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Original Article

miR-200c affects the mRNA expression of E-cadherin by regulating the mRNA level of TCF8 during post-natal epididymal development in juvenile rats

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The unique temporal expression pattern of miR-200c in epididymis during post-natal development in juvenile rats was revealed by our home-made miRNA microarray in this paper. It was found that miR-200c expressed in the lowest level at Day 7 and then increased to the highest at Day 36 followed by a dramatic decrease. The pattern was exactly inverse to that of mRNA expression of transcription factor 8 (TCF8) revealed by quantitative real-time polymerase chain reaction (qRT-PCR), providing an extra evidence that TCF8 is one degradation target of miR-200c even in epididymis. Moreover, the qRT-PCR study on expression of E-cadherin and interleukin-2 indicated that miR-200c does exert an obvious effect on the mRNA expression of E-cadherin by directly regulating the mRNA level of TCF8, although the effect on interleukin-2 is not obvious as on E-cadherin, which implicates that interleukin-2 may be also regulated by other factors besides TCF8 in rat epididymis.

Keywords epididymis; miR-200c; TCF8; E-cadherin; interleukin-2

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Introduction

MicroRNAs (miRNAs) are a class of small, non-coding, regulatory RNA molecules. Since the discovery of miRNAs in *Caenorhabditis elegans* [1], miRNA-dependent gene regulation has been widely investigated in a variety of eukaryotic organisms [2–4]. By December 2009, the miRNA registry (Release 14) had 10,883 entries representing hairpin precursor miRNAs, expressing 10,581 mature miRNA products, in 115 species. Mounting evidence shows the important regulatory roles for miRNAs in various developmental, differentiation, cell proliferation, and apoptosis pathways of diverse organisms [5–10]. However, few studies concern the roles for miRNAs in post-natal developing mammalian epididymis, a highly convoluted tubule that performs a variety of functions including sperm protection, maturation, concentration, and storage, which is critical to the acquisition of the fertilizing ability [11,12].

Recently, we profiled 350 miRNA expression in post-natal rat epididymis from Days 7 to 49, a vital period for maturation of male fertility, by using a home-made miRNA microarray. We found that the levels of 25 miRNAs changed significantly with time during this period. In these 25 miRNAs, miR-200c, which has not been reported in epididymis study so far, expressed in the lowest level at Day 7 and then increased to the highest at Day 36 followed by a dramatic decrease. Such a specific temporal expression pattern caught our attention. Hurteau *et al*. [13,14] reported that miR-200c can cause mRNA degradation of transcription factor 8 (TCF8, also termed as ZEB1, δEF1, and Nil-2-α) and consequently affects the mRNA level of E-cadherin and interleukin-2 which are repressed by TCF8 in their transcription according to the results from overexpression or inhibition of miR-200c in human cell lines. Park *et al*. [15] also reported that miR-200c can target the E-cadherin repressor, TCF8, so that it can determine the epithelial phenotype of cancer cells as E-cadherin is involved in epithelial-to-mesenchymal transition. As known, E-cadherin is a junctional protein and interleukin-2 is a cytokine that can activate macrophages concerning endocytosis [16,17]. It was also revealed that these two kinds of molecules are related to post-natal epididymal development in the rat [11]. More interesting, some studies reported that it is at round Day 36 that the highest expression level of E-cadherin mRNA and more active endocytosis presented in post-natal rat epididymis [11,16]. These previous results and the specific temporal expression pattern of miR-200c mentioned above strongly suggest that miR-200c should relate to some events in post-natal epididymal development of rat. However, few studies have focused on the expression of miR-200c and its roles in mammalian epididymal development so far. In this paper, we explored the relation between miR-200c and *TCF8* mRNA as well as E-cadherin and interleukin-2 in juvenile rat epididymis.

Materials and Methods

Construction of small RNA library

Whole epididymis from 3 to 18 SD rats was pooled and the total RNAs were extracted with Trizol reagent (Invitrogen, Carlsbad, USA). Thereafter, 20 μg total RNA for each time point (Days 7, 14, 21, 28, 36, and 49) was dephosphorylated, then separated on a 15% denaturing polyacrylamide gel with 70 fmol synthetic RNA (5′-AUCAGGCUCAUGACCUGAAG-3′) as internal control, and then 14–30 nucleotide RNAs which contained miRNAs were recovered. The steps described above were done independently twice for each time point.

