target downregulation scores. Experimentally observed expression patterns (target sites by: miRanda, scores by: mirSVR).
PicTar	http://www.pictar.org	PicTar is an algorithm for the identification of microRNA targets.
miRNA – Target Gene Prediction at EMBL	http://www.russelllab.org/miRNAs	The website provides access to miRNA-Target predictions for *Drosophila* miRNAs
DIANA Lab	http://diana.cslab.ece.ntua.gr	The activities of the DIANA lab range from the analysis of expression regulation from deep sequencing data, the annotation of miRNA regulatory elements and targets to the interpretation of the role of miRNAs in various diseases.
TarBase	http://diana.cslab.ece.ntua.gr/tarbase/	Contains more than 1300 experimentally supported miRNA target interactions.
miRGen	http://diana.cslab.ece.ntua.gr/mirgen/	miRGen is an integrated database of miRNA gene transcripts, Transcription Factor Binding Sites, miRNA expression profiles and Single Nucleotide Polymorphisms associated with miRNAs
TargetScan	http://www.targetscan.org	On the TargetScan website one can search for predicted microRNA targets in mammals (human, mouse, worm, fly)

Table III (continued)

Database name	Website	Description
microCosm (miRBase Targets)	http://www.ebi.ac.uk/enright-srv/microcosm/htdocs/targets/v5/	MicroCosm is a web resource developed by the Enright Lab at the EMBL-EBI containing computationally predicted targets for microRNAs across many species.
miRDB	http://mirdb.org/miRDB/	miRDB is an online database for miRNA target prediction and functional annotations in animals.
miR2Disease	http://www.mir2disease.org/	Provides a comprehensive resource of miRNA deregulation in various human diseases.
Human MiRNA & Disease Database (HMDD)	http://202.38.126.151/hmdd/mirna/md/	Contains miRNA names, disease names, dysfunction evidences, and the literature PubMed ID. The tissue expression pictures of some miRNAs in 40 tissues are also provided.
RegRNA	http://regrna.mbc.nctu.edu.tw/index.php	RegRNA is an integrated web server for identifying the homologs of Regulatory RNA motifs and elements against an input mRNA sequence.
miRNAMap	http://mirnamap.mbc.nctu.edu.tw/index.php	Collects experimental verified microRNAs and experimental verified miRNA target genes in human, mouse, rat, and other metazoan genomes.
miRacle	http://miracle.igib.res.in/miracle/	Predicts microRNA targets based on RNA secondary structure.
miRU	http://bioinfo3.noble.org/miRNA/miRU.htm	Predicts plant miRNA target genes.
RNA22	http://cbcsrv.watson.ibm.com/rna22.html	microRNA target detection and precursor prediction.
miRTar	http://mirtar.mbc.nctu.edu.tw/human/	An integrated web server for identifying miRNA-target interactions in human. (Mouse, rat and dog are in progress.)
RNAhybrid	http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/	RNAhybrid is a tool for finding the minimum free energy hybridisation of a long and a short RNA. The tool is primarily meant as a means for microRNA target prediction.
ViTa	http://vita.mbc.nctu.edu.tw/index.php	ViTa is a database which collects virus data from miRBase and ICTV, VirGne, VBRC., etc., including known miRNAs on virus and supporting predicted host miRNA targets by miRanda and TargetScan.

Function of microRNAs

MiRNAs have crucial role in divergent mechanisms, such as embryonic (*miR-51* family) (43, 56) and haematopoietic (*miR-155*) (16) development, apoptosis (*miR-15, miR-16*) (6), genesis and progression of malignancies (*miR-17–92* cluster, *miR-21*, *miR-372*) (42), electric functions of the heart (*miR-1, miR-2*) (69), cardiomyopathies (upregulated: *miR-23a* and *b, miR-24, miR-125b, miR-195, miR-199a, miR-214*, downregulated: *miR-93, miR-133a, miR-150, miR-181b*) (61) or endocrine mechanisms (*miR-375*) (51). Thus, miRNAs are associated to many physiological (5) and pathophysiological mechanisms (13).

