RESEARCH HIGHLIGHT

Detection of intracellular microRNA using a self-assembling magnetic resonance beacon

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MicroRNAs (miRNAs or miRs) are implicated in several biological processes such as proliferation, differentiation, apoptosis, development and disease. A number of miRNA imaging tools have been developed due to the scientific and clinical significance of miRNA. Current miRNA imaging techniques are based on reporter gene and molecular beacon systems, and most of these techniques utilize fluorescent probes. These techniques have been successfully used to study the function and interaction of miRNAs in the cellular environment. However, they have limited clinical applicability as imaging systems, mainly due to the inability of fluorescent probes to penetrate tissue. Magnetic resonance imaging (MRI) provides high resolution in three dimensions to analyze the anatomy and structure of tissues. We developed a self-assembling magnetic nanoparticle based molecular beacon (miR124a MR beacon) to detect miR124a in mammalian cells and tissues during neuronal differentiation by T2-weighted magnetic resonance (MR). The self-assembled structure of the miR124a MR beacons was induced by incubating 3'-adaptor, 5'-adaptor and miR linker containing the miR124a binding sequence and adaptor binding sequence. When the miR124a expression level was low in cells and tissues, the MR signal of the miR124a MR beacons was quenched. As the concentration of miR124a increased during neuronal differentiation, selective hybridization occurred between miR124a and the miR124a binding sequence of the miR124a MR beacon. This ultimately induced nanoparticle disassembly and a gradual increase in the MR signal. Our findings suggest that this method can be used to monitor the expression of miRNAs, as well as other intracellular genetic and cellular processes, by MR imaging *in vivo*.

Keywords: microRNA; magnetic resonance imaging; self-assembly; magnetic nanoparticle; molecular beacon

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MicroRNAs (miRNAs or miRs) are small noncoding RNAs consisting of 18-25 nucleotides. MiRNAs are clinically important because they simultaneously regulate expression of several genes and have crucial roles in proliferation [1], differentiation [2], development [3], apoptosis [4] and diseases [5]. Therefore, the development of a novel miRNA molecular imaging method would be of great value for practical diagnostic and therapeutic applications.

For conventional miRNA analysis, microarray [6],

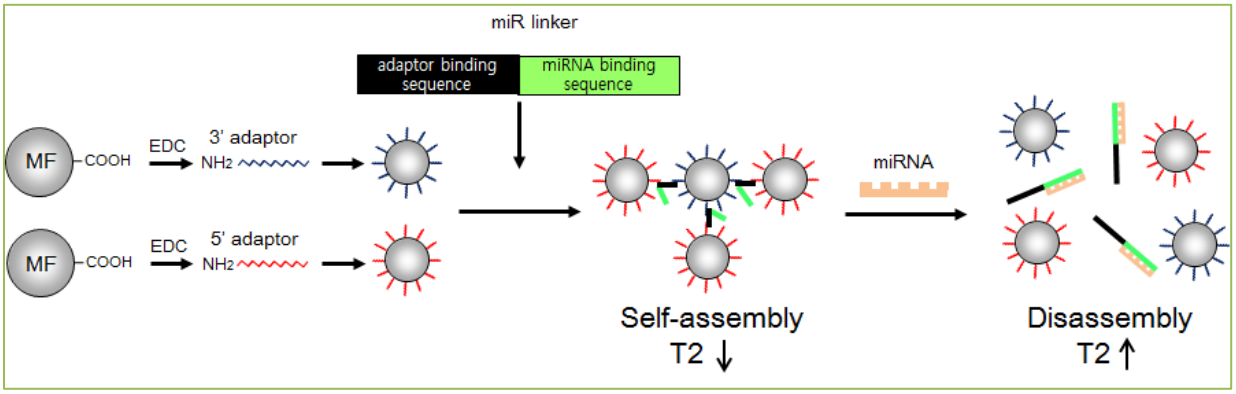

Figure 1. Schematic diagram of the self-assembling miR124a MR beacon for detecting intracellular miR124a expression by MRI. Reprinted with permission [16].

real-time PCR [7], RT-PCR [8], northern blot [9] and in situ hybridization [10] have been used. However, these methods have low sensitivity, are laborious and time-consuming, and require expensive reagents and instruments. The main drawback of these methods is the invasive way of sample preparation and analysis, which requires cell lysis or fixation. During sample preparation, target miRNAs degrade. This makes it impossible to repeat analysis of the same samples or to obtain precise and accurate data from living cells.

