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MicroRNA Biomarkers in Neurodegenerative Diseases and Emerging Nano-Sensors Technology

Pratik Shah,[1,2] Seok Keun Cho,[3] Peter Waaben Thulstrup,[4] Morten Jannik Bjerrum,[4] Phil Hyu Lee,[5] Ju-Hee Kang,[6] Yong-Joo Bhang,[7] Seong Wook Yang[1,3]

[1]UNIK Center for Synthetic Biology, University of Copenhagen, Copenhagen, Denmark
[2]Department of Biomedical Engineering, University of California Irvine, Irvine, CA, USA
[3]Department of Systems Biology, College of Life Science and Biotechnology, Yonsei University, Seoul, Korea
[4]Department of Chemistry, University of Copenhagen, Copenhagen, Denmark
[5]Department of Neurology, Severance Biomedical Science Institute, Yonsei University College of Medicine, Seoul, Korea
[6]Department of Pharmacology, Hypoxia-related Disease Research Center, Inha University School of Medicine, Incheon, Korea
[7]Seoulin Bioscience Co., Ltd., Seongnam, Korea

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Corresponding author: Seong Wook Yang, PhD, Department of Systems Biology, College of Life Science and Biotechnology, Yonsei University, 50 Yonsei-ro, Seodaemun-gu, Seoul 03722, Korea
Tel: +82-2-2123-2655 **Fax:** +82-2-2123-2655 **E-mail:** yangsw@yonsei.ac.kr

Corresponding author: Pratik Shah, PhD, Department of Biomedical Engineering, University of California Irvine, Irvine 92697, CA, USA
Tel: +1-949-501-6270 **E-mail:** pshah2@uci.edu

ABSTRACT

MicroRNAs (miRNAs) are essential small RNA molecules (20–24 nt) that negatively regulate the expression of target genes at the post-transcriptional level. Due to their roles in a variety of biological processes, the aberrant expression profiles of miRNAs have been identified as biomarkers for many diseases, such as cancer, diabetes, cardiovascular disease and neurodegenerative diseases. In order to precisely, rapidly and economically monitor the expression of miRNAs, many cutting-edge nanotechnologies have been developed. One of the nanotechnologies, based on DNA encapsulated silver nanoclusters (DNA/AgNCs), has increasingly been adopted to create nanoscale bio-sensing systems due to its attractive optical properties, such as brightness, tuneable emission wavelengths and photostability. Using the DNA/AgNCs sensor methods, the presence of miRNAs can be detected simply by monitoring the fluorescence alteration of DNA/AgNCs sensors. We introduce these DNA/AgNCs sensor methods and discuss their possible applications for detecting miRNA biomarkers in neurodegenerative diseases.

Key Words

miRNAs; neurodegenerative diseases; silver nanoclusters; DNA sensor.

INTRODUCTION

As the fundamental unit of living organisms, the cell runs thousands of biochemical reactions with high accuracy, precision and efficiency. The systemic regulation of cellular processes involves a variety of regulatory components and pathways. To study these sophisticated systems, researchers rely on cellular biomarkers as the representatives of any given cellular reaction. The biomarkers provide key information about the normal biological processes, as well as the pathogenic or disease conditions that arise in response to external signals or internal imbalances. One highly attractive class of biomarkers comprises the nucleic acids (NAs), i.e., DNA and RNA, because of their easy extraction from non-invasive samples such as blood, urine and stool for prognosis and diagnosis.[1,2] One of the most widely used biomarkers, with a huge potential for practical applications, is a class of small RNA molecules known as microRNAs (miRNAs).[3] miRNAs are short non-coding RNAs that are 21–22 nucleotides in length and are involved in the regulation of gene expression at the post-transcriptional level.[4] Detailed studies have revealed the spatial and temporal occurrence of many miRNAs, as well as their unique biological roles in messenger RNA (mRNA) cleavage and translational suppression. More than 2000 miRNAs have been identified in humans, and many of them are functionally involved in diseases, showing great potential as biomarkers.[4-6] However, for the practical applications of miRNAs as biomarkers, highly reliable, economic, and convenient detection methods are required in addition to the currently available methods. Thus, there has been a surge in the interest of developing improved miRNA detection methods, especially using nanotechnological tools.[5-9] One such advanced tool that has drawn particular attention is the use of the fluorescent properties of silver nanoclusters (AgNCs).[10-12] When clustered silver atoms approach a size less than 2 nm, due to quantum confinement, AgNCs exhibit unique properties such as strong and stable fluorescence.[12,13] The synthesis of AgNCs in aqueous solutions, using stabilizing scaffolds such as NAs, has allowed for a large number of applications for the detection of biomolecules.[14-16] The fundamental understanding of the role and properties of NAs, as well as their influence on the fluorescent synthesis of AgNCs, has increased in recent years.[17-21] By exploiting such new technology, research groups have developed several reliable methods for NA/AgNCs-based miRNA detection.[10,22] In this review, we discuss the current findings of miRNAs as biomarkers for human diseases, especially neurodegenerative diseases, and how miRNAs can be efficiently determined, with an emphasis on novel nanosensor methods.

