Chinese Journal of Cancer

Editorial

MicroRNA therapeutics: principles, expectations, and challenges

Rajesha Rupaimoole[1,4], Hee-Dong Han[1,4], Gabriel Lopez-Berestein[2,3,4] and Anil K. Sood[1,3,4]

Abstract

MicroRNAs (miRNAs) are a class of highly abundant non-coding RNA molecules that are involved in several biological processes. Many miRNAs are often deregulated in several diseases including cancer. There is substantial interest in exploiting miRNAs for therapeutic applications. In this editorial, we briefly review current advances in the use of miRNAs or antisense oligonucleotides (antagomirs) for such therapies. One of the key issues related to therapy using miRNAs is degradation of naked particles *in vivo*. To overcome this limitation, delivery systems for miRNA-based therapeutic agents have been developed, which hold tremendous potential for improving therapeutic outcome of cancer patients.

Key words MicroRNA, nanoparticles, miRNA therapeutics, DOPC, chitosan

MicroRNAs (miRNAs) are small stretches of non-coding RNA molecules that negatively regulate gene expression and are implicated in several cellular processes including cell proliferation and differentiation. Since the discovery of *Lin-4* and *Let-7* in *C. elegans*, several hundred miRNAs have been discovered in vertebrates and implicated in many diseases including cancer. These key regulators of gene expression are often found to be misexpressed in cancer and are involved in promoting cancer growth and progression. Recent studies have also shed light on alterations in key components of the miRNA biogenesis machinery [1,2]. Deregulation of these components in cancer has been attributed to altered expression of miRNAs. Since abnormal expression of miRNAs is a key component of cancer pathogenesis, there is growing interest in restoring miRNA expression to normal levels. Although some delivery approaches look promising, one of the challenges is the development of effective and selective delivery systems for reliable delivery of miRNAs to the tumor site. Here, we briefly review contemporary information related to the role of miRNAs for new therapeutic approaches for cancer.

Authors' Affiliations: Department of [1]Gynecologic Oncology, [2]Experimental Therapeutics, and [3]Department of Cancer Biology, [4]Center for RNA Interference and Non-Coding RNA, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030, USA.

Corresponding Author: Anil K. Sood, Departments of Gynecologic Oncology and Cancer Biology, Unit 1362; P.O. Box 301439; University of Texas M.D. Anderson Cancer Center, Houston, Texas 77230-1439, USA. Tel: +1-713-745-5266; Fax: +1-713-792-3643; Email: asood@mdanderson.org.

MiRNAs: Key Regulators of Gene Expression

miRNAs are small non-coding RNAs of approximately 22 nucleotides in length. They bind to the 3'-untranslated region (3'UTR) of target mRNA based on sequence complementarity and result in target mRNA degradation or suppression of translation [3,4]. Initial discoveries of miRNAs were made in *Caenorhabditis elegans*, but have now been extended to majority of vertebrates and several hundred miRNAs have been identified. miRNA biogenesis starts with transcription of the miRNA gene into precursor miRNA, which gets cleaved by RNA polymerase Drosha. This step yields primary miRNA (pri-miRNA), which gets exported from the nucleus into cytosol by Exportin 5. These pri-miRNAs are further processed by Dicer to form mature miRNAs, which undergo incorporation of RNAi-induced silencing complex (RISC) and result in binding with target mRNAs to effectively suppress the gene expression by mRNA degradation or translational inhibition [4]. There has been growing interest in understanding the implications of miRNA in regulating cellular processes, such as cell growth, proliferation, and differentiation in cancer and other diseases. As such, mutation of miRNAs, dysfunction of miRNA biogenesis and deregulation of miRNAs have been shown to play a key role in the development of cancer and other diseases. For example, Let-7 family of miRNAs is down-regulated in several types of cancer and associated with poor patient

outcomes[5]. Alterations in miRNA biogenesis machinery may also contribute to global decreases in miRNA expression, which also correlates with poor patient outcomes[1,2,6]. However, some miRNAs, such as *miR-17-5p*, *miR-20a*, *miR-21*, *miR-92*, *miR-106a*, and *miR-155*, have been shown to be overexpressed in cancer, pointing to the complexity of miRNA involvement in cancer pathogenesis. Current therapeutic methods such as small molecule inhibitors or monoclonal antibodies have shown promise; however, there are many genes that are not "drugable" using these methods. Advantages of using miRNA-based therapy include the ability to target several genes in a given pathway and the ability to rapidly develop new therapies. Here, we briefly discuss advances in miRNA-based therapy.

MiRNA as A Therapeutic Modality

miRNAs work by highly specific binding to the complementary site on the mRNA target and are being considered as new therapeutic strategies. Specific oligomers, called antagomirs, have a complementary sequence to a specific miRNA and ultimately compete with the target mRNA to bind to miRNA. This concept has shown promise in cell culture models as well as initials *in vivo* experiments. For example, Krutzfeldt *et al.*[7] demonstrated that miRNA antagomirs were successfully delivered *in vivo* and had higher stability showing target modulation in specific tissues where particular miRNAs were expressed. For example, miR-122 antagomir in the liver showed up-regulation of several genes targeted by miR-122[7]. Conversely, it is possible to restore miRNAs that are often down-regulated in cancer by external delivery using suitable carrier systems. One of the key issues for this modality is to target the specific sites or tissues of interest. Efficient delivery systems for *in vivo* delivery of miRNA are highly desirable and several are currently under investigation. There are also emerging studies involving restoration of tumor suppressive miRNAs in tumor by *in vivo* delivery.