Altering miRNA expression *in vivo*

Since miRNA expression is altered in many diseases, therapeutic influence on miRNA function may be beneficial. One major problem of *in vivo* nucleic acid (siRNA, miRNA, antisense oligodesoxynucleotide, plasmid DNA) therapy is the delivery of the nucleic acid to target organs and into cells (49). The issues of *in vivo* therapy are assessed in Table IV.

Table IV. Problems of *in vivo* nucleotide therapy

Instability of nucleic acids in extracellular fluids
Short half-life (seconds) due to nuclease degeneration in body fluids (22)
Fast filtration by the kidney (15)
Low cell membrane penetration due to negative charge (52)

Several methods have been investigated to support *in vivo* delivery of nucleotides, the main groups of which are summarized below:

Physical forces (high pressure, high volume): Local or systemic injection of nucleic acids dissolved in a large volume helps them to cross their cellular uptake (19), since the high volume diminishes the efficacy of nucleases, while high pressure presses the nucleotides into the interstitium of parenchymal organs, and then through the cell membrane into the cytoplasm, the place of RNAi. Similar pore openings can be achieved with sonoporation (60) or electroporation (21, 66).

Chemical modifications: Altering the structure of a nucleic acid with the aim of therapeutic application includes the modification of the ribose backbone (35) the terminals (by addition of methyl, alkyl (11) or cholesterol group (59) or synthetic molecules: non-ionic DNA analogues=morpholinos) (55) or locked nucleic acids (LNA) (62).

Vectors, plasmid DNA (pDNA): Viral and non-viral vectors, polycations (polyplexes (57): poly-ethylene-imine (PEI) (17), polyethylene glycol (PEG) or poly-L-lysine), complexing with molecules of lipid nature (developing liposomes with for instance Lipofectamine RNAiMax®) can enhance cellular nucleic acid uptake (68).

Conjugation with cell surface receptor ligands: enables cell specific uptake (45).

Depo-products (**carriers**, such as gelatine (46), hydrogels (33), atelocollagen (3) or citosan) can elongate silencing effect.

Diagnostic value of miRNAs

Many disease-specific miRNA expression patterns and/or miRNA amounts could be clinically used in diagnosing and evaluating prognosis, for instance with real-time PCR (34). Human malignant tumor-specific miRNA chips are already commercially available (48) Huang and colleagues described that the plasma level of some miRNAs (*miR-29a, miR-92a*) have diagnostic value in advanced colorectal cancer (26). Other miRNAs (*miR-146a, miR-223*) have been shown to be significantly associated with sepsis (*miR-146a, miR-223*) (64). Furthermore, *miR-1* is a new biomarker of cardiac muscle ischemia, independent of many factors (age, sex, blood pressure, diabetes mellitus) (1).

Conclusions

MiRNAs play a crucial role in the development and course of diseases. Multiplex understanding of the miRNome may have diagnostic and therapeutic relevance.

Acknowledgements

Support was provided to P. H. from the Hungarian Research Fund: OTKA T049022, NF69278, K 81972 and ETT 07-011/2009. This work was supported by NIH Research Grant #R03 TW07069 funded by the Fogarty International Center and the National Institute of Diabetes and Digestive and Kidney Diseases.

