Chemical Tools for Spatiotemporal Regulation of microRNAs

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MicroRNAs (miRNAs) are short noncoding RNAs (20-25 nucleotides) that regulate diverse cellular processes. Dysregulation of miRNAs has been associated with various human diseases, including cancer, cardiovascular diseases, metabolic diseases, infectious diseases, and neurodegenerative diseases among others [1]. They have emerged as a new class of important therapeutic target. Their biological functions in regular cellular events and their contributions to the onset or the progression of diseases have been under intensive studies in recent years.

miRNAs are encoded in genes that are transcribed to give primary miRNAs, which are then processed by a ribonuclease, Drosha, in the nucleus to generate pre-miRNAs (~70 nts). Pre-miRNAs are transported to the cytoplasm and then further processed by another ribonuclease, Dicer that eventually gives mature miRNAs to be incorporated into the RNA-induced silencing complex (RISC). The most common mechanism of miRNA-mediated gene regulation is at the post-transcriptional level. Through pairing with perfectly or partially matched complementary sites in 3'-untranslated regions (UTRs) of messenger RNAs (mRNAs), miRNA-RISCs can either block translation or degrade targeted mRNAs [2]. miRNAs is estimated to mediate post-transcriptional silencing of 30% of protein-coding genes in mammals. Each miRNA can target hundreds of different mRNAs bearing partially complementary sequences and each miRNA typically has multiple recognition sequences for different miRNAs. This suggests the roles played by miRNAs are likely dynamic, combinatorial, and cellular context-dependent. Given the critical and diverse roles that miRNAs play in cells and in diseases, methods that allow the manipulation of activities or levels of miRNAs will contribute to the understanding of miRNA functions and the development of novel therapies. Because of the dynamic nature in miRNA's roles in cellular processes, spatiotemporal-specific methods that enable rapid manipulations of chosen miRNAs at desired cellular locations and time will be invaluable to reveal their functions for biological processes in various cellular and environmental contexts.

Several strategies have been developed to regulate the activities of miRNAs. Small interfering RNAs (siRNAs) are designed to target the pri-miRNA in a similar way as siRNA-mediated mRNA silencing through RNA degradation. The miRNA sponge method presents multiple copies of miRNA-targeted sequences as decoys to quench the chosen miRNAs [3]. Both methods require the introduction of DNA plasmids encoding either the designed siRNAs or miRNA sponges into the cells and then require the transcription process to generate the desired RNA species. Other methods employ chemically synthesized antisense oligonucleotide derivatives including peptide nucleic acids (PNAs), morpholinos (MOs), locked nucleic acids (LNAs) or antagonirs [4,5]. Most of these molecules, such as MOs, act as steric-blockers, which physically disrupt interactions between mature miRNAs (or their precursors) and their associated protein partners. Others, such as antagonirs, mask miRNA-binding sites on mRNAs. These molecules are chemically modified to give unnatural structures that resist the degradation by endogenous nucleases and provide higher binding affinities towards the targeted RNA sequences. Although a diverse array of approaches has been developed to regulate miRNA levels or activities, available methods for their spatiotemporal-specific controls are limited.

A few novel methods have been reported to allowing spatiotemporal controls of gene expression through regulating miRNA translation. These methods are expected be applicable in regulating miRNAs. They mainly apply the design of incorporating photolabilechemical groups as masks or linkers into the chemically modified antisense molecules complementary to the desired RNA targets. Installing photochemical controllable handles enables using optical methods to activate or inactivate the modified antisense molecules in high spatial and temporal precisions and in turn regulate the activities of targeted RNAs corresponding. In the Chen laboratory, a novel strategy was developed that a MO designed to inhibit the no tail (ntl) gene in zebrafish was tethered to a short complementary oligomer through a photo-cleaveable linker with a dimethoxynitrobenzyl(DMNB) or a bromohydroxyquinoline (BHQ) group [6,7]. Before light activation, the MO was masked by the complementary oligomer and therefore cannot target the ntl mRNA. Applying the UV or two-photon excitation, a spatial specific cleavage of the linker released the MO from the complementary mask and blocked the ntl expression selectively at the chosen location in the fish. In the Deiters et al. and Yoder groups, a 6-nitropropionylomethyl group (NPOM) was chemically linked to the MO monomer [8]. This chemical modification disrupted the base recognitions between MOs and RNAs. With this caging group installed, the modified MO remained inactive until NPOM groups were removed by the UV excitation. This approach was shown to be effective to control eGFP expression in zebrafish. In the Mayer group, photo-activatable MOs were generated by hybridizing the functional MO sequences with complementary caging strands containing a photocleavablenitrobenzyl linkage [9]. The caging strand masked the morpholino activity until applying the UV irradiation, which released the functional MOs to knockdown different genes in zebrafish. A similar approach reported by the Tallafuss and Washbourne groups, on the other hand, allowed spatial-specific inactivation of MOs by light [10]. Using MOs containing a photocleavablenitrobenzyl linkage, these molecules could be cleaved and inactivated by the UV excitation to reverse the knockdown effects by MOs.

These photochemical tools have contributed tremendously to the capability of dissecting complex biological events through spatiotemporal controls of gene expression. These methods should be readily applicable for miRNA regulations to reveal their diverse functions in the ever-changing cellular contexts. There are still several limitations encountered by current methods for miRNA regulations.

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such as undesired off-target effects and inefficient cellular deliveries. Additional novel strategies and methods to address these difficulties and expand the toolbox for miRNA research are highly desired.

References