MiRNA *In Vivo* Delivery—Strategies and Challenges

One of the key issues in delivering miRNAs *in vivo* relates to nuclease-mediated degradation before achieving target modulation. To overcome this limitation, several chemical modifications have been tried such as replacing the phosphodiester group with phosphorothioate and the introduction of a fluoro, an O-methyl group, or a 2-methoxyethyl group [8,9]. However, chemical modifications may lead to off-target effects, reduced miRNA activity, and production of toxic metabolites as a result of degradation of these molecules. These concerns call for a suitable delivery system, which will protect the naked miRNAs from nucleases and conditions *in vivo*. The ideal systemic delivery system for miRNA antagomirs or miRNAs is expected to provide robust target binding, and will require a biodegradable and non-immunogenic carrier. In addition, an ideal carrier will have target tissue specificity based on tumor-specific receptors. One of the strategies is the use of nanocarriers that are submicron in size and biocompatible, which are usually made up of natural or synthetic lipids or polymers. Advantages of using nanocarriers are that they can be coated with high-affinity ligands for tumor-specific receptors and can be controlled- and/or sustained-release.

Among the various options for miRNA delivery, the neutral nanoliposome, 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine (DOPC), is particularly attractive[10]. Advantages of DOPC nanoliposome include lack of toxicity, biocompatible and biodegradable characteristics, and lack of any apparent toxicity. Other nanoparticles such as chitosan carry a positive charge, and are highly effective and safe for *in vivo* delivery of siRNA and miRNA[11]. In addition, reconstituted high density lipoprotein (rHDL) nanoparticles are also promising delivery carriers and are being evaluated further[12].

While several nanoparticle platforms have been developed for passive delivery, methods enabling selective delivery of siRNA or miRNA are highly desirable to minimize the risk of unwanted side effects. Tumor-specific or active targeting of nanoparticles to tumor tissues and tumor cells can be achieved by coupling ligands to the exterior surface and could potentially increase delivery of miRNA. Some of the conjugates under investigation include antibodies, aptamers, and peptides targeted against specific surface receptors in the tumor microenvironment.

Light at The End of Tunnel

The substantial body of research over the last 10 years in the area of miRNA and cancer has increased our knowledge regarding the key roles of miRNA in cancer growth and progression. We believe that in this current era of genomics and targeted therapy, miRNA-based therapeutics hold great potential for cancer management. It is through a better understanding of the systemic behavior of nanoparticles and non-coding RNAs that we will see the light at the end of the tunnel. Although challenges such as development of suitable delivery systems remain, it is likely that these will be

overcome in the near future to realize the full therapeutic potential of miRNAs.

Acknowledgements

Rajesha Rupaimoole is supported by the Hawkins Biomarker Discovery fellowship. Portions of this research were supported by grants from the National Institutes of Health (CA109298, CA110793, CA128797, RC2GM092599, U54 CA151668), Department of Defense (OC073399, OC093146, BC085265), a Program Project Development Grant from the Ovarian Cancer Research Fund, Inc, the Ward Family, the Zarrow Foundation, the Marcus Foundation, the M. D. Anderson Cancer Center SPORE in Ovarian Cancer (P50 CA083639) and Uterine Cancer (P50 CA098258), the Laura and John Arnold Foundation, and the Betty Ann Asche Murray Distinguished Professorship.

Received: 2011-05-05; accepted: 2011-05-09.

References

[1] Merritt WM, Lin YG, Han LY, et al. Dicer, Drosha, and outcomes in patients with ovarian cancer [J]. N Engl J Med, 2008,359(25):2641–2650.
[2] Lin RJ, Lin YC, Chen J, et al. MicroRNA signature and expression of Dicer and Drosha can predict prognosis and delineate risk groups in neuroblastoma [J]. Cancer Res, 2010,70(20):7841–7850.
[3] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function [J]. Cell, 2004,116(2):281–297.
[4] Kim VN. Small RNAs: classification, biogenesis, and function [J]. Mol Cells, 2005,19(1):1–15.
[5] Lu J, Getz G, Miska EA, et al. MicroRNA expression profiles classify human cancers [J]. Nature, 2005,435(7043):834–838.
[6] Martello G, Rosato A, Ferrari F, et al. A microRNA targeting dicer for metastasis control [J]. Cell, 2010,141(7):1195–1207.
[7] Krutzfeldt J, Rajewsky N, Braich R, et al. Silencing of microRNAs in vivo with "antagomirs" [J]. Nature, 2005,438 (7068):685–689.
[8] Chiu YL, Rana TM. SiRNA function in RNAi: a chemical modification analysis [J]. RNA, 2003,9(9):1034–1048.
[9] Harborth J, Elbashir SM, Vandenburgh K, et al. Sequence, chemical, and structural variation of small interfering RNAs and short hairpin RNAs and the effect on mammalian gene silencing [J]. Antisense Nucleic Acid Drug Dev, 2003,13(2): 83–105.
[10] Landen CN Jr, Chavez-Reyes A, Bucana C, et al. Therapeutic EphA2 gene targeting in vivo using neutral liposomal small interfering RNA delivery [J]. Cancer Res, 2005,65 (15):6910–6918.
[11] Lu C, Han HD, Mangala LS, et al. Regulation of tumor angiogenesis by EZH2 [J]. Cancer Cell, 2010,18(2):185–197.
[12] Shahzad MM, Mangala LS, Han HD, et al. Targeted delivery of small interfering RNA using reconstituted high-density lipoprotein nanoparticles [J]. Neoplasia, 2011,13(4):309–319.