Bioimaging of transcriptional activity of microRNA124a during neurogenesis

Hae Young Ko, Jonghwan Lee, Yong Seung Lee, Youngsok Choi, Bahy A. Ali, Abdulaziz A. Al-Khedhairy & Soonhag Kim

Biotechnology Letters

ISSN 0141-5492
Volume 37
Number 11

Biotechnol Lett (2015) 37:2333-2340
DOI 10.1007/s10529-015-1912-3

Springer

Biotechnol Lett (2015) 37:2333–2340
DOI 10.1007/s10529-015-1912-3

ORIGINAL RESEARCH PAPER

Bioimaging of transcriptional activity of microRNA124a during neurogenesis

Hae Young Ko · Jonghwan Lee · Yong Seung Lee · Youngsok Choi · Bahy A. Ali · Abdulaziz A. Al-Khedhairy · Soonhag Kim

Received: 4 May 2015 / Accepted: 9 July 2015 / Published online: 15 July 2015

Abstract
Objectives A special vector system was developed to monitor the in vitro and in vivo endogenous level of a primary transcript of miR124a during neuronal differentiation
Results The upstream regions of miR124a were fused with luciferase (Gluc) and their activity was measured. During neurogenesis of P19 cells, the primary transcript level of miR124a was increased 1.5-times compared to the undifferentiated P19 cells. P19 cells grafted to nude mice exhibited the same pattern of luciferase activity in vivo as they did in vitro.
Conclusion The expression of primary miR124a during neurogenesis was successfully imaged by in vitro and in vivo luciferase reporter gene-based method.

Keywords Bioluminescence imaging · In vivo imaging · MicroRNA124a · Neurogenesis · Promoter

Introduction

MicroRNAs (miRNAs or miRs) are single-stranded, non-coding RNA sequences comprised of 18–25 nucleotides (nt). They are found in animals, plants, microorganisms and viruses (Pfeffer et al. 2004).

Hae Young Ko and Jonghwan Lee contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s10529-015-1912-3) contains supplementary material, which is available to authorized users.

H. Y. Ko · J. Lee · Y. S. Lee · S. Kim (✉)
Institute for Bio-Medical Convergence, College of Medicine, Catholic Kwandong University, Gangneung-si, Gangwon-do 270-701, Republic of Korea
e-mail: kimsoonhag@empal.com

H. Y. Ko · J. Lee · Y. S. Lee · S. Kim
Catholic Kwandong University International St. Mary's Hospital, Incheon Metropolitan City 404-834, Republic of Korea

Y. Choi
Department of Biomedical Science, CHA Unversity, 335 Pangyo-ro, Bundang-gu, Seongnam-si, Gyeonggi-do 463-400, Republic of Korea

B. A. Ali
Al-Jeraisy DNA Research Chair, Department of Zoology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia

B. A. Ali
Department of Nucleic Acids Research, Genetic Engineering and Biotechnology Research Institute, City for Scientific Research and Technological Applications, Alexandria 21934, Egypt

A. A. Al-Khedhairy
Department of Zoology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia

MiRNAs are first produced as a form of primary miRNA (pri-miRNA) with a hairpin structure in the nucleus. Approximately 70-nt precursor miRNAs (pre-miRNAs) are formed in the nucleus by the cleavage of pri-miRNAs by the nuclease Drosha (Lee et al. 2003). Pre-miRNAs are translocated to the cytoplasm by exportin-5 and cleaved by Dicer to single-stranded mature miRNA. In general, mature miRNAs are combined by the RNA-induced silencing complex (RISC) and bind to the 3′-untranslated region (UTR) of their target mRNAs. This binding leads to either mRNA cleavage or translational inhibition (Hutvagner and Zamore 2002).

Diverse miRNAs, such as miR9, miR9*, miR124a, miR134, miR23a, miR132 and miR128 have been identified in mammalian neurons and appear to be regulated during neuronal differentiation (Smirnova et al. 2005). In particular, miR124a is expressed at very low or undetectable levels in neural progenitors, but is highly expressed in mature or differentiating neurons, and is associated with neuronal differentiation (Makeyev et al. 2007). miR124a has been used as a biomarker for neurogenesis of P19 cells (mouse embryonic carcinoma cells) in non-invasive imaging systems (Lee et al. 2015).