BIOLOGY OF MICRORNAS

The biological impacts of the small RNA molecules remained unnoticed until the last part of the 20th century, but the discovery of miRNA by the group of Victor Ambros opened up a whole new avenue of research.[4] The miRNAs turned out to be very important regulators of gene expression.[4] Recent studies on their origin, biogenesis and mechanisms of action have greatly advanced our knowledge of the molecular biology of miRNAs.[4] miRNAs are generated through two step-wise processes involving the coordinated functions of a multi-protein complex known as the microprocessor.[23-25] Primary miRNAs are transcribed from *MIRNA* genes and then processed into precursor miRNAs and further to mature miRNAs by the microprocessor.[23] Mature miRNAs are then loaded onto the so-called RNA-Induced Silencing Complex to recognize target mRNAs through sequence complementarity. The complex of the protein machinery with a loaded miRNA can either remove target mRNA or repress the translation of mRNA.[26,27] The levels of miRNAs are often differentially modulated by the growth and developmental stage of organisms, specific cell and tissue types, and environmental stimuli.[3,28-33]

MIRNA BIOMARKERS IN HUMAN NEURODEGENERATIVE DISEASES

Many studies have suggested that miRNAs can be important biomarkers in a variety of diseases, including cancer, diabetes, cardiovascular disease, aging, asthma, autoimmune disease, kidney diseases, and neurodegenerative diseases.[3,34-46] Due to the potential of miRNA as biomarkers, several studies have predicted that monitoring methods for miRNAs will be extensively useful for curing those diseases in the near future.[42,47-50] Among the defined miRNAs, we focused our discussion on miRNAs associated with

neurodegenerative diseases in this review. Neurodegenerative disorders–Alzheimer's disease (AD), Huntington's disease (HD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and prion diseases–are defined by the progressive loss of specific neuronal cells in the central nervous system. These diseases commonly cause significant defects in motor and cognitive ability. Recently, a number of studies have revealed that specific miRNAs are differentially expressed in the human brain and, more importantly, some of the miRNAs modulate genes associated with specific neurodegenerative disorders. For instance, Harraz et al.[51] showed that among 224 miRNAs sequenced, miR-133b, miR218-2, miR-15b, miR101-1, miR107, miR-335, and miR-345 were notably down-regulated in PD patients. miR-133b seems to be functional as a negative regulator of *Pitx3*, an important transcriptional factor that is involved in the differentiation of dopaminergic neurons.[52-54] Interestingly, miR-133 and *Pitx3* modulate the expression of each other through a negative feedback regulation. *Pitx3* induces *MIR-133* gene expression, which in turn targets *Pitx3* to be degraded.[55] The profiling of miRNAs in PD brains uncovered decreased expression of miR-34b and miR-34c in the affected brain areas.[56] The reduction of miR-34b/34c inversely enhanced the expression of α-synuclein in human dopaminergic SH-SY5Y cells.[57] The sequence variations in the miR-433 recognition site of *fibroblast growth factor 20* (*FGF20*) may disrupt the miR-433-mediated silencing of *FGF20*, and that results in the increased expression of α-synuclein, which confers risk for PD.[58] Three differentially regulated miRNAs–miR-1, miR-22*, and miR-29–were identified in the blood samples of PD patients compared to healthy subjects, showing the feasibility of miRNAs as biomarkers.[59]

