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Differential expression of components of the microRNA machinery during mouse organogenesis

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Abstract

MicroRNA (miRNA)-mediated gene silencing has recently emerged as a major mechanism of gene expression regulation during development in a variety of species. Little is known, however, about the presence of components of miRNA machinery in mammalian organogenesis. In this study, we report that members of the *Argonaute* (*Ago*) gene family are expressed in restricted of the day 11.5 and 14.5 embryo, including the brain, neural tube, limb, lungs, and hair follicles. In the developing lung, we found expression of *Ago1* and *Ago2* localized to branching regions, in distal epithelium and mesenchyme, respectively. These were sites undergoing the most dynamic changes in gene expression and rapid remodeling. We show that *Ago1* transcripts are enriched in neural structures at these stages, consistent with the reported role of *Drosophila Ago1* in the development of the central nervous system. Our results suggest a role for miRNAs in organogenesis.

Keywords: MicroRNA; Organogenesis; *Argonaute*; *Dicer*; Lung development

MicroRNAs (miRNAs) are 21 to 22-nucleotide RNAs that are transcribed by RNA polymerase II as large precursor non-protein-coding transcripts, sometimes from intronic regions of a known gene [17,10]. The precursor is processed into a stem–loop intermediate of about 70 bp, and then to the mature miRNA by Drosha, Dicer, and other currently uncharacterized components of the RNA interference (RNAi) machinery [8,9]. The mature miRNAs are incorporated into a multi-protein complex called RNA-induced silencing complex (RISC), which guides the specific translational repression, or mRNA cleavage of their target mRNAs [5,14,4,15].

Deficiency in the components of miRNA-mediated gene silencing machinery in animals or plants results in developmental abnormalities. *Drosophila Dicer1* mutants have small eyes with defective optical structures [11]. Interestingly, *Caenorhabditis elegans Dicer* mutant has defects in developmental timing [7]. In *Arabidopsis*, mutation of the *Dicer* homolog, *carpel factory* (*caf*), results in defects in floral determination as well as in carpel leaf and floral morphogenesis [16]. In zebrafish, *Dicer* deficiency also results in early developmental arrest [20]. Knocking out *Dicer1* in mice results in early embryonic lethality and depletion of stem cells [1]. The defects observed in *Ago* deficient animals or plants also strongly suggest a role for miRNAs in development. *Drosophila Ago1* mutants have central nervous system abnormalities [6]. In *C. elegans*, deficiency in *alg-1* or *alg-2* mimics the phenotype of *lin-4* and *let-7* by displaying abnormal developmental timing [3]. *Arabidopsis Ago1* is required for leaf morphogenesis [2], and expression of *Ago1* is under the control of the miRNA *mir-168*. In plants, suppression of AGO1 expression by *mir-168* is required for proper plant development [18]. Most recently, it

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has been reported that *Ago2*-null mice die early in development, and show severe neural tube defects [12]. Little is known about the expression of *Ago* and *Dicer* during mammalian organogenesis. Here we show that these genes are differentially transcribed in several developing structures of the E11.5–14.5 embryo. Our results suggest that miRNA-mediated mechanisms are at work at sites undergoing dynamic changes in gene expression and rapid remodeling.

Materials and methods

In situ hybridization. Whole mount in situ hybridization was performed in 96-well plates in freshly isolated E11.5 lungs and E11.5–14.5 embryos, as previously described [19,13]. Isotopic in situ hybridization was performed according to protocols detailed in [21]. The DNA templates for the labeling of RNA probes were generated by PCR, using GenBank sequences as described [13]: *Ago1*, NM_153403; *Ago2*, NM_153178; *Ago3*, NM_153402; *Ago4*, NM_153177; *Dicer1*, NM_148948. The primers containing T7 or T3 promoters sequences used to generate the DNA templates for probes were:

Ago1:
5′ primer: AATTAACCCTCACTAAAGGGGCCATTCGAGATGCATGCAT
3′ primer: TAATACGACTCACTATAGGGCCATTGCTTTGCCCTGATAT
Ago2:
5′ primer: AATTAACCCTCACTAAAGGGTATCAGCCAGGAATCACGTT
3′ primer: TAATACGACTCACTATAGGGGGTCAAGCAAAGTACATGGT
Ago3:
5′ primer: AATTAACCCTCACTAAAGGGGTCCTTCACACTATCATGTT
3′ primer: TAATACGACTCACTATAGGGTTGTCACGGAGAATAAACTT
Ago4:
5′ primer: AATTAACCCTCACTAAAGGGCCTCTGTAGTCATGCAGGAA
3′ primer: TAATACGACTCACTATAGGGGAGGATACGTGAGATGCCAA
Dicer1:
5′ primer: AATTAACCCTCACTAAAGGGTCCGATGATGCAGCCTCTAA
3′ primer: TAATACGACTCACTATAGGGCTGTTCTCTCTCAGCCCGAA