Generally, molecular regulation of endogenous miRNAs has been studied by northern blotting, in situ hybridization, and real-time RT-PCR. However, these methods are not entirely reproducible and have limited clinical applications because they require lysing or fixation of samples, which can lead to miRNA degradation. Bioluminescent reporter protein-based optical imaging techniques offer non-invasive and repeatable imaging of stem cells and tumors in living animals (Ottobrini et al. 2006). For bioluminescent imaging of living small animals, Renilla luciferase (Rluc), Gaussia luciferase (Gluc) and Firefly luciferase (Fluc) are commonly used as reporter proteins. Rluc and Gluc react with colenterazine as a substrate and emit light maximally at 480 nm with a wide spectrum extending to 600 nm. Fluc emits light maximally at 562 nm by the oxidation of its substrate, D-luciferine. Compared with Rluc and Fluc, the intensity of the reaction with Gluc is about 1000-fold greater. These luciferase systems have been applied to image miRNA expression both in vitro and in vivo (Ko et al. 2008; Lee et al. 2008; Kim et al. 2008; Yoo et al. 2014).

miRNA expression is mainly regulated at the transcription level by RNA polymerase II and other transcription factors (Cullen 2004; Libri et al. 2013). Controlling miRNA expression at the transcription level offers a useful way to study the mechanisms and pathways of miRNA biogenesis, cellular development and miRNA-related diseases. However, no studies to date have investigated the promoter activity and expression level of pri-miRNA transcripts. In this study, we have investigated the gene expression pattern of pri-miR124a during neurogenesis of P19 cells using the luciferase (Gluc) reporter gene. The upstream regions of miR124a were fused with Gluc. Highly expressed pri-miR124a was monitored during neurogenesis by in vitro and in vivo luciferase reporter gene assays.

Materials and methods

Construction and analysis of a promoter-fused reporter gene

Five nested deletion fragments of the upstream region of miR124a1 from X to +39 bp (the first nucleotide in the 5′ end of pre-miR-124a1 was defined as +1) were cloned by PCR and inserted into a pGLuc-Basic vector containing promoterless Gaussia luciferase (Targeting Systems, San Diego, CA, USA). The amplified fragments were first cloned into the T/A cloning vector pCR2.1 (Invitrogen, Grand Island, NY, USA), and then sequenced. The orientation of the promoter fragments at the EcoRI sites of pGLuc-Basic was determined by sequencing analysis to drive Gluc activity. The resulting plasmids were designated miR124a1P1084/Gluc (−1045 to +39 bp), miR124a1P893/Gluc (−854 to +39 bp), miR124a1P692/Gluc (−653 to +39 bp), miR124a1P379/Gluc (−340 to +39 bp) and miR124a1P116/Gluc (−77 to +39 bp). These plasmids were then transfected into P19 cells using lipofectamine and Plus reagent (Invitrogen), and Gluc activity was measured according to the manufacturer's protocol during neuronal differentiation. The amount of cell extract in each luciferase assay was calibrated to be equal to protein content using the Bradford protein assay.

Cell culture

HeLa cells (a cervical carcinoma cell line) were cultured in RPMI supplemented with 10 % (v/v) fetal bovine serum (FBS) and 1 % (w/v) penicillin/streptomycin at 37 °C. P19 cells were from ATCC, and cultured in α-MEM supplemented with 7.5 % (v/v) bovine calf serum 2.5 % (v/v) FBS, and 1 % penicillin/streptomycin solution at 37 °C in a 5 % CO_2-humidified chamber. To induce neurogenesis, P19 cells were seeded on gelatin-coated culture dishes at $5 \times 10^3/cm^2$ and cultured under serum-free conditions in DMEM/12 (1:1) media supplemented with 1 % penicillin/streptomycin and 1 % insulin-transferrin-selenium (Gibco). After adhesion of the cells, P19 cells were treated with 5×10^{-7} M all-*trans*-retinoic acid (RA). Two days after RA-treatment, the RA was removed from the culture medium and the cells were further cultured for three days under serum-free conditions.