β-amyloid cleavage enzyme 1 (BACE-1) is required for the cleavage of *amyloid precursor protein* (*APP*), which thereby generates toxic Aβ species.[60] The expression of *BACE-1/β-secretase* is inversely correlated with the levels of miRNAs from the miR-29a/b-1 family, showing that *BACE-1/β-secretase* is a target of this family miRNA.[61] Interestingly, in a group of AD patents, the expression of miR-29a/b-1 clusters was significantly down-regulated.[61,62] Two additional miRNAs, miR-298 and miR-328, also seem to play essential roles in down-regulating *BACE-1/β-secretase* expression.[61,63] The level of miR-107 is reduced in the brains of AD patients and that is inversely correlated with the increased levels of *BACE-1/β-secretase* and *cofilin*, showing the role of miR-107 in human AD.[64,65] Cyclin-dependent kinase 5, a tau kinase which is dysregulated in AD, and the metalloproteinase ADAM10, which is important for APP processing, are also targeted by miR-107.[66] miR-15 targets a pathological hallmark gene of AD, extracellular signal-regulated kinase 1, a tau kinase. The level of miR-15 is also decreased in AD patiens.[67] Other studies have demonstrated that the miR-29 family seems to play a role in the modulation of microglial activity. The miR-29 family also targets the microglial modulators insulin-like growth factor-1 (IGF-1) and fractalkine ligand (CX3CL1). Indeed, the increased level of miR-29b was inversely correlated with the expression of IGF-1 and CX3CL1 in human cortical tissue, implying the role of the miR-29 family in brain aging.[68] p53 is highly upregulated in AD and induces tau hyperphosphorylation. The miR-34 family is transcriptionally induced by p53 and is also considered a critical mediator of p53 functions. Accordingly, miR-34 is found at high levels in the hippocampus of AD patients, and the down-regulation of miR-34 rescues some cognitive defects.[69,70] miR-106a and miR-106b are complementary to the transcript of *APP*, which is increased in AD patients. Consistently, these miRNAs are reduced in the temporal lobe of AD patients. The expression of the transporter *ABCA1* is also regulated by miR-106a and miR-106b, confirming the roles of the miR-106 family in AD processes.[71] An miRNA dysfunction in AD could be a cause, but in some cases, it could also be a result of AD progress. For example, miR-181c is down-regulated by Aβ in *in vitro* hippocampal cultures. This reduction of miR-181c is recapitulated in Aβ-depositing APP23 transgenic mice and in human AD tissue.[72] In contrast, miR-9, miR-125b, and miR-128 are increased in the hippocampal region of AD-affected brains.[73] In the case of miR-146a, it is upregulated in the temporal cortex of AD patients, and complement factor H is a target of miR-146a, indicating that miR-146a mediates AD-related inflammation.[74] Other miRNAs have been associated with AD, such as miR-124, miR-132, and miR-153. Most of them seem to be implicated in APP processing, neuro-inflammation, tau hyper-phosphorylation, and ApoE-lipidization.[66,75]

The pathogenic poly-glutamine expanded hun-

tingtin (HTT) causes HD, a hereditary neurodegenerative disorder. In normal neurons, HTT forms a complex with repressor element 1 silencing transcription factor (REST) in the cytoplasm, which suppresses the translocation of REST into the nucleus. The REST-HTT interaction can be abolished by the pathogenic modification of HTT, which consequently leads to neuronal death.[76,77] The transcript of *REST* is targeted by miR-9; therefore, the reduction of miR-9 in HD brains could cause the accumulation of free REST protein. Indeed, the cerebral cortex of HD brains have considerably reduced levels of miR-9, showing the importance of miR-9 in HD.[78] miR-132 is notably down-regulated in the post-mortem brains of HD patients. The reduction of miR-132 is known to increase its target, *p250GAP*, encoding a member of the group of GTPase-activating proteins, which inhibit neurite outgrowth, resulting in HD progress.[79,80]

Although we have hitherto discussed miRNAs dysregulation in AD, PD, and HD, there are many miRNAs that are directly or indirectly involved in other neurological disabilities (Table 1), such as epilepsy, ALS, traumatic brain injury, and prion diseases. Recent studies have shed light on the impacts of numerous miRNAs on the pathogenesis and progression of neurodegenerative diseases. However, comprehensive profiles of the effects of different sets of miRNAs that are related to a specific disease still remain elusive. Not only for disease diagnosis but also for prognosis, the expression profile of miRNAs must be precisely and efficiently characterized. To meet the demand, a variety of methods for miRNA profiling have been developed, some of which are discussed below.