Labeling of miRNA polymerase chain reaction products

The miRNA polymerase chain reaction (PCR) products were prepared as described for miRNA cloning [18]. Briefly, the 3′ adaptor [5′-(Pu)uuAACCGCGAATTCCAG(idT)-3′] was ligated to the 3′ end of the 14–30 nucleotide RNAs. The ligated RNAs were then subjected to reverse transcription in the presence of the real-time (RT) primer (5′-GACTAGCTGGAATTCGCGGTTAAA-3′) and the 5′ adaptor (5′-GACCACGCGTATCGGGCACCACGTATGCTATCGATCGTGAGATGGG-3′). After annealing of the 5′adaptor to the poly(C) overhang at the 3′ end of synthesized cDNAs, the cDNA products were amplified by PCR for 24 cycles (30 s at 94°C, 30 s at 55°C, and 10 s at 72°C) with the 5′ PCR primer [5′(Cy5)-GCGTATCGGGCACCACGTATGC-3′] and the 3′ PCR primer (5′-GACTAGCTTGGTGCCGAATTCGCGGTTAAA-3′). The steps described above were done independently twice for each small RNA library.

Fabrication of miRNA microarray

The miRNA microarrays were fabricated as described in the previous papers [19–21]. Briefly, standard 1′ × 3′ microscope glass slides from Sigma were activated with glycidyloxipropyltrimethoxysilane. The activated glass slides can immobilize amine-containing molecules such as amino-modified oligonucleotide DNA. Then, 5′ amine-modified oligonucleotide probes were immobilized on amine-reactive glass slides and each probe contained 10 deoxyadenosines in the 5′ terminus to minimize the spatial obstacle in hybridization and detection. We printed spots with 350 rat miRNA probes on each microarray which consisted of six submicroarrays. The 350 rat miRNA probes were perfectly matched for all miRNAs that were registered and annotated in the miRBase at Wellcome Trust Sanger Institute. Besides, we also printed spots with 21 negative control probes and 7 same internal control probes on each microarray, which were located in different submicroarrays. Each miRNA probe or control probe had three replicated spots printed on a microarray. **Table 1** shows part of oligonucleotide probes used in the microarray.

Hybridization and detection of microarray

The labeled PCR products of miRNA obtained from epididymis at Days 7, 14, 21, 28, 36, and 49, respectively, were precipitated, washed, resuspended in 10 μl of 1× hybridization buffer (8× SSC/0.2% SDS, pH 7.0) that had been heated to 45°C. Heat the mixture at 100°C for 2 min to denature and chill on ice immediately. Thereafter, inject the mixture into the hybridization chamber on the microarray slide. Hybridization was carried out at 45°C for 16 h. After hybridization, the microarray slides were washed (2× SSC/0.02% SDS, 2 min; 0.2× SSC, 2 min; and 0.1× SSC, 30 s) at 25°C. Then, the hybridized microarray slides were scanned with Innoscan700 (Innopsys, Carbonne, France) with a scan resolution of 5 μm.

For each time point, two independent small RNA libraries (biological replications) were generated and two independent reverse transcriptions/amplifications/hybridizations (technical replications) were performed for each small RNA library. Thus, for each time point, data were collected from four independent hybridizations.

Microarray data analysis

The images were quantified by MAPIX (Innopsys) using the fixed circle quantification methods. Signal intensities of

Table 1 Sequences of part of oligonucleotide probes used in the miRNA microarray

miRNAs	Oligonucleotide probes
rno-let-7c	Amino-5′-$(A)_{10}$AACCATACAACCTACTACCTCAC-3′
Rno-miR-296	Amino-5′-$(A)_{10}$GAGAGCCTCCACCCAACCCT-3′
Rno-miR-335	Amino-5′-$(A)_{10}$ACATTTTTCGTTATTGCTCTTGACC-3′
Rno-miR-200c	Amino-5′-$(A)_{10}$CCATCATTACCCGGCAGTATTA-3′
Rno-miR-98	Amino-5′-$(A)_{10}$AACAATACAACTTACTACCTCACC-3′
Negative control 1	Amino-5′-$(A)_{10}$CGTAATACGACTCACTATAGGG
Internal control	Amino-5′-$(A)_{10}$CTTCAGGTCATGAGCCTGAT-3′

spots were calculated by subtracting local background from total intensities. Further analyses were completed by visual basic for application in Microsoft Excel. We used a synthetic RNA which was complementary to the internal control probe printed on each microarray to normalize all hybridizations and then analysis of variance was performed for the values. For each probe at one time point, an arithmetic mean of 12 replicates from four independent hybridizations was calculated. Those probes whose mean values were <1000 at all time points were filtered as undetectable. The probes that changed >2 folds at least at one time point and whose P-value was <0.01 were selected for further study. The following cluster analysis was performed using the CLUSTER/TreeView software [22].