Transfection and luciferase assay

HeLa cells and P19 cells were seeded at 5×10^5 per well in 24-well plates one day prior to transfection. Transient transfection of each plasmid was conducted using 0.6 μg DNA, 3 μl Plus reagent and 1.5 μl Lipofectamine per well. After 3 h, the transfection media was replaced with culture media. After two days, cells were washed three times with PBS and lysed with 200 μl/well of passive lysis buffer. Next, 100 μl cell lysate was utilized for the Gaussia luciferase assay kit (Targeting Systems) according to the manufacturer's instructions. Luciferase activity was then measured using a Wallac 1420 VICTOR 3 V plate reader (PerkinElmer). The data are presented as the mean ± standard error of the mean (SEM) calculated from triplicate wells.

RT-PCR (reverse transcription PCR)

Total RNA was isolated from P19 cells each day after RT treatment using TRIzol. For cDNA synthesis, reverse transcription was carried out using random-hexamers and SuperScript II reverse transcriptase (Invitrogen) in 20 μl. Taq polymerase was used to amplify 1 μl cDNA (25-30 cycles). The primer sequences are presented in Supplementary Table 1.

qRT-PCR (quantitative reverse transcription-PCR) of mature miR124a

The *mir*Vana miRNA isolation kit (Ambion, Austin, TX, USA) was used to isolate small RNA during neurogenesis of P19 cells. qRT-PCR was conducted using the *mir*Vana qRT-PCR primer Set (Ambion) and *mir*Vana qRT-PCR miRNA detection kits (Ambion). The PCRs were conducted in triplicate using iCycer (Bio-Rad, CA, USA) with SYBR Premix Ex Taq (2x) (Takara, Japan) according to following conditions: 95 °C for 3 min and 40 cycles (95 °C for 15 s, 60 °C for 30 s). The relative amounts of mature miR124a were normalized versus the U6 snRNA primer set (Ambion, USA) using the equation $2^{-\Delta\Delta CT}$, where $\Delta CT = (C_{TmiRNA} - C_{TU6RNA})$, $\Delta\Delta C_T = (\Delta C_T - \Delta C_{TmiRNA\ of\ P19\ cell})$.

Grafting of cells with reporter gene constructs into nude mice and in vivo visualization of miR124a

P19 cells were transiently transfected with miR124a 1P379/Gluc. After 48 h transfection, 2.5×10^6 cells were resuspended in 100 μl PBS and implanted subcutaneously into the flanks of male Balb/c nude mice (6 weeks old, 25–27 g). Cells containing miR124a1P379/Gluc were implanted in both thighs. The right thigh was treated with RA simultaneously with cell injection for neuronal differentiation evaluation, while the left thigh was not treated with RA and was used as a negative control. Three mice in each experiment group were anesthetized with isofluorane, and then transferred into the light-tight chamber of an IVIS 100 imaging system (Xenogen, CA, USA). To obtain images of Gluc, mice were injected with 5 μg of coelenterazine. The Gluc images were acquired 2 min later. Region of interest (ROI) analysis was performed for a quantitative evaluation of the signals from all mice.

Statistical analysis

Data are displayed as mean ± standard deviations (SD) and differences were evaluated using the student's *t* test (*$P < 0.05$ and **$P < 0.01$).

Results

Neuronal-specific expression pattern of three different primary miR124as during neurogenesis

Bioinformatic analysis of the genomic context for intergenic miR124a showed that mature miR124a might originate from three different loci in human, mouse, rat and opossum species. In the case of the mouse, mature miR124a can be produced from chromosome 14D1 (miR124a1), chromosome 3A2 (miR124a2) and chromosome 2H4 (miR124a3) (Fig. 1a).