INTRODUCTION TO SILVER NANOCLUSTERS

Metal nanoclusters (MNCs) exhibit size-dependent distinct optical properties unlike their bulk counterpart material.[12,13,81] When the metal size approaches the Fermi wavelength, the continuous density of states becomes discrete and all the electronic wavefunctions are overlapped.[82] In this size regime, MNCs may behave as molecular systems, where energy levels are separated to allow direct electronic transitions due to quantum confinement (Figure 1). Because of their behavior as "pseudo-atoms," the metal clusters exhibit unique properties such as strong photoluminescence.[83,84] Among MNCs, AgNCs have gained more prominence due to their brighter fluorescence emission. AgNCs are unstable in solution, and they readily aggregate to form large particles or aggregates lacking any fluorescence properties. For biological application purposes, a water soluble and biocompatible encapsulating ligand is required to restrict the growth of AgNCs in the specific size regime (< 2 nm).[11,12] In recent years, many studies have shown the development of AgNCs using different stabilizing templates, such as dendrimers, peptides, and thiolates.[81,85-88] Among the various stabilizing templates

Table 1. List of miRNAs which are related to neurodegenerative diseases

Neurodegenerative diseases	Expression	Profiled miRNAs
AD	Increased	miR-146a, miR-197, miR-320, miR-423, miR-511
	Decreased	let-7i, miR-9, miR-15a, miR-22, miR-26b, miR-29a.b-1, miR-30a-5p, miR-93, miR-98, miR101, miR-106b, miR-107, miR-181c, miR-210, miR-363
PD	Increased	miR-1, miR-22
	Decreased	miR-7, miR-15b, miR-16-2*, miR-19b, miR-26a/a2*, miR-28-5p, miR-29, miR-29b/c, miR-30a/b/c, miR-34b/c, miR-101-1, miR-107, miR-126, miR-126*, miR-133b, miR-147, miR-151-5p, miR-133, miR-199a-3p, miR-199a-5p, miR-218-2, miR-301a, miR-335, miR-345, miR-374a/b
HD	Increased	miR-29a, miR-330, miR-132, miR-196, miR-486, miR-100, miR-151-3p, miR-16, miR-219-2-3p, miR-27b, miR-451, miR-92a, miR-34b
	Decreased	miR-9/9*, miR-22, miR-29c, miR-124a, miR-128, miR-138, miR-132, miR-128, miR-222, miR-344, miR-674*
ALS	Increased	miR-338-3p, miR-27a, miR-155, miR-146a, miR-32-3p miR-146*, miR-524-5p miR-582-3p, miR-24-2*, miR-142-3p, miR-142-5p, miR-1461, miR-146b
	Decreased	miR-451, miR-1275, miR-328, miR-638, miR-149, miR-665, miR-583

miRNAs: microRNAs, AD: Alzheimer's disease, PD: Parkinson's disease, HD: Huntington's disease, ALS: amyotrophic lateral sclerosis.

for emissive AgNCs formation, we here focus on a type of biocompatible template, NAs, for generating AgNCs. Due to the biological implications of DNA and RNA, NAs have attracted great attention as scaffold templates for emissive AgNCs. The first major advancement in this direction was achieved by Dickson's group.[11] Not only could a strong emission be measured from the DNA AgNCs, but as shown by Dickson's group, the emission wavelength of the AgNCs could also be altered (Figure 2).[89,90] Furthermore, the structure and sequences of NAs can create an excellent interface between the fluorescent properties of AgNCs and biological applications. Therefore, a whole new avenue of applications was opened with the findings from Dickson's group, proving the potential of NAs as a stabilizing template for AgNCs.[11] In subsequent studies, many research groups focused on understanding the fundamental mechanisms underlying the utility of AgNCs as fluorophores. Although these studies suggested many interesting findings on the nature of AgNCs that are worthwhile to discuss in detail, we here focus on discussing the applications of DNA/AgNCs sensors for miRNAs.

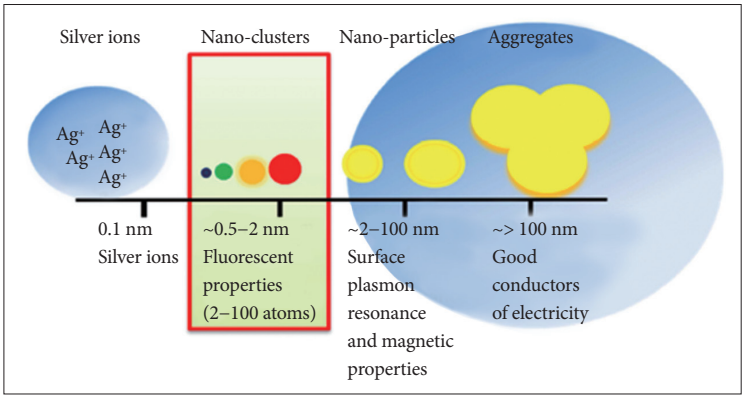

Figure 1. Size-dependent properties of different metallic states. Modified from Pettibone et al. ACS Nano 2013;7:2491-2499, with permission of American Chemical Society.[13]