Quantitative RT-PCR of miR-200c

To validate the result of miR-200c expression detected by microarray, miR-200c was quantified with a stem–loop primer-based quantitative RT (qRT)-PCR as described previously [23]. For each time point (Days 7, 14, 21, 28, 36, and 49), 1 μg total RNA was reverse transcribed with the stem–loop RT primer (5′-GTCGTATCCAGTGCAGGGT CCGAGGTATTCGCACTGGATACGACCCATCA-3′) by ReverTra Ace-α^{TM} kit (Toyobo, Osaka, Japan). The reaction solution was incubated for 30 min at 16°C, 30 min at 42°C, 5 min at 85°C, and then held at 4°C. All reverse transcriptase reactions were run in duplicate. Then, the expression level of miR-200c was determined by RT-PCR using an SYBR Green Realtime PCR Master Mix protocol (Toyobo) on a Rotor gene 6500HRM RT-PCR machine (Corbett Life Science, Sydney, Australia). The primers used were listed as follows: miR-200c, 5′-CGCTGTAA TACTGCCGGGTAA-3′ (F) and 5′-GTGCAGGGTCCG AGGTATTC-3′ (R); and GAPDH, 5′-TACAAGGAGTAA GAAACCGTGGAC-3′ (F) and 5′-GTTATTATGGGGTC TGGGATGG-3′ (R).

qRT-PCR of TCF8, E-cadherin, and interleukin-2

To qualify the mRNA expressions of TCF8, E-cadherin, and interleukin-2, 1 μg total RNA was reverse transcribed with oligo$(dT)_{20}$ by ReverTra Ace-α^{-TM} kit for each time point (Days 7, 14, 21, 28, 36, and 49). All reverse transcriptase reactions were run in duplicate. Then, the expression levels of TCF8, E-cadherin, and interleukin-2 were determined by RT-PCR using an SYBR Green Realtime PCR Master Mix on a Rotor gene 6500HRM machine. The primers used were listed as follows: TCF8, 5′-GCCCAGTTACCCACAATCGT-3′ (F) and 5′-CATTTG GCTGGATCACCTTCAC-3′ (R); E-cadherin, 5′-ATGAGG TCGGTGCCCGTATT-3′ (F) and 5′-CTCGTTGGTCTTG GGGTCTGT-3′ (R); interleukin-2, 5′-CACTGACGCTTGT CCTCCTTGT-3′ (F) and 5′-GTTTCAATTCTGTGGCCT GCTT-3′ (R); and GAPDH, 5′-TACAAGGAGTAAGAAA CCGTGGAC-3′ (F) and 5′-GTTATTATGGGGTCTGGGA TGG-3′ (R).

Results

Temporal expression of 350 miRNAs in juvenile rat epididymis detected by miRNA microarray

The images of miRNA expression microarray slides of rat epididymis at post-natal Days 7, 14, 21, 28, 36, and 49 were obtained, respectively, after hybridization and detection by a laser confocal scanner, etc., as described in the 'Materials and Methods' section. **Figure 1** only shows the image of a miRNA microarray of rat epididymis at Day 36 and other remained images are not shown. Analysis of these images indicated that the expression level of 25 of 350 miRNAs was significantly time-dependent in juvenile rat epididymis according to the standard described in the 'Materials and Methods' section. We grouped these 25 miRNAs using a hierarchical clustering algorithm (**Fig. 2**) and classified their expression patterns as given in **Table 2** [22].

Time dependence of miR-200c expression in juvenile rat epididymis

Figure 3 shows the time dependence of miR-200c expression in epididymis obtained from the miRNA microarray analysis, indicating that the miR-200c concentration was at the lowest level at Day 7 and reached its peak at Day 36 followed by a dramatic decrease. To validate the microarray results, a stem–loop primer-based qRT-PCR was performed and the results are shown in **Fig. 4**. A comparison between **Figs. 3** and **4** indicated that the miR-200c expression patterns obtained by both microarray and qRT-PCR were consistent.