Results and discussion

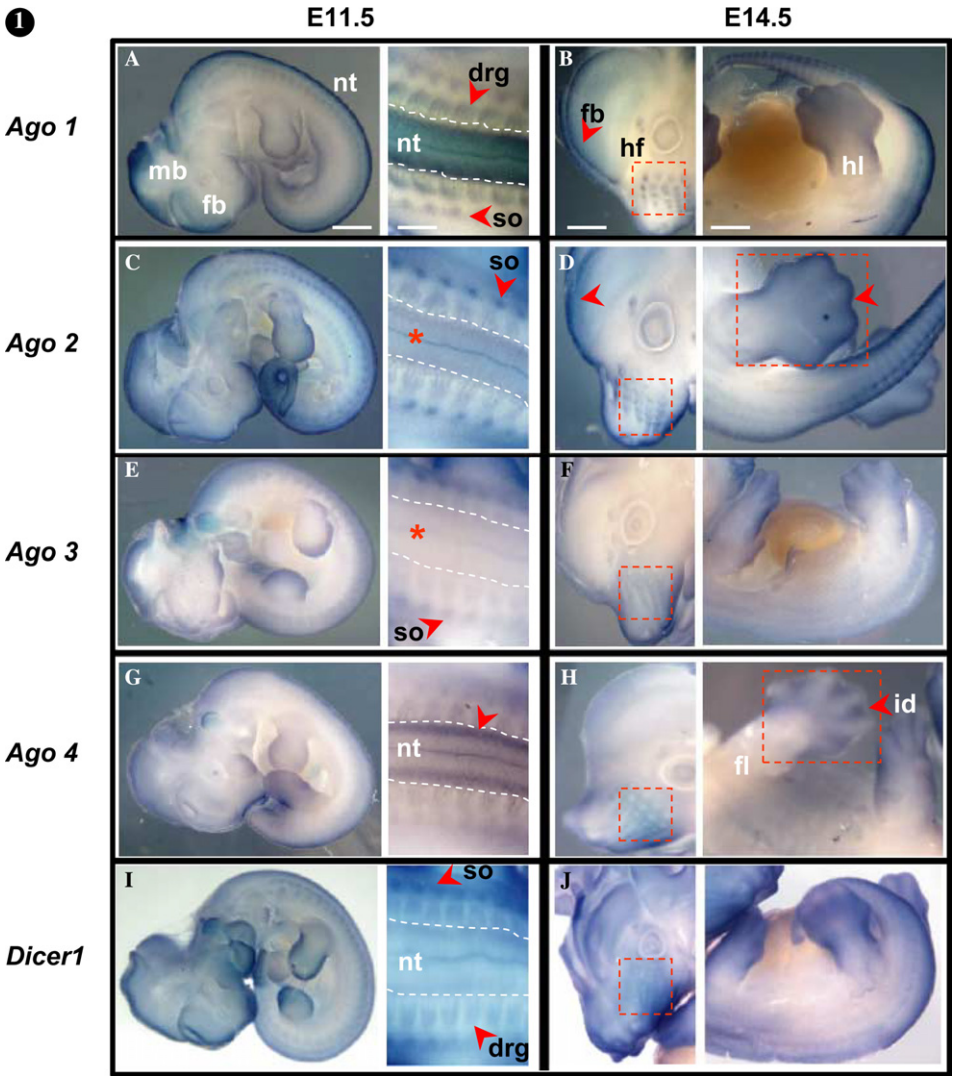
We assessed the expression of *Ago1–4* and *Dicer1* in E11.5 and E14.5 embryos by whole mount in situ hybridization. Our analysis revealed defined patterns of expression of these components in the embryo, in some cases with region-specific distribution of transcripts within an organ. For example, at E11.5, the neural tube showed only low levels of expression of *Ago2* and *3* transcripts (Figs. 1C and E). By contrast, this structure strongly expressed *Ago1* and *Ago4* (Figs. 1A and G). While *Ago4* signals were restricted to the lateral column of neurons, *Ago1* was present in the medial column and dorsal root ganglia. The latter was further confirmed by isotopic in situ hybridization and is shown in Figs. 2A and B. *Ago1* is also strongly expressed in the neuroepithelium of the forebrain and midbrain (Figs. 1A and 2A). The highly enriched expression of *Ago1* in E11.5 neural structures may be functionally relevant, since in *Drosophila*, *Ago1* is required for central nervous system development [6]. Differential expression of *Ago* family members was also observed in the developing limb. As shown in Figs. 1A, C, E, and I, *Ago1*, *2*, *3*, and *Dicer1* were present in the E11.5 limb bud. *Ago 4*, however, was typically seen at E14.5 in the interdigital region of the limb. These contrasted with the more or less uniform pattern of staining of the other *Ago* genes in the limb at this stage (compare Fig. 1H with Fig. 1D). Our results are consistent with the recently reported expression pattern of *Ago2* in limb buds, forebrain, branchial arches, and other structures of the embryo [12].