DNA/AGNCS-BASED METHODS FOR MIRNA DETECTION

For the first time, Petty et al.[91] demonstrated the potential value of DNA/AgNCs for the detection of DNA, where a bi-functional DNA sensor was used. One component of the sensor was composed of a

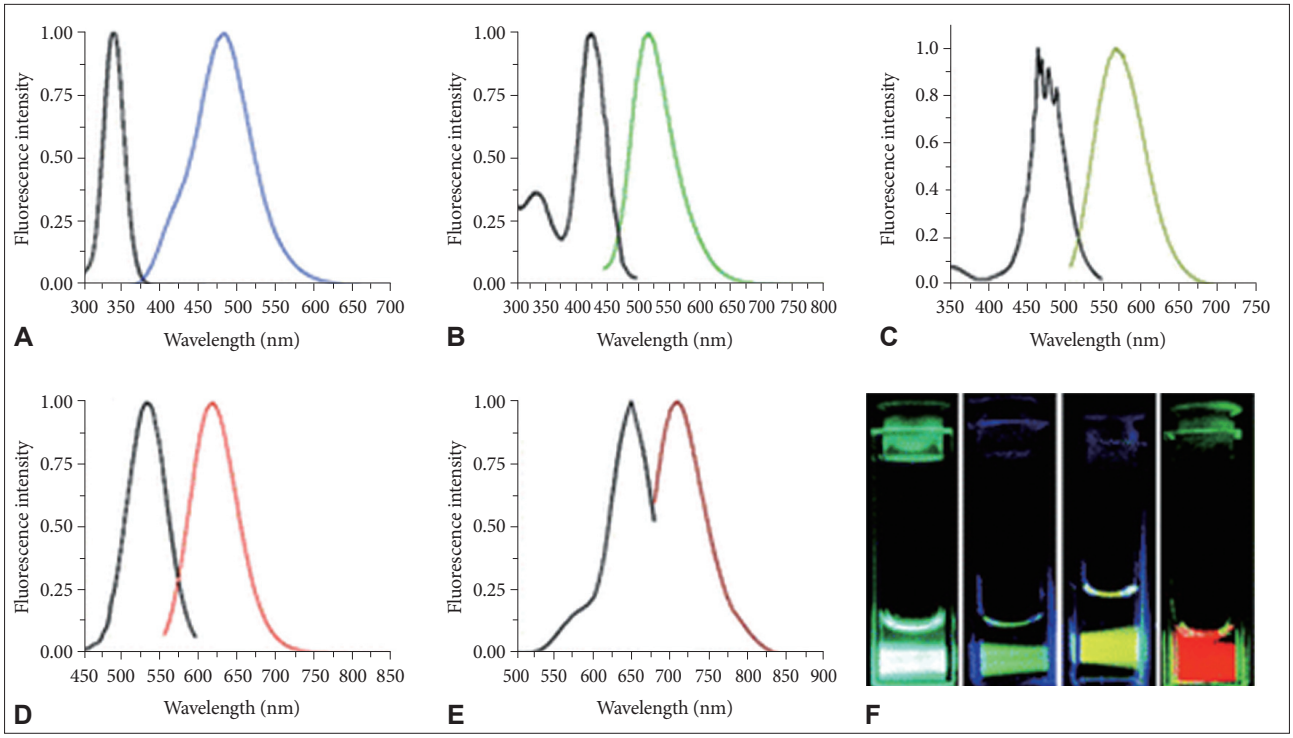

Figure 2. Tuning of the oligonucleotide base sequence in a 12-mer DNA, resulted in generating AgNCs emitters with different fluorescence values. Steady-state excitation and emission spectra for five distinct ss-DNA-encapsulated Ag nanoclusters. A: Blue emitters created in 5′-CCCTTTAACCCC-3′. B: Green emitters created in 5′-CCCTCTTAACCC-3′. C: Yellow emitters created in 5′-CCCTTAATCCCC-3′. D: Red emitters created in 5′-CCTCCTTCCTCC-3′. E: Near IR emitters created in 5′-CCCTAACTCCCC-3′. F: Pictures of emissive solutions in A-D. Adapted from Richards et al. J Am Chem Soc 2008;130:5038-5039, with permission of American Chemical Society.[90] AgNCs: silver nanoclusters.