To monitor gene expression of the primary transcripts of miR124a from three different loci during neurogenesis of P19 cells, a sequence-specific RT-PCR analysis was conducted. Sequences around the pri-miR124a from three different loci were aligned to identify nucleotide polymorphisms. PCR primers were designed at the polymorphic sites to match each sequence without amplifying any alternative sequences. In this design, the forward primer had partial homology among three different pri-miR124as. In addition, each reverse primer could only amplify a specific pri-miR124a (Fig. 1a). Up-regulation of the neuronal marker gene, MAP2, and down-regulation of the stem cell marker gene, Oct4, indicated that standard protocol RA treatment of P19 cells reliably induced neuronal differentiation (Fig. 1b). The RT-PCR analysis of primary miR124a1 (pri-miR124a1), primary miR124a2 (pri-miR124a2) and primary miR124a3 (pri-miR124a3) showed a similar expression pattern that increased gradually after induction of neuronal differentiation and peaked at the fourth day after RA treatment. Unlike the other two primary transcripts, the expression of pri-miR124a3 showed a sudden drop-off after reaching its peak level.

High expression of mature miR124a during neurogenesis

Intergenic mature miR124a is a small sequence of RNA composed of 18 ~ 25 nucleotides and is well conserved from humans to aquatic species. To confirm endogenous expression levels of mature miR124a during neurogenesis of P19 cells, real-time RT-PCR was conducted using cDNA synthesized from isolated small RNA of differentiated P19 cells. The concentrations of mature miR124a for each differentiated day were normalized to U6 snRNA. Similar to the endogenous expression of pri-miR124a, the expression pattern of mature miR124a showed a gradually increase during neurogenesis, up to more than 10-fold after RA treatment (Fig. 2).

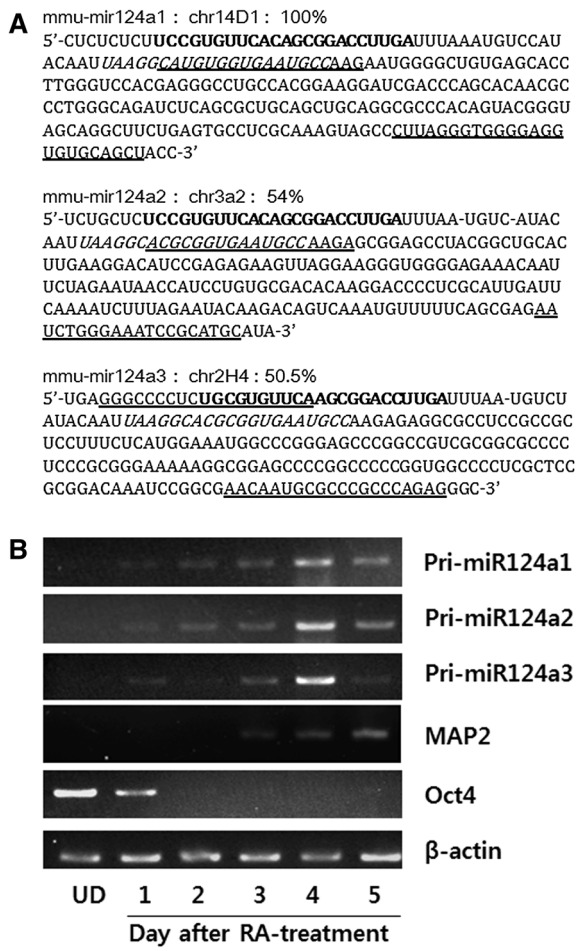

Fig. 1 Sequences and gene expression of three different primary transcripts of miR124a. **a** Three types of primary miR124a, located in different mouse chromosomes (miR124a1: Chromosome14D1, miR124a2: Chromosome 3A2, miR124a3: Chromosome.2H4). Mature sequences of miR124a are highlighted by *bold letters* and complementary paired sequences are italicized. *Underlining* indicates the location of the primer used to amplify each primary miR124a transcript. The percentage of sequence homology of pri-miR124a2 and pri-miR124a3 with pri-miR124a1 is provided next to the chromosome number. **b** RT-PCR analysis of primary miR124a1, a2 and a3 during neurogenesis of P19 cells. (*UD* undifferentiated P19 cells)