For non-invasive miRNA imaging, reporter gene systems have been developed using bioluminescence [11], radioisotope [12], fluorescence [13] and magnetic resonance (MR) [14] techniques. In this system, target miRNA imaging is based on a signal-off system, i.e., the presence of target miRNA reduces the reporter signal. Thus, low target miRNA concentration can produce a false-positive signal from its autofluorescence background. Indeed, low sensitivity and autofluorescence have led to limited *in vivo* applications of reporter gene systems.

Molecular beacon (MB) has been developed as a signal-on strategy for non-invasive miRNA imaging with high sensitivity. MB utilizes a fluorescent probe and a quencher molecule. The signal-on strategy is useful when the target miRNA expression level is low because of its low autofluorescence. However, imaging is achieved by a fluorescence signal. The critical drawback of fluorescence-based imaging is its low penetration in tissue, which results in low signal-to-noise contrast [15]. Thus, MB is not appropriate for clinical applications.

We have developed a new way of using magnetic resonance imaging (MRI) to detect intracellular miRNA by using self-assembling magnetic nanoparticles [16]. MRI provides high resolution and sensitivity for imaging anatomy and tissues and is widely used clinically. Magnetic nanoparticles were employed as the MRI contrast agent, and the MR signal-on was induced by binding of target miRNAs and subsequent nanoparticle disassembly. The target miRNA binding-responsive contrast change has a high potential as a novel MR imaging tool. For imaging and future clinical applications, this work aimed at detecting miR124a, which is highly expressed during neurogenesis [17].

For construction of a miR124a detecting MR beacon (miR124a MR beacon), self-assembling magnetic nanoparticles were prepared using 50-nm carboxyl-group functionalized cobalt ferrite magnetic fluorescence nanoparticles. The miR124a MR beacon self-assembly was initiated by incubation of magnetic nanoparticles with an amine-group functionalized 3'-adaptor or 5'-adaptor and a miR linker containing an adaptor-binding sequence and miR124a-binding sequence at a molar ratio of 1:16:16 (Fig. 1). In brief, 3'- or 5'-adaptor conjugated nanoparticles were hybridized together by a miR linker to form self-assembled nanoparticles. In the absence of target miR124a, the self-assembled nanoparticles did not show any conformational change and generated a low T2 value and a weak MRI signal. However, in the presence of miR124a, the self-assembled nanoparticles hybridized with the miR124a binding sequence of the miR linker. The specific hybridization between the target miR124a and the miR linker induced detachment of the miR linker and nanoparticle disassembly, resulting in MRI intensity signal-on and an increased T2 value.

Transmission electron microscopy (TEM) images showed the target miR124a concentration-dependent disassembly of the miR124 MR beacons (Figure 2A). In the presence of miR124a (500 and 1000 pmol), the miR124a MR beacons disassembled due to the specific hybridization of the target miR124a to the miR linker and subsequent miR linker detachment from the miR124a MR beacons. However, the miR124a mt MR beacon, a negative control containing a mutated miR124a binding sequence in the miR linker, did