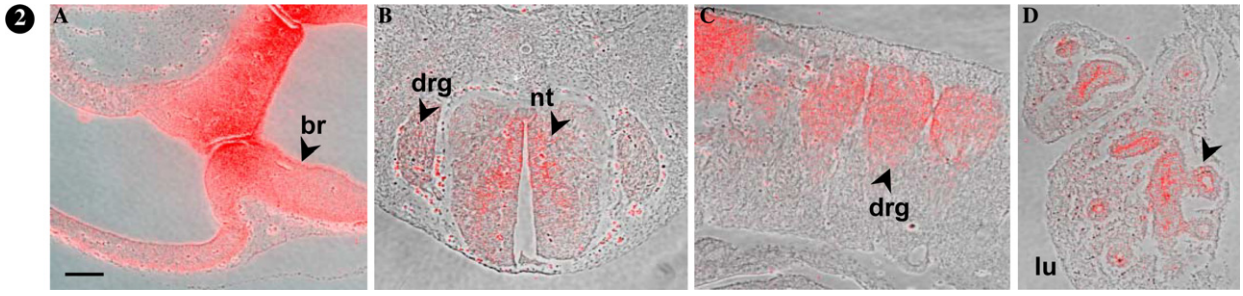
In the developing hair follicles, *Ago1* and *2* transcripts were present in the epithelium (Figs. 1B and D), while *Ago4* was detected in the surrounding mesenchyme (Fig. 1H). Another striking example of regional distribution of *Dicer* and *Ago* genes was found in the developing lung during branching morphogenesis. Whole mount in situ hybridization of the E11.5 lung showed *Dicer1* and *Ago* genes expressed in somewhat overlapping, but also distinct

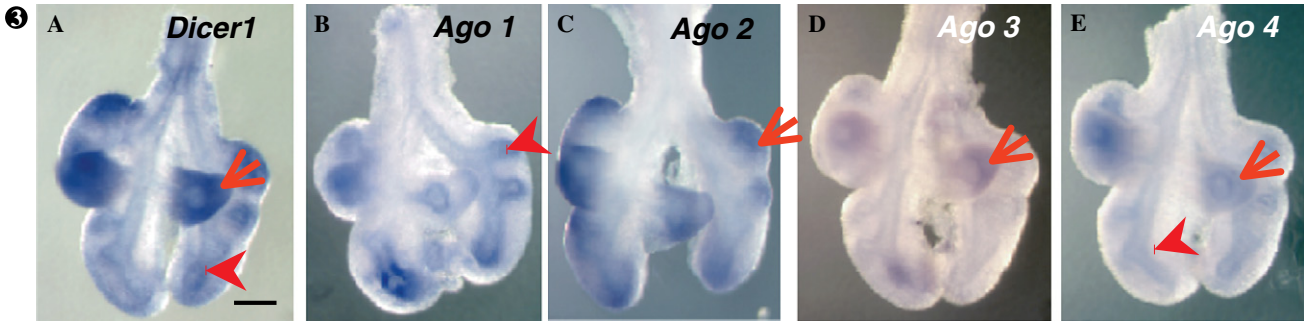
Fig. 1. Expression pattern of *Ago1–4* (A–H) and *Dicer1* (I,J) at E11.5 (A,C,E,G,I) in whole embryo (left panel), neural tube (nt), dorsal root ganglia (drg), and somites (so) (right panel), and at E14.5 (B,D,F,H,J) in forebrain (fb), midbrain (mb), hair follicles (hf) (left panel), limbs (fl, forelimbs; hl, hindlimbs; id, interdigital region), and tail (tl). Arrowheads and asterisks depict the presence or absence of signals, respectively; dotted boxes highlight transcript distribution in hair follicles or limb. Bars in left panel of (A) and right panel of (B) represent 1 mm. Bars in right panel of (A) and left panel of (B) represent 150 μm.