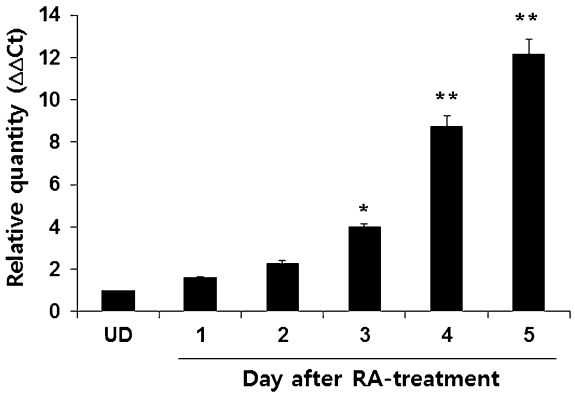

Fig. 2 Expression of mature miR124a during neurogenesis of P19 cells. qRT-PCR analysis of the expression of mature miR124a. Data are displayed as mean ± SD of triplicate samples (*$P < 0.05$ and **$P < 0.01$). (*UD* undifferentiated P19 cells)

In vitro luciferase assay of the miR124a1 promoter

To monitor gene expression of pri-miR124a transcripts in vitro and in vivo, pri-miR124a1 was randomly chosen and cloned about 1.1 kb upstream of miR-124a1. The 5 fragments of the upstream region were fused to a promoterless reporter gene Gluc and further analyzed (Fig. 3a). The five gene constructs containing five different regions of the promoter, −1045 to +39 (miR124a1P1084/Gluc), −854 to +39 (miR124a1P893/Gluc), −653 to +39 (miR124a 1P692/Gluc), −340 to +39 (miR124a1P379/Gluc) and −77 to +39 bp (miR124a1P116/Gluc), were transfected into P19 cells and neurogenesis was induced by RA treatment. Promoter activity was determined by comparing luciferase activity during neurogenesis of P19 cells. In all gene constructs, the expression level of luciferase was higher than that of the negative control, the pGLuc-basic vector. A similar expression pattern of luciferase from the 5 gene constructs was found, where luciferase activity was relatively strong on the second and third day after induction of neuronal differentiation and decreased thereafter (Fig. 3b). Gluc expression of miR12 4a1P379/Gluc was significantly higher than it was in the other constructs. Gluc activity from miR12 4a1P379/Gluc showed an approx. 1.5-fold increase after the third day of neuronal differentiation compared to the undifferentiated Gluc expression of miR124a1P379/Gluc, reflecting the increased endogenous level of the pri-miR124a1 transcript. A further deletion to −77 bp almost completely abolished the promoter activity, indicating a minimal core promoter element of miR124a1. The excellent promoter activity of miR124a1P379/Gluc implies that negative promoter elements, such as neuronal repressors, may be present between −653 and −340 bp, and neuronal differentiation-related positive elements may be present between −340 and −77 bp.

In addition, no differences in the promoter activity of miR124a1P1084/Gluc further suggest there are additional unknown factors between −1045 and −653 bp. The five fragments of the upstream region of the pri-miR124a2 were also fused to a promoterless reporter gene Gluc and transfected into P19 cells and neurogenesis was induced by RA treatment. However, in all gene constructs, the expression level of luciferase was not significant different from that of the pGLuc-basic vector before and after neurogenesis of P19 cells, indicating that the cloned upstream region of the pri-miR124a2 did not have the capacity to visualize the transcriptional activity of pri-miR124a during neurogenesis (Supplementary Fig. 1). Accordingly, a miR124a1P379/Gluc construct would be good for further in vivo imaging of gene expression of the pri-miR124a1 during neurogenesis.

In vivo imaging of primary miR124a1 during neuronal differentiation of P19 cells

To monitor in vivo endogenous expression levels of pri-miR124a1 during neurogenesis, mice were grafted with P19 cells co-transfected with miR12 4a1P379/Gluc and Fluc regulated by a CMV promoter (CMV/Fluc, internal control), and imaged for 2 days (Fig. 3c). Gluc activity of miR124a1P379/Gluc after treatment with RA, from the right thigh, was significantly higher during neuronal differentiation of P19 cells compared with that of the left thigh without RA treatment. Fluc activity of CMV/Fluc was constant, irrespective of RA treatment as the positive control but, due to the transient transfection, Fluc activity decreased over time. The fold ratio of the ROI analysis of Gluc activity of both thighs showed nearly a 2-fold increase of promoter-directed Gluc activity regulated by neuronal differentiation, representing an increased level of the pri-miR124a1 transcript (Fig. 3d).