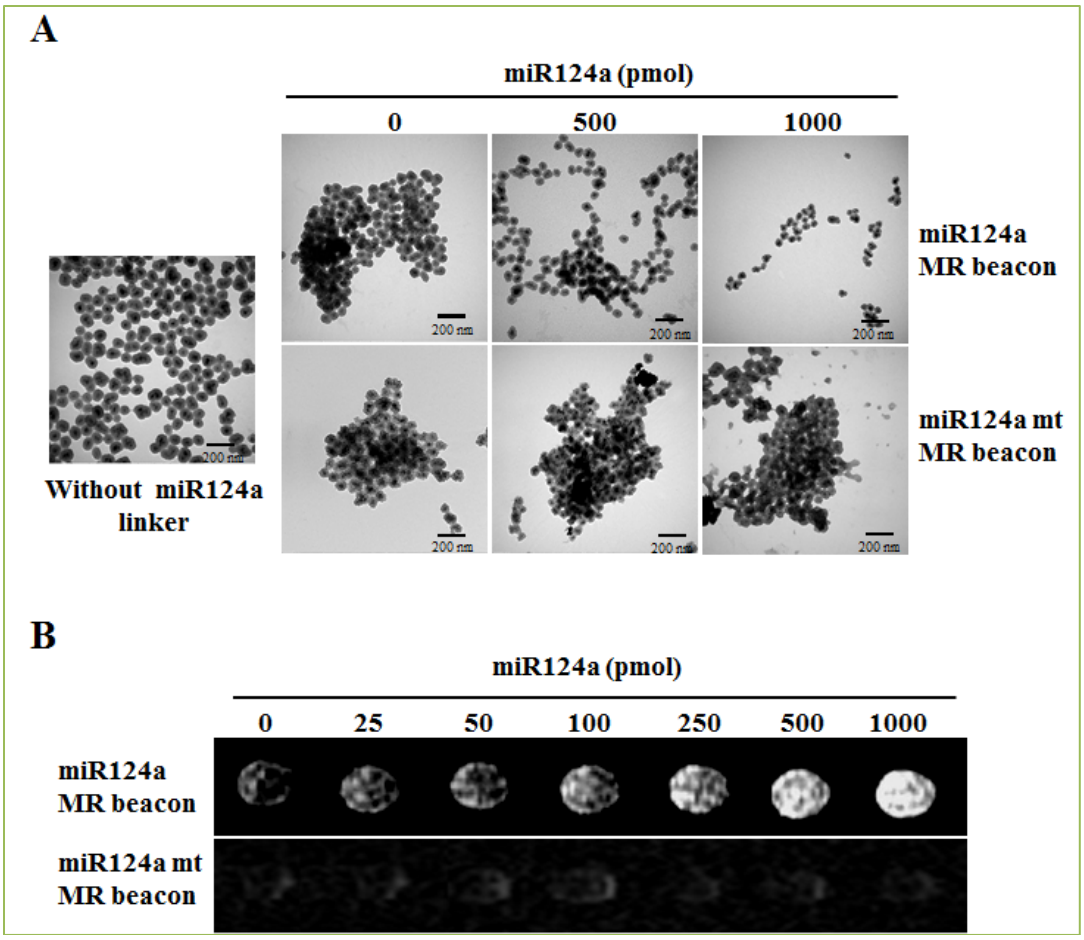

Figure 2. Characterization of the miR124a MR beacons in test tube. (A) TEM images and (B) T2-weighted MR images of the miR124a MR beacons and the miR124a mt MR beacons incubated with different concentrations of exogenous miR124a. Reprinted with permission [16].

not show nanoparticle disassembly. This demonstrates the higher specificity of the designed miR124a binding sequence in the miR124a MR beacon. The T2-weighted MR images of the miR124a MR beacons further demonstrated a miR124a concentration-dependent MR signal increase (Figure 2B). In contrast, the MR signal of the miR124a mt MR beacon showed no response to different miR124a concentrations. These results suggest high specificity of the miR124a MR beacon for detecting miR124a by MR imaging.

The *in vitro* MR imaging of endogenous miR124a expression during neuronal differentiation was confirmed using the mouse embryonic teratocarcinoma P19 cell line. Neuronal differentiation of P19 cells was induced by treatment with retinoic acid (RA). Magnetofection was used to efficiently transfect the miR124a MR beacons into the P19 cells. In undifferentiated P19 cells, the transfected miR124a MR beacons were highly aggregated. During neuronal differentiation, the expression of endogenous miR124a triggered disassembly of the miR124a MR beacons via selective hybridization to the miR124a binding sequence of the miR124a MR beacons. The T2-weighted images showed a gradual increase of the MR signal in P19 cells transfected with the miR124a MR beacons (Figure 3A). In contrast, P19 cells transfected with the miR124a mt MR beacons showed no change in MR signal during neuronal differentiation.