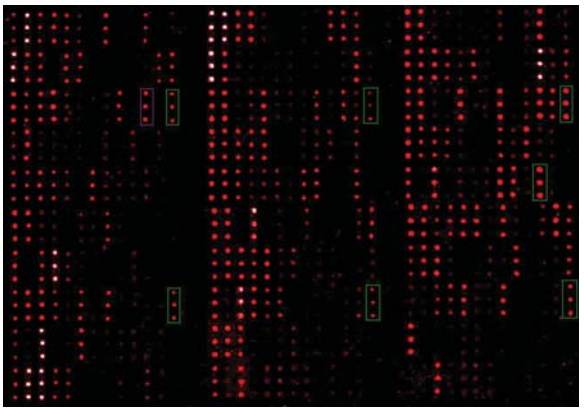

Figure 1 Image of miRNA microarray of rat epididymis at Day 36
Purple box, miR-200c; green boxes, internal control.

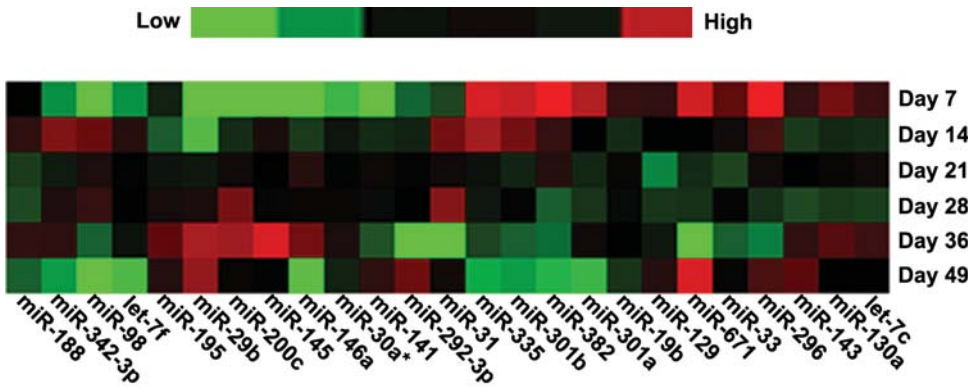

Figure 2 Cluster analysis of the temporal expression of 25 miRNAs in juvenile rat epididymis Relative expression levels for the 25 miRNAs are shown in six rows corresponding to the six time points. The miRNA expression profile was sorted using a hierarchical clustering method. Colors indicate relative signal intensities.

Table 2 Expression patterns of the 25 miRNAs during post-natal epididymal development in juvenile rats

miRNAs	Expression patterns
miR-19b, miR-301a, miR-301b, miR-382, miR-335	miRNA expression always decreased with time from Days 7 to 49
miR-30a*, miR-145, miR-200c, miR-29b, miR-195, let-7f	miRNA expression increased with time from Day 7 and reached their peaks before Day 49
miR-296, miR-671, miR-129	miRNA expression decreased with time from Day 7 and reached their lowest points before Day 49
Other 11 miRNAs	miRNA expression experienced fluctuation between Days 7 and 49

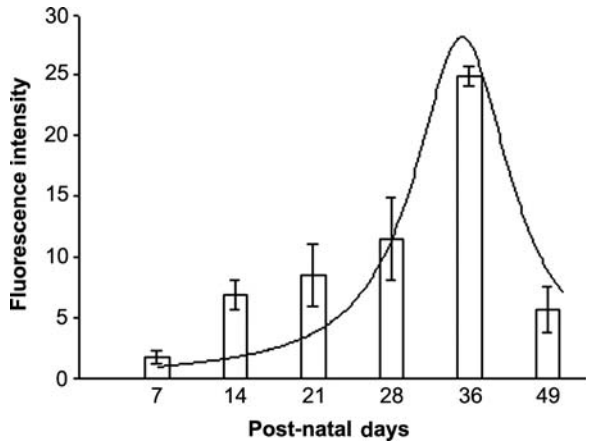

Figure 3 Expression of miR-200c in rat epididymis between Days 7 and 49 obtained from miRNA microarray results.

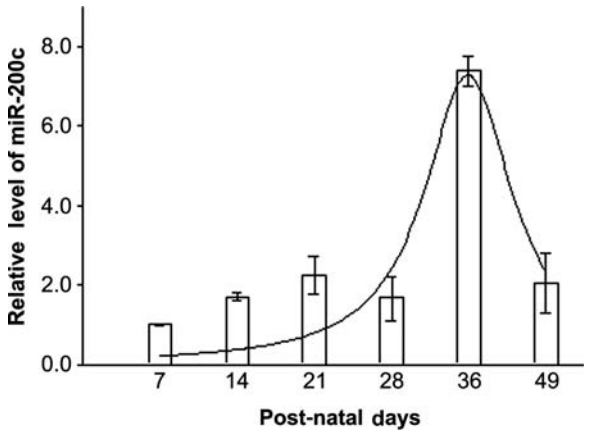

Figure 