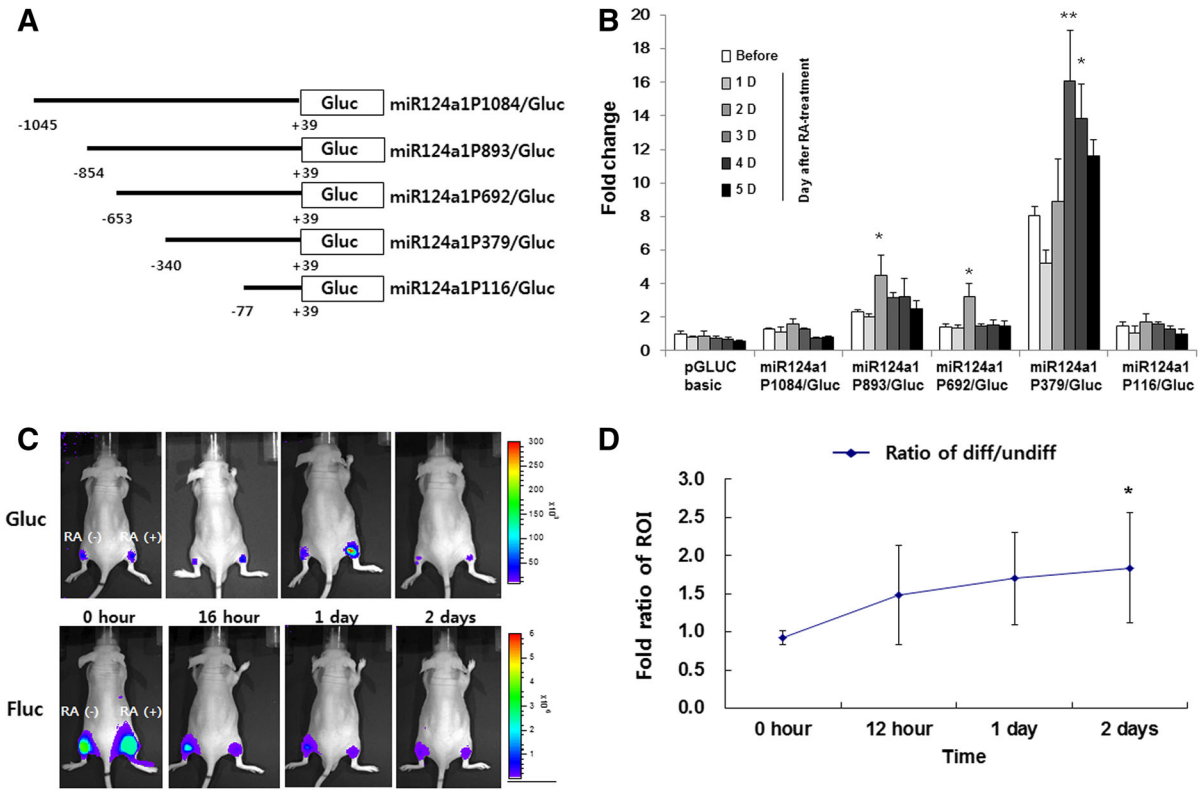

Fig. 3 In vitro and in vivo expression of pri-miR124a1 during neuronal differentiation. **a** Schematic diagram of promoter-fused reporter gene constructs. Five different fragments of the miR124a1 upstream region were inserted into a promoterless vector, pGLuc-basic. The promoter size of each construct is indicated by the number in the name of each construct. **b** Promoter analysis of pri-miR124a1. **c** Bioluminescence image of the expression of pri-miR124a1 in nude mice. **d** ROI analysis of bioluminescence image. Data are expressed as the mean ± SD in triplicate samples (*$P < 0.05$; **$P < 0.01$)