Fig. 2. . Isotopic in situ hybridization of *Ago1* in the neuroepithelial wall of midbrain (A), neural tube (nt), and dorsal root ganglion (drg) (B,C), and lung epithelium (D) at E11.5. Scale bar in (A): 60 μm.

Fig. 3. . Expression of *Dicer1* (A) and *Ago* family members (B–E) in E11.5 embryonic lung by whole mount in situ hybridization. Arrowhead and arrow depict expression in epithelium and mesenchyme, respectively. Scale bar in (A): 100 μm.


1
E11.5
E14.5
Ago 1
Ago 2
Ago 3
Ago 4
Dicer1
A
nt
mb
fb
drg
nt
so
B
fb
hf
hl
C
so
*
D
E
*
so
F
G
nt
H
id
fl
I
so
nt
drg
J


2
A
br
B
drg
nt
C
drg
D
lu


3
A
Dicer1
B
Ago 1
C
Ago 2
D
Ago 3
E
Ago 4

domains. Strong *Dicer1* signals could be detected in the distal mesenchyme and epithelium, a region that is morphogenetically very active (Fig. 3A). *Ago1* was mainly expressed in distal epithelial tubules, although low-level signals could be detected in more proximal regions (Figs. 2D and 3B). *Ago2* was predominantly expressed in the distal lung mesenchyme, with only low levels present in the epithelium (Fig. 3C). Overall expression of *Ago3* was the weakest of all *Ago* gene family members, and appeared to be restricted to the distal mesenchyme (Fig. 3D). *Ago4* was expressed in both epithelial and mesenchymal layers and without an obvious proximal–distal gradients (Fig. 3E). Other structures in which *Ago* gene family members and *Dicer1* were differentially expressed include tail, mandible, somites, and branchial arches (Figs. 1 and 2).

The importance of miRNA-mediated processes in mouse embryonic development is supported by the observation that *Dicer1* and *Ago2* knockout mice die very early in development [12,1]. Nevertheless, to date, information about the function of miRNAs in mammalian organogenesis has not been reported. In this study, we present novel evidence that components of miRNA machinery are present during mouse organogenesis. We show that *Dicer1* and several *Ago* family members are differentially transcribed at discreet sites in structures such as the developing neural tube, lung, brain, limbs, and others. The selectively enriched expression of *Ago1* in neural structures is consistent with the reported function of *Ago1* in *Drosophila* central nervous system development [6]. We found that in the developing lung, most of these components are expressed in branching regions, sites undergoing the most dynamic changes in gene expression and rapid remodeling of distal structures. The restricted expression of *Ago1* and *Ago2* in distal epithelium and mesenchyme, respectively, is suggestive of their distinct roles in regulating genes selectively expressed in each of these layers. The differential expression of *Agos* revealed by this study in several other morphogenetically active regions of the embryo also suggest that miRNA-mediated gene regulation is involved in the establishment of dynamic and localized gene activities in developmental processes.

