MicroRNA therapeutics: principles, expectations, and challenges

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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. 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 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.

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. 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 miRNAs to effectively suppress the gene expression by mRNA degradation or translational inhibition. 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 antagonirs, 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 initial in vivo experiments. For example, Krutzfeldt et al.\(^7\) demonstrated that miRNA antagonirs were successfully delivered in vivo and had higher stability showing target modulation in specific tissues where particular miRNAs were expressed. For example, miR-122 antagonir 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 antagonirs 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.

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