cytosine-rich sequence, and the other part was a sequence complementary to the target DNA sequences. The presence of the target DNA sequence enhanced the fluorescence of the DNA/AgNCs sensor approximately 2–3-fold, enabling the sensor to be used in a turn-on method for detecting a specific DNA fragment. In a further development of the system, the same group showed that the wavelength shift, rather than altered fluorescence intensity, can be used to detect a target DNA fragment.[91] By applying the potential of DNA/AgNCs-based methods, Yang and Vosch[10] reported for the first time a DNA/AgNCs-based method for miRNA detection. A DNA-sensor consists of two components, a cytosine-rich scaffold and a DNA sequence complementary to the target miRNA. The DNA sensor generates a bright red fluorescence within 1 hour and drops the fluorescence in the presence of target miRNA. This "turn-off"-based method allows the quantitative detection of miRNA down to a picomole level (Figure 3). Because of the huge diversity of miRNA sequences in Arabidopsis and humans, Shah et al.[20] attempted to systemically design DNA/AgNCs sensors by changing only the target miRNA sensing sequences. However, they found that the systemic designing of DNA/AgNCs sensors was not simply established by replacing the target sensing sequences for each miRNA. Through detailed analysis, they suggested that the secondary structure of DNA/AgNCs sensors, including the silver encapsulating C-rich scaffold, is essential for the generation of fluorescence and target recognition.[20] Based on this finding, they reconstituted the C-rich scaffold and target recognition sequences of the non-functional DNA/AgNCs sensor (for miR172 in plants) to be structured, and that was eventually functional in detecting target miRNA. Next, Shah et al.[92] demonstrated that RNA and a DNA/RNA chimera can be an efficient sensor for a specific group of human miRNAs because of the rotational freedom of RNA backbones. By introducing RNA backbones, they further showed that non-functional DNA/AgNCs sensors (for let-7a and miR-200 in humans) can be successfully converted into functional RNA/DNA-chimera/AgNCs sensors.[93] The newly developed chimera sensors were highly sensitive to recognize let-7a and miR-200 in human