Discussion

MiRNAs are evolutionally well conserved across species and associated with various diseases. Neuronal-specific miRNAs such as miR9, miR124a, miR125b, miR128, miR131, and miR178 have been shown to be highly expressed during brain development. Neuronal miRNAs interact with transcription factors including REST (repressor element silencing transcription factor), CREB (cAMP response element-binding protein), Nanog and Oct4. However, no previous studies have examined miRNAs to determine the gene expression of pri-miRNA transcripts using upstream promoter regions. These genomic regions are important for coding and non-coding gene transcription activities, which are regulated by tissue or environmental stimuli-specific transcription factors and RNA polymerase II, except for the miR23a ~ 27a ~ 24-2 cluster, wherein the promoters are transcribed by RNA polymerase II. Previously, studies have demonstrated in vitro and in vivo imaging of the pri-miR23a transcript (Lee et al. 2008).

In this study, promoter analysis indicated that the upstream region of pri-miR124a1 transcripts, –340 to +39, showed strong promoter activity. This finding was confirmed by a gradual increase in pri-miR124a expression during neurogenesis of P19 cells. Other constructs containing three longer fragments produced very weak Gluc signals, likely due to negative promoter elements in the −653- and −340-bp regions. The promoter was analyzed to identify the transcription factor binding site using the JASPAR program (http://jaspar.binf.ku.dk/) (Supplementary Fig. 2). Two putative transcription factor binding sites for paired-related homeodomain protein (Prx) and upstream stimulatory factor (USF) were found between positions −448 and −470. Prx family members Prx1 and Prx2 contain the homeobox domain and

play a role in development during embryogenesis (Susa et al. 2012). USF transcription factor recognizes and binds to symmetrical DNA sequences (E-boxes) (5′-CACGTG-3′) that are frequently found in several viral and cellular promoters. The functional role of Prx1 and Prx2 is unclear, but USF family members USF1 and USF2 are known to be transcriptional repressors that regulate the intestinal monocarboxylate transporter 1 promoter (Hadjiagapiou et al. 2005). The specificity of putative binding sites for Prx and USF family members requires further study.

The miRNAMap genome database of humans, mice and other species using experimental miRNA clones shows tens of miRNAs with multiple copies throughout the genome. The pri-miRNA from each locus producing the same mature miRNA has its own upstream region with regulation by promoter for intergenic miRNAs or enhancer-like regulator for intragenic miRNAs. This arrangement suggests that different pri-miRNA transcripts of a mature miRNA may regulate at different times and/or in different cellular spaces. For example, two different types of pri-miR1, pri-miR1-1 and pri-miR1-2, are expressed in different regions of the heart. In the hearts of transgenic mice after embryonic day 8.5, pri-miR1-1 was strongly expressed in the inner curvature of the looping heart tube and atria while pri-miR1-2 had high expression in embryonic ventricles, but not the atria. The three different pri-miR124a transcripts showed a similar expression pattern of gradual increase of neuronal differentiation over time of P19 cells treated by RA. However, pri-miR124a1 and pri-miR124a2 showed a stronger and more similar expression pattern compared to pri-miR124a3. These variations of expression level among the three different types of pri-miR124a indicate that they have their own regulatory systems involved in producing mature miR124a. Moreover, compared with the expression pattern of three types of pri-miR124a, the expression level of mature miR124a constantly increased in a time-dependent manner without decline. Such discrepancy in expression levels between primary and mature miRNAs is induced by an unknown mechanism associated with posttranscriptional regulation (Slezak-Prochazka et al. 2010; Newman and Hammond. 2010).

Both in vitro and in vivo luciferase reporter systems of miR124a1P379/Gluc successfully and specifically monitored a PCR-confirmed increased level of endogenous primary transcript of miR124a during neurogenesis of P19 cells. Unfortunately, after 3 days of neurogenesis, the in vivo Gluc activity was no longer retained. For long-term and noninvasive imaging of miR124a biogenesis, a stabilized cell line or viral vectors are needed to capture dynamic changes of miR124a-mediated neurogenesis.

Conclusion

We developed a luciferase reporter gene system to monitor the endogenous level of primary transcript of miR124a during neurogenesis of P19 cells in vitro and in vivo. The upstream promoter regions of the miR124a1 were used to construct a promoter-fused Gluc reporter vector. Molecular imaging of endogenously expressed pri-miR124a was achieved using a special vector system. This method can be used to study the regulation pathways of miRNA biogenesis and miRNA-mediated mechanisms.