REFERENCES

1. Ai J, Zhang R, Li Y, Pu J, Lu Y, Jiao J, Li K, Yu B, Li Z, Wang R, Wang L, Li Q, Wang N, Shan H, Yang B: Circulating microRNA-1 as a potential novel biomarker for acute myocardial infarction. Biochem. Biophys. Res. Commun. 391, 73–77 (2010)
2. Ambros V, Bartel B, Bartel DP, Burge CB, Carrington JC, Chen X, Dreyfuss G, Eddy SR, Griffiths-Jones S, Marshall M, Matzke M, Ruvkun G, Tuschl T: A uniform system for microRNA annotation. RNA 9, 277–279 (2003)
3. Ashihara E, Kawata E, Maekawa T: Future prospect of RNA interference for cancer therapies. Curr. Drug. Targets 11, 345–360 (2010)
4. Baek D, Villen J, Shin C, Camargo FD, Gygi SP, Bartel DP: The impact of microRNAs on protein output. Nature 455, 64–71 (2008)
5. Bartel DP: MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116, 281–297 (2004)
6. Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferracin M, Shimizu M, Wojcik SE, Aqeilan RI, Zupo S, Dono M, Rassenti L, Alder H, Volinia S, Liu CG, Kipps TJ, Negrini M, Croce CM: miR-15 and miR-16 induce apoptosis by targeting BCL2. Proc. Natl. Acad. Sci. USA 102, 13944–13949 (2005)
7. Crick FH: On protein synthesis. Symp. Soc. Exp. Biol. 12, 138–163 (1958)
8. Djuranovic S NA, Green R: A parsimonius model for gene regulation by miRNAs. Science 331, 550–553 (2011)
9. Du T, Zamore PD: microPrimer: the biogenesis and function of microRNA. Development 132, 4645–4652 (2005)
10. Ecker JR, Davis RW: Inhibition of gene expression in plant cells by expression of antisense RNA. Proc. Natl. Acad. Sci. USA 83, 5372–5376 (1986)
11. Engels JW, Odadzic D, Smicius R, Haas J: Chemical synthesis of 2'-O-alkylated siRNAs. Methods Mol. Biol. 623, 155–170 (2010)
12. Esau CC: Inhibition of microRNA with antisense oligonucleotides. Methods 44, 55–60 (2008)
13. Esau CC, Monia BP: Therapeutic potential for microRNAs. Adv. Drug Deliv. Rev. 59, 101–114 (2007)
14. 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 391, 806–811 (1998)