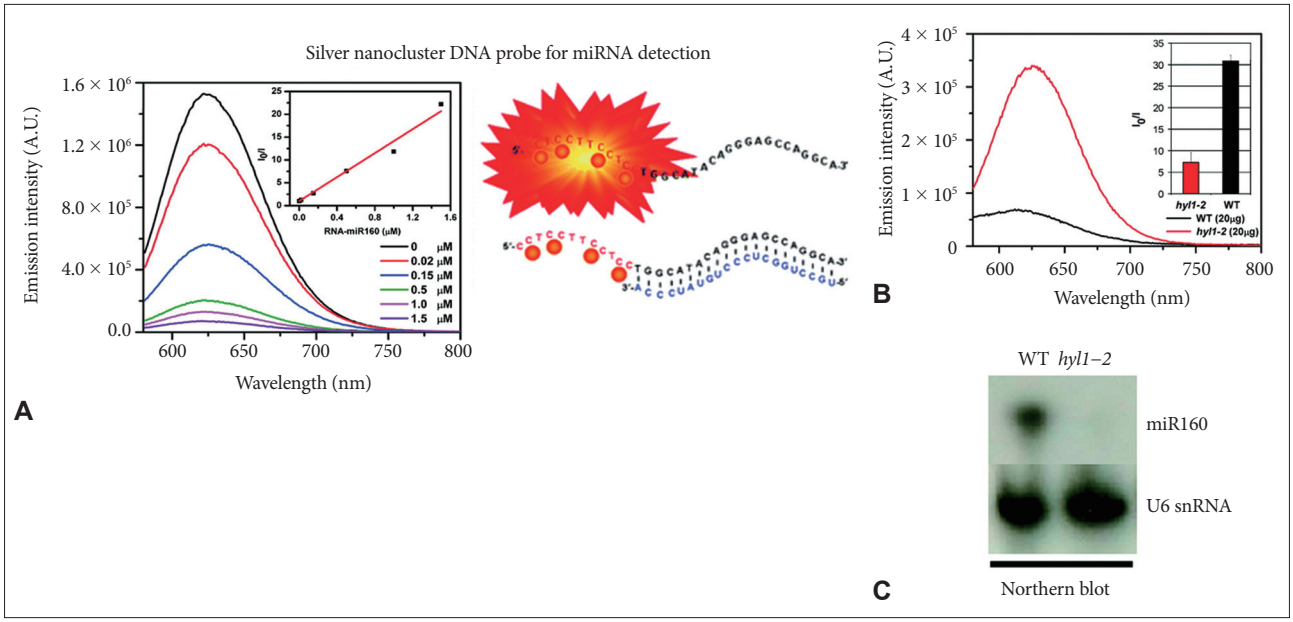

Figure 3. Fluorescence intensity of the AgNCs formed in 12 nt-RED-160 probe with RNA-miR160 target in a concentration ranging from 0 to 1.5 μM. The fluorescence spectra were recorded, exciting at 560 nm. Emission spectra (excited at 560 nm) of AgNCs emission from a solution containing 1.5 μM of DNA-12 nt-RED-160 probe and 20 μg of endogenous RNA from WT (black curve) or *hyl1-2* mutant Arabidopsis thaliana plants. Northern blot analysis shows the presence and absence of RNA miR160 in WT and *hyl1-2* mutant plants. A: Fluorescence intensity of the AgNCs formed in 12 nt-RED-160 probe with RNA-miR160 target in a concentration ranging from 0 to 1.5 μM. The fluorescence spectra were recorded, exciting at 560 nm. B: Emission spectra (excited at 560 nm) of AgNCs emission from a solution containing 1.5 μM of DNA-12 nt-RED-160 probe and 20 μg of endogenous RNA from WT (black curve) or hyl1-2 mutant Arabidopsis thaliana plants. C: Northern blot analysis shows the presence and absence of RNA miR160 in WT and hyl1-2 mutant plants, respectively. Adapted from Yang et al. Anal Chem 2011;83:6935-6939, with permission of American Chemical Society.[10] AgNCs: silver nanoclusters, WT: wild-type.

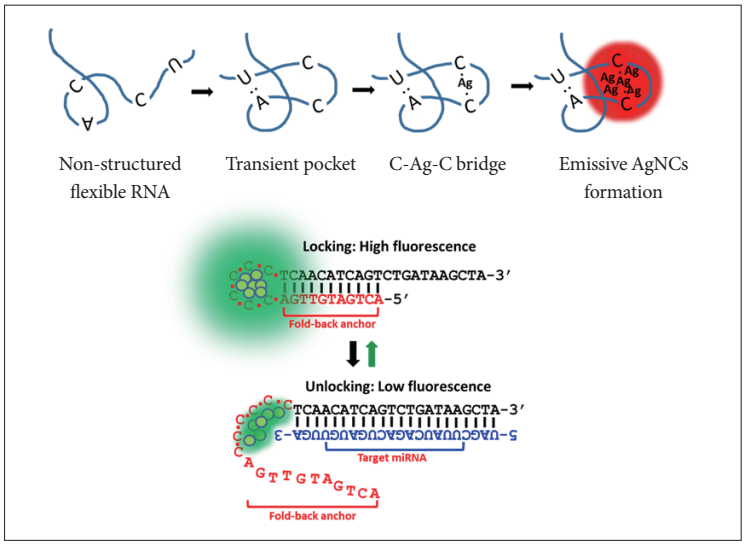

Figure 4. Schematic cartoon for the generation of highly emissive AgNCs in DNA-RNA chimera sensor. A working model of locking-to-unlocking DNA/AgNCs sensor in detecting miRNAs. Adapted from Shah et al. Chem Commun (Camb) 2014;50:13592-13595, with permission of Royal Society of Chemistry.[92] Adapted from Shah et al. Nucleic Acids Res 2016;44:e57, with permission of Oxford University Press.[94] AgNCs: silver nanoclusters.

cell lines. Furthermore, to improve the designing system for DNA/AgNCs sensors, Shah et al.[94] suggested a locking-to-unlocking system by which several DNA/AgNCs sensors were successfully constructed for miR-21, miR-18a, and miR-27b (Figure 4).