Acknowledgments This work was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Korean government (MEST) (No. 2011-0019270 and No. 2013R1A2A2A01068140), the Next-Generation BioGreen 21 program (#PJ010002), Rural Development Administration and a grant of the Korean Health Technology R&D Project, Ministry of Health & Welfare (HI14C3297), and the Visiting Professor Program (VPP) at King Saud University, Kingdom of Saudi Arabia.

Supporting information Supplementary Table 1—A list of primer sequences.

Supplementary Fig. 1—In vitro expression of pri-miR124a2 during neuronal differentiation.

Supplementary Fig. 2—Analysis of miR124a promoter.

References

Cullen BR (2004) Transcription and processing of human microRNA precursors. Mol Cell 16:861–865

Furuse Y, Finethy R, Saka HA, Xet-Mull AM, Sisk DM, Smith KL, Lee S, Coers J, Valdivia RH, Tobin DM, Cullen BR (2014) Search for microRNAs expressed by intracellular bacterial pathogens in infected mammalian cells. PLoS ONE 9:e106434

Hadjiagapiou C, Borthakur A, Dahdal RY, Gill RK, Malakooti J, Ramaswamy K, Dudeja PK (2005) Role of USF1 and USF2 as potential repressor proteins for human intestinal

monocarboxylate transporter 1 promoter. Am J Physiol Gastrointest Liver Physiol 288:G1118–G1126

Hutvagner G, Zamore PD (2002) A microRNA in a multiple-turnover RNAi enzyme complex. Science 297:2056–2060

Lee Y, Ahn C, Han J, Choi H, Kim J, Yim J, Lee J, Provost P, Rådmark O, Kim S, Kim VN (2003) The nuclear RNase III Drosha initiates microRNA processing. Nature 425: 415–419

Lee JY, Kim S, Hwang DW, Jeong JM, Chung JK, Lee MC, Lee DS (2008) Development of a dual-luciferase reporter system for in vivo visualization of MicroRNA biogenesis and posttranscriptional regulation. J Nucl Med 49:285–294

Lee J, Jin YA, Ko HY, Lee YS, Heo H, Cho S, Kim S (2015) Magnetic resonance beacon to detect intracellular microRNA during neurogenesis. Biomaterials 41:69–78

Libri V, Miesen P, van Rij RP, Buck AH (2013) Regulation of microRNA biogenesis and turnover by animals and their viruses. Cell Mol Life Sci 70:3525–3544

Makeyev EV, Zhang J, Carrasco MA, Maniatis T (2007) The MicroRNA miR-124 promotes neuronal differentiation by triggering brain-specific alternative pre-mRNA splicing. Mol Cell 27:435–448

Newman MA, Hammond SM (2010) Emerging paradigms of regulated microRNA processing. Genes Dev 24: 1086–1092

Ottobrini L, Ciana P, Biserni A, Lucignani G, Maggi A (2006) Molecular imaging: a new way to study molecular processes in vivo. Mol Cell Endocrinol 246:69–75

Pfeffer S, Zavolan M, Grässer FA, Chien M, Russo JJ, Ju J, John B, Enright AJ, Marks D, Sander C, Tuschl T (2004) Identification of virus-encoded microRNAs. Science 304: 734–736

Slezak-Prochazka I, Durmus S, Kroesen BJ, van den Berg A (2010) MicroRNAs, macrocontrol: regulation of miRNA processing. RNA 16:1087–1095

Smirnova L, Gräfe A, Seiler A, Schumacher S, Nitsch R, Wulczyn FG (2005) Regulation of miRNA expression during neural cell specification. Eur J Neurosci 21: 1469–1477

Susa T, Kato T, Yoshida S, Yako H, Higuchi M, Kato Y (2012) Paired-related homeodomain proteins Prx1 and Prx2 are expressed in embryonic pituitary stem/progenitor cells and may be involved in the early stage of pituitary differentiation. J Neuroendocrinol 24:1201–1212