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Up- and downregulation of mature miR-1587 function by modulating its G-quadruplex structure and using small molecules

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ABSTRACT: Using bioinformatics analysis, we found some mature human miRNAs containing G-rich sequences with four G-tracts that had a high probability of forming G-quadruplex structures. Here, we chose G-rich miR-1587 as a model to characterize the function and regulation of miRNAs. Using electrospray ionization mass spectrometry, magnetic resonance imaging, circular dichroism spectrometry, we had confirmed that miR-1587 folded into a stable parallel G-quadruplex structure. By microarray, Q-RT-PCR and 3'UTR luciferase assay, TAGLN, an early marker of smooth muscle differentiation and tumor suppressor, was identified as a target gene of miR-1587, thus providing a direct target to study miR-1587 functions. We identified three aspects of miR-1587 regulation: 1) KCl induced miR-1587 G-quadruplex formation, reducing the interaction between miR-1587 and the target gene, and inhibiting miR-1587 function; 2) pseudopalmatine ligand further inhibited miR-1587 binding to TAGLN mRNA, which disrupted its function and increased the TAGLN expression; 3) the addition of TMPyP4 ligand interfered G-quadruplex formation, and significantly enhanced miR-1587 regulation of TAGLN expression. This study has revealed the possibility of using the G-quadruplex structure as a strategy to regulate miR-1587 function, showing potential for the development of up- and downregulation of mature G-rich microRNA function by modulating its G-quadruplex and using small molecules.

1. Introduction

G-quadruplex structure, a specific type of secondary structure formed by DNA or RNA strands found in telomeres, promoters, exons, and UTR regions, have been proven to play an important role in replication and translation processes [1]. Recently, G-quadruplexes have also been researched as a potential target for cancer treatment [2]. New small molecules have been developed to selectively recognize and bind G-quadruplexes to modulate gene expression [3-6].

MicroRNAs (miRNAs) were naturally occurring, highly conserved, and short noncoding RNA molecules. They were 17-27 nucleotides long and normally regulated gene expression at the post-transcriptional level by binding to complementary 3'UTR in the mRNA sequence, which in turn led to translational inhibition and gene silencing. One miRNA might regulate the functions of multiple mRNAs, and these mRNAs were usually associated with the same disease or occurred in the same signal transduction pathway [7]. The development of a miRNA inhibitor associated with a specific disease would allow the simultaneous regulation of multiple associated mRNAs targeting various signaling pathways and functions. Therefore, miRNA-targeted disease treatment has attracted growing research interests [8]. At present, several strategies for regulating mRNA function through miRNA targeting have been described. An exogenously introduced anti-miR could complementarily pair with an overexpressed miRNA and competed with the target gene mRNA, thereby inhibiting miRNA binding to the mRNA 3'UTR region [9], which might also lead to sequence-dependent [10] or non-sequence-specific off-target effects [11]. For these reasons, regulating miRNAs function with small molecules has attracted considerable research interest [12-13].

Very little research has focused on the relationship between miRNAs function and the G-quadruplex structure. Some studies have shown that G-quadruplexes produced during miRNA formation had an impact on the maturation process [14]. Besides, the G-rich sequence in the 3'UTR region of the FADS2 gene affected the binding of mRNA to miR-331-3p after the formation of the G-quadruplex structure [15].

Recently, by bioinformatics analysis, we found that 152 mature human miRNAs containing G-rich sequences such as miR-197-5p, miR-765, miR-1587 [16], miR-3620 [17], miR-4507 and miR-5196 (Table S1), had a high probability of forming intramolecular G-quadruplexes. Among them, miR-1587 contained regular four G-tracts and exhibited high expression in HeLa cells. Recent studies have also shown that Glioma-associated mesenchymal stem cells secreted miR-1587 by releasing exosomes, targeting and inducing downregulation of NCOR1 expression in Glioma stem cells and thereby promoting proliferation and colony formation and increasing cells tumorigenicity [18]. The results of this study highlighted the importance of miR-1587. Therefore, miR-1587 was selected as a model to test G-quadruplex formation in mature human miRNAs and targeted functional regulation by G-quadruplex structure and its ligands.