For *in vivo* MR imaging of endogenous miR124a, P19 cells (1×10^6) transfected with the miR124a MR beacons or the miR124a mt MR beacons were incorporated within a poly-L-lactic acid (PLLA) scaffold and subcutaneously implanted into both thighs of nude mice. The right thigh was treated with retinoic acid to induce neuronal differentiation, and the left thigh was used as a untreated control. The T2-weighted MRI signals in both thighs were dark right after implantation of P19 cells (Figure 3B). During neuronal differentiation, the MRI signal of the miR124 MR beacons in the right thigh was increased, whereas those of the miR124a MR beacons in the left thigh and of the miR124a mt MR beacons in both thighs were constant. This demonstrated that the specific MRI signal change in the right thigh came from endogenous miR124a expression in P19 cells during

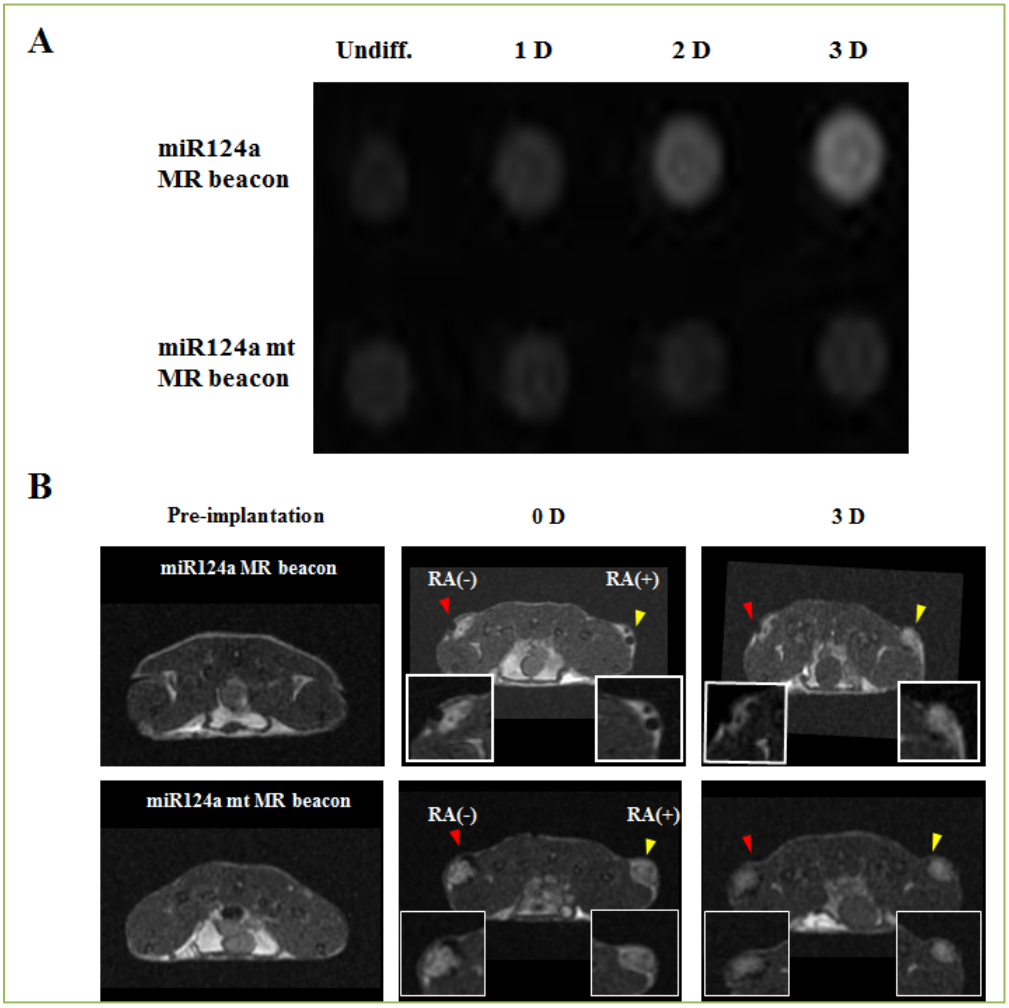

Figure 3. *In vitro* and *in vivo* MR detection of endogenous miR124a expression during neuronal differentiation in P19 cells using the miR124a MR beacons. T2-weighted MRI of the miR124a MR beacons and the miR124a mt MR beacons (A) prior to and during neuronal differentiation of P19 cells and (B) in an undifferentiated state (Undiff.) and 3 days after neuronal differentiation of P19 cells. The P19 cell-scaffold complexes were treated with (+) or without (-) RA to induce neuronal differentiation. Reprinted with permission [16].

neuronal differentiation.