These studies also demonstrated the pragmatic potential of DNA/AgNCs-based sensors by using biological samples. For instance, the levels of target miR160 in wild-type and in *hyl1-2* mutant of Arabidopsis were determined with a DNA/AgNCs sensor.[10] Let-7a is now used as a biomarker for many cancers in humans, such as colorectal cancer and breast cancer. By using chimera/AgNCs sensors, the expression of let-7a was successfully observed in HT-29 (a colorectal adenocarcinoma) and MDA-MB-231 (a breast cancer cell line); miR-200c was monitored only in the HT-29 cell line.[93] Likewise, miR-21 and miR-27b are considered as specific biomarkers of cancers such as MCF-7 (a breast cancer cell line) and PANC-1 (a non-endocrine pancreatic cancer cell line), respectively. The differential expression of miR-21 and miR-27b in the cell lines was successfully monitored by the locking-to-unlocking DNA/AgNCs methods.[94] Yang's group further applied the method to study the processing pathway of miRNA in plants and identified that the deficiency of CONSTITUTIVE PHOTOMORPHOGENETIC 1, a negative regulator of photomorphogenesis, led to a dramatic reduction of miRNAs.[95] The detection of miRNA levels by the DNA/AgNCs-based method allowed them to rapidly screen out a novel component in plant miRNA biogenesis.

In addition to these methods, Zhang et al.[96] suggested the use of target-assisted isothermal exponential amplification coupled with fluorescent DNA/AgNCs. By using two enzymes, DNA polymerase and nicking endonuclease, the polymerization of a designed DNA sensor can be initiated upon the binding of target miRNA. They presented that miR-21 and miR-141 could be detected with a high specificity and sensitivity, and claimed that the sensitivity was similar to the routinely used quantitative real-time reverse transcription PCR (qRT-PCR)-based method. The outcome of this method was verified by qRT-PCR in parallel. Xia et al.[97] suggested a new type of AgNCs/hairpin DNA sensor where a 5-TCC/CCC-3'-overhang for embedding emissive AgNCs was attached to a target sensing DNA sequence. The fluorescence of the sensor was diminished in the presence of the target RNA sequence with complementarity to the sensing DNA sequence. Two hairpin DNA-templated AgNCs (AgNCs/HpDNA) sensors were combined with a strand-displacement amplification (SDA) procedure to detect miR-16-5p and miR-19b-3p, which are known as biomarkers of gastric cancer. This method demonstrated that the effect of G-rich fluorescence enhancement can be successfully implemented in the SDA reaction for the rapid and specific detection of miRNAs. Dong et al.[98] demonstrated that a conformational molecular beacon coupled with target recycling amplification can be a facile and sensitive miRNA biosensor. In the method, the competition displacing between target miRNA and Hg^{2+} provides the biosensor with high sequence specificity. Although the functionality of a DNA/AgNCs-based method has been proven to date with only miRNA biomarkers in cancer cells and tissue samples from cancer patients, and because some of them are also functional in neurodegenerative diseases, the potential of the method is evidently promising for detecting miRNA biomarkers in neurodegenerative diseases.

DISCUSSION

Each of the conventional methods for miRNA detection, such as small RNA blotting, qRT-PCR, and

microarrays, has its own individual drawbacks. For instance, small RNA blotting is labor intensive and requires radioactive-labeling or very complicated DIG-labeling. In general, small RNA blot analysis takes at least 2 days to evaluate the levels of miRNAs in samples, and its sensitivity is relatively lower than other methods. However, this method has a major advantage that reflects the actual levels of miRNAs in samples, as it reads the signals from direct hybridization between a probe and target miRNA. qRT-PCR and microarrays are costly in terms of operational materials and equipment and have non-specific readout problems. The qRT-PCR-based method detects target miRNAs by PCR, and it extensively relies on the success of cDNA synthesis, which is initiated by priming miRNAs with a short adaptor. Failure of the primers to conjugate with the miRNAs could result in false negatives. In the case of microarray analysis, a probe must hybridize with the miRNA in a fixed temperature, even though miRNAs vary in their melting temperatures. This intrinsic limitation often causes readouts to be false positives or false negatives.[99] Therefore, many research groups have extensively investigated ways to build efficacious tools for rapid, simple and specific miRNA detection as alternatives to the conventional methods. To date, many new methods have been suggested, such as nanomaterial-derived methods, non-PCR-based amplification methods, electrochemical methods, etc. The newly developed methods focus on simplicity, lower cost, and better sensitivity and specificity as the common concepts. Here, we have discussed a class of the miRNA detection methods by exploiting the photoluminescent characteristics of AgNCs. Compared to other methods, the AgNCs-based methods have many advantages in monitoring miRNAs, such as the ease of applicability, the extremely low cost of materials, a wide range of fluorescence properties, and the feasibility of both on-and-off signal platforms. Therefore, we suggest that by applying DNA/AgNCs sensor methods, miRNAs biomarkers and their expression profiles in neurodegenerative diseases could be easily investigated without cost or labor problems.

Conflicts of Interest

The authors have no financial conflicts of interest.

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