15. Gao S, Dagnaes-Hansen F, Nielsen EJ, Wengel J, Besenbacher F, Howard KA, Kjems J: The effect of chemical modification and nanoparticle formulation on stability and biodistribution of siRNA in mice. Mol. Ther. 17, 1225–1233 (2009)
16. Georgantas RW, 3rd, Hildreth R, Morisot S, Alder J, Liu CG, Heimfeld S, Calin GA, Croce CM, Civin CI: CD34+ hematopoietic stem-progenitor cell microRNA expression and function: a circuit diagram of differentiation control. Proc. Natl. Acad. Sci. USA 104, 2750–2755 (2007)
17. Grzelinski M, Urban-Klein B, Martens T, Lamszus K, Bakowsky U, Hobel S, Czubayko F, Aigner A: RNA interference-mediated gene silencing of pleiotrophin through polyethylenimine-complexed small interfering RNAs *in vivo* exerts antitumoral effects in glioblastoma xenografts. Hum. Gene Ther. 17, 751–766 (2006)
18. Guo H, Ingolia NT, Weissman JS, Bartel DP: Mammalian microRNAs predominantly act to decrease target mRNA levels. Nature 46, 835–840 (2010)
19. Hamar P, Song E, Kokeny G, Chen A, Ouyang N, Lieberman J: Small interfering RNA targeting Fas protects mice against renal ischemia-reperfusion injury. Proc. Natl. Acad. Sci. USA 101, 14883–14888 (2004)
20. He L, Hannon GJ: MicroRNAs: small RNAs with a big role in gene regulation. Nat. Rev. Genet. 5, 522–531 (2004)
21. Heller LC, Heller R: Electroporation gene therapy preclinical and clinical trials for melanoma. Curr. Gene Ther. 10, 312–317 (2010)
22. Higuchi Y, Kawakami S, Hashida M: Strategies for *in vivo* delivery of siRNAs: recent progress. BioDrugs 24, 195–205 (2010)
23. http://rfam.janelia.org/
24. http://www.isical.ac.in/~bioinfo_miu/TF-miRNA.php
25. http://www.mirbase.org/
26. Huang Z, Huang D, Ni S, Peng Z, Sheng W, Du X: Plasma microRNAs are promising novel biomarkers for early detection of colorectal cancer. Int. J. Cancer 127, 118–126 (2010)
27. Huntzinger E IE: Getting to the root of miRNA-mediated gene silencing. Cell 132, 9–14 (2008)
28. Hutvagner G, McLachlan J, Pasquinelli AE, Balint E, Tuschl T, Zamore PD: A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. Science 293, 834–838 (2001)
29. Jinek M, Doudna JA: A three-dimensional view of the molecular machinery of RNA interference. Nature 457, 405–412 (2009)
30. Ke XS, Liu CM, Liu DP, Liang CC: MicroRNAs: key participants in gene regulatory networks. Curr. Opin. Chem. Biol. 7, 516–523 (2003)
31. Kim DH, Behlke MA, Rose SD, Chang MS, Choi S, Rossi JJ: Synthetic dsRNA Dicer substrates enhance RNAi potency and efficacy. Nat. Biotechnol. 23, 222–226 (2005)
32. Kim VN: Small RNAs: classification, biogenesis, and function. Mol. Cells 19, 1–15 (2005)
33. Krebs MD, Jeon O, Alsberg E: Localized and sustained delivery of silencing RNA from macroscopic biopolymer hydrogels. J. Am. Chem. Soc. 131, 9204–9206 (2009)
34. Kroh EM, Parkin RK, Mitchell PS, Tewari M: Analysis of circulating microRNA biomarkers in plasma and serum using quantitative reverse transcription-PCR (qRT-PCR). Methods 50, 298–301 (2010)
35. Kumar VA, Ganesh KN: Structure-editing of nucleic acids for selective targeting of RNA. Curr. Top. Med. Chem. 7, 715–726 (2007)
36. Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T: Identification of novel genes coding for small expressed RNAs. Science 294, 853–858 (2001)
37. Li CL (2008): Small RNA-Mediated Gene Activation. RNA and the Regulation of Gene Expression: A Hidden Layer of Complexity. Caister Academic Press
38. 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)
39. Lee Y, Kim M, Han J, Yeom KH, Lee S, Baek SH, Kim VN: MicroRNA genes are transcribed by RNA polymerase II. EMBO J. 23, 4051–4060 (2004)
40. Lund E, Guttinger S, Calado A, Dahlberg JE, Kutay U: Nuclear export of microRNA precursors. Science 303, 95–98 (2004)
41. Mattick JS, Makunin IV: Non-coding RNA. Hum. Mol. Genet. 15 Spec No 1, R17–29 (2006)
42. McManus MT: MicroRNAs and cancer. Semin. Cancer Biol. 13, 253–258 (2003)
43. Morita K, Han M: Multiple mechanisms are involved in regulating the expression of the developmental timing regulator lin-28 in Caenorhabditis elegans. EMBO J. 25, 5794–5804 (2006)
44. Muhonen P, Holthofer H: Epigenetic and microRNA-mediated regulation in diabetes. Nephrol. Dial. Transplant. 24, 1088–1096 (2009)