There is a growing need for molecular imaging methods that are noninvasive and have high sensitivity. Reporter gene and MB based imaging systems have successfully been used as imaging tools for miRNA research. However, these methods employ fluorophores and are therefore limited by poor tissue penetration. Thus, there is still a need for a better, highly sensitive method of detecting miRNAs. Our study demonstrates the potential of MRI to track or monitor many miRNAs of interest during differentiation *in vivo*. This is the first report of an *in vivo* MRI method to image intracellular miRNAs during cellular development. Previous MRI methods have been limited with regards to imaging intracellular gene expression because of nonspecific binding of targeting probes, difficulty of target discrimination from surrounding molecules, and phagocytosis of magnetic nanoparticles. Our findings suggest that the self-assembling magnetic resonance beacon method can lead to a new way of miRNA imaging for *in vivo* studies and clinical applications.

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References

1. Dostie J, Mourelatos Z, Yang M, Sharma A, Dreyfuss G. Numerous microRNAs in neuronal cells containing novel micro-RNAs. RNA 2003; 9:180-186.
2. Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, *et al*. The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans. Nature 2000;

403:901-906.

3. Brennecke J, Hipfner DR, Stark A, Russell RB, Cohen SM. Bantam encodes a developmentally regulated microRNA that controls cell proliferation and regulates the proapoptotic gene hid in Drosophila. Cell 2003; 113:25-36.
4. Xu P, Vernooy SY, Guo M, Hay BA. The Drosophila microRNA Mir-14 suppresses cell death and is required for normal fat metabolism. Curr Biol 2003; 13:790-795.
5. Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D. MicroRNA expression profiles classify human cancers. Nature 2005; 435:834-838.
6. Yan LX, Huang XF, Shao Q, Huang MY, Deng L, Wu QL, *et al*. MicroRNA miR-21 overexpression in human breast cancer is associated with advanced clinical stage, lymph node metastasis and patient poor prognosis. RNA 2008; 14:2348-2360.
7. Nikiforova MN, Tseng GC, Steward D, Diorio D, Nikiforov YE. MicroRNA expression profiling of thyroid tumors: biological significance and diagnostic utility. J Clin Endocrinol Metab 2008; 93: 1600-1608.
8. Huang GL, Zhang XH, Guo GL, Huang KT, Yang KY. Shen X, *et al*. Clinical significance of miR-21 expression in breast cancer: SYBR-Green I-based real-time RT-PCR study of invasive ductal carcinoma. Oncol Rep 2009; 21:673-679.
9. Iorio MV, Ferracin N, Liu GG, Veronese A, Spizzo R, Sabbioni S, *et al*. MicroRNA gene expression deregulation in human breast cancer. Cancer Res 2005; 65:7065-7070.
10. Yamamichi N, Shimomura R, Inada KI, Sakurai K, Haraguchi T, Ozaki Y, *et al*. Locked nucleic acid in situ hybridization analysis of miR-21 expression during colorectal cancer development. Clin Cancer Res 2009; 15:4009-4016.
11. Giraldez AJ, Cinalli RM, Glasner ME, Enright AJ, Thomson JM, Baskerville S, *et al*. MicroRNAs regulate brain morphogenesis in zebrafish. Science 2005; 308: 833-838.
12. Lee JY, Kim S, Hwang DW, Jeong JM, Chung J, Lee MC *et al*. Development of a dual-luciferase reporter system for in vivo visualization of microRNA biogenesis and posttranscriptional regulation. J Nucl Med 2008; 49:285-294.
13. Driskell JD, Tripp RA. Label-free SERS detection of microRNA based on affinity for an unmodified silver nanorod array substrate. Chem Comm 2010; 46:3298-3300.
14. Jo M, Jeong MS, Ko HY, Lee CH, Kang WJ, Kim S. Radioisotope imaging of microRNA-9-regulating neurogenesis using sodium iodide sympoter. Biomaterials 2013; 34:4803-4809.
15. Troy T, Jekic-McMullen D, Sambucetti J, Rice B. Quantitative comparison of the sensitivity of detection of fluorescent and bioluminescent reporters in animal models. Mol Imaging 2004; 3:9-23.
16. Lee J, Jin YA, Ko HY, Lee YS, Heo HJ, Cho S, *et al*. Magnetic resonance beacon to detect intracellular microRNA during neurogenesis. Biomaterials 2015; 41:69-78.
17. Hwang DW, Song IC, Lee DS, Kim S. Smart magnetic fluorescent nanoparticle imaging probes to monitor microRNAs. Small 2010; 6:81-88.