Acknowledgments

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References

[1] E. Bernstein, S.Y. Kim, M.A. Carmell, E.P. Murchison, H. Alcorn, M.Z. Li, A.A. Mills, S.J. Elledge, K.V. Anderson, G.J. Hannon, Dicer is essential for mouse development, Nat. Genet. 35 (2003) 215–217.
[2] K. Bohmert, I. Camus, C. Bellini, D. Bouchez, M. Caboche, C. Benning, AGO1 defines a novel locus of *Arabidopsis* controlling leaf development, EMBO J. 17 (1998) 170–180.
[3] A. Grishok, A.E. Pasquinelli, D. Conte, N. Li, S. Parrish, I. Ha, D.L. Baillie, A. Fire, G. Ruvkun, C.C. Mello, Genes and mechanisms related to RNA interference regulate expression of the small temporal RNAs that control *C. elegans* developmental timing, Cell 106 (2001) 23–34.
[4] S.M. Hammond, E. Bernstein, D. Beach, G.J. Hannon, An RNA-directed nuclease mediates post-transcriptional gene silencing in *Drosophila* cells, Nature 404 (2000) 293–296.
[5] G. Hutvagner, P.D. Zamore, A microRNA in a multiple-turnover RNAi enzyme complex, Science 297 (2002) 2056–2060.
[6] Y. Kataoka, M. Takeichi, T. Uemura, Developmental roles and molecular characterization of a *Drosophila* homologue of *Arabidopsis* Argonaute1, the founder of a novel gene superfamily, Genes Cells 6 (2001) 313–325.
[7] R.F. Ketting, S.E. Fischer, E. Bernstein, T. Sijen, G.J. Hannon, R.H. Plasterk, Dicer functions in RNA interference and in synthesis of small RNA involved in developmental timing in *C. elegans*, Genes Dev. 15 (2001) 2654–2659.
[8] Y. Lee, C. Ahn, J. Han, H. Choi, J. Kim, J. Yim, J. Lee, P. Provost, O. Radmark, S. Kim, V.N. Kim, The nuclear RNase III Drosha initiates microRNA processing, Nature 425 (2003) 415–419.
[9] Y. Lee, K. Jeon, J.T. Lee, S. Kim, V.N. Kim, MicroRNA maturation: stepwise processing and subcellular localization, EMBO J. 21 (2002) 4663–4670.
[10] Y. Lee, M. Kim, J. Han, K.H. Yeom, S. Lee, S.H. Baek, V.N. Kim, MicroRNA genes are transcribed by RNA polymerase II, EMBO J. 23 (2004) 4051–4060.
[11] Y.S. Lee, K. Nakahara, J.W. Pham, K. Kim, Z. He, E.J. Sontheimer, R.W. Carthew, Distinct roles for *Drosophila* Dicer-1 and Dicer-2 in the siRNA/miRNA silencing pathways, Cell 117 (2004) 69–81.
[12] J. Liu, M.A. Carmell, F.V. Rivas, C.G. Marsden, J.M. Thomson, J.J. Song, S.M. Hammond, L. Joshua-Tor, G.J. Hannon, Argonaute2 is the catalytic engine of mammalian RNAi, Science 305 (2004) 1437–1441.
[13] J. Lu, J. Qian, K.I. Izvolsky, W.V. Cardoso, Global analysis of genes differentially expressed in branching and non-branching regions of the mouse embryonic lung, Dev. Biol. 273 (2004) 418–435.
[14] J. Martinez, A. Patkaniowska, H. Urlaub, R. Luhrmann, T. Tuschl, Single-stranded antisense siRNAs guide target RNA cleavage in RNAi, Cell 110 (2002) 563–574.
[15] P.H. Olsen, V. Ambros, The lin-4 regulatory RNA controls developmental timing in *Caenorhabditis elegans* by blocking LIN-14 protein synthesis after the initiation of translation, Dev. Biol. 216 (1999) 671–680.
[16] W. Park, J. Li, R. Song, J. Messing, X. Chen, CARPEL FACTORY, a Dicer homolog, and HEN1, a novel protein, act in microRNA metabolism in *Arabidopsis thaliana*, Curr. Biol. 12 (2002) 1484–1495.
[17] A. Rodriguez, S. Griffiths-Jones, J.L. Ashurst, A. Bradley, Identification of mammalian microRNA host genes and transcription units, Genome Res. (2004).
[18] H. Vaucheret, F. Vazquez, P. Crete, D.P. Bartel, The action of ARGONAUTE1 in the miRNA pathway and its regulation by the

miRNA pathway are crucial for plant development, Genes Dev. 18 (2004) 1187–1197.
[19] K. Wertz, B.G. Herrmann, Large-scale screen for genes involved in gonad development, Mech. Dev. 98 (2000) 51–70.
[20] E. Wienholds, M.J. Koudijs, F.J. van Eeden, E. Cuppen, R.H. Plasterk, The microRNA-producing enzyme Dicer1 is essential for zebrafish development, Nat. Genet. 35 (2003) 217–218.
[21] D.G. Wilkinson, M.A. Nieto, Detection of messenger RNA by in situ hybridization to tissue sections and whole mounts, Methods Enzymol. 225 (1993) 361–373.