45. Nakagawa O, Ming X, Huang L, Juliano RL: Targeted intracellular delivery of antisense oligonucleotides via conjugation with small-molecule ligands. J. Am. Chem. Soc. 132, 8848–8849 (2010)
46. Nezhadi SH, Choong PF, Lotfipour F, Dass CR: Gelatin-based delivery systems for cancer gene therapy. J. Drug Target 17, 731–738 (2009)
47. NobelPrize.org. Stanley B. Prusiner – Autobiography (2007)
48. Partners R. SM: Febit's miRBase 14 Geniom-Biochip now with 58 additional new sequences available for cancer research. (2010)
49. Pathak A, Patnaik S, Gupta KC: Recent trends in non-viral vector-mediated gene delivery. Biotechnol. J. 4, 1559–1572 (2009)
50. Petersen CP, Bordeleau ME, Pelletier J, Sharp PA: Short RNAs repress translation after initiation in mammalian cells. Mol. Cell 21, 533–542 (2006)
51. Poy MN, Eliasson L, Krutzfeldt J, Kuwajima S, Ma X, Macdonald PE, Pfeffer S, Tuschl T, Rajewsky N, Rorsman P, Stoffel M: A pancreatic islet-specific microRNA regulates insulin secretion. Nature 432, 226–230, (2004)
52. Racz Z, Hamar P: RNA interference in research and therapy of renal diseases. Contrib. Nephrol. 159, 78–95 (2008)
53. Rácz Z, Hamar P: SiRNS-technológia, a jövő génterápiája? Orv. Hetil. 149, 153–159 (2008)
54. Robert Olby FC: DNA and the central dogma. Daedalus 99, 950 (1970)
55. Shan G: RNA interference as a gene knockdown technique. Int. J. Biochem. Cell Biol. 42, 1243–1251 (2010)
56. Shaw WR, Armisen J, Lehrbach NJ, Miska EA: The conserved miR-51 microRNA family is redundantly required for embryonic development and pharynx attachment in Caenorhabditis elegans. Genetics 185, 897–905 (2010)
57. Shen Y: Advances in the development of siRNA-based therapeutics for cancer. Drugs 11, 572–578 (2008)
58. Slezak-Prochazka I, Durmus S, Kroesen BJ, van den Berg A: MicroRNAs, macrocontrol: regulation of miRNA processing. RNA 16, 1087–1095
59. Soutschek J, Akinc A, Bramlage B, Charisse K, Constien R, Donoghue M, Elbashir S, Geick A, Hadwiger P, Harborth J, John M, Kesavan V, Lavine G, Pandey RK, Racie T, Rajeev KG, Rohl I, Toudjarska I, Wang G, Wuschko S, Bumcrot D, Koteliansky V, Limmer S, Manoharan M, Vornlocher HP: Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs. Nature 432, 173–178 (2004)
60. Suzuki R, Oda Y, Utoguchi N, Maruyama K: Progress in the development of ultrasound-mediated gene delivery systems utilizing nano- and microbubbles. J. Control Release 149, 36–41 (2010)
61. van Rooij E, Sutherland LB, Liu N, Williams AH, McAnally J, Gerard RD, Richardson JA, Olson EN: A signature pattern of stress-responsive microRNAs that can evoke cardiac hypertrophy and heart failure. Proc. Natl. Acad. Sci. USA 103, 18255–18260, (2006)
62. Veedu RN, Wengel J: Locked nucleic acids: promising nucleic acid analogs for therapeutic applications. Chem. Biodivers. 7, 536–542 (2010)
63. Wang B, Yanez A, Novina CD: MicroRNA-repressed mRNAs contain 40S but not 60S components. Proc. Natl. Acad. Sci. USA 105, 5343–5348 (2008)
64. Wang JF, Yu ML, Yu G, Bian JJ, Deng XM, Wan XJ, Zhu KM: Serum miR-146a and miR-223 as potential new biomarkers for sepsis. Biochem. Biophys. Res. Commun. 394, 184–188 (2010)
65. Watson JD, Crick FH: Molecular structure of nucleic acids; a structure for deoxyribose nucleic acid. Nature 171, 737–738 (1953)
66. Wells DJ: Electroporation and ultrasound enhanced non-viral gene delivery *in vitro* and *in vivo*. Cell Biol. Toxicol. 26, 21–28 (2010)
67. Wu L, Fan J, Belasco JG: MicroRNAs direct rapid deadenylation of mRNA. Proc. Natl. Acad. Sci. USA 103, 4034–4039 (2006)
68. Zhao M, Yang H, Jiang X, Zhou W, Zhu B, Zeng Y, Yao K, Ren C: Lipofectamine RNAiMAX: an efficient siRNA transfection reagent in human embryonic stem cells. Mol. Biotechnol. 40, 19–26 (2008)
69. Zhao Y, Ransom JF, Li A, Vedantham V, von Drehle M, Muth AN, Tsuchihashi T, McManus MT, Schwartz RJ, Srivastava D: Dysregulation of cardiogenesis, cardiac conduction, and cell cycle in mice lacking miRNA-1-2. Cell 129, 303–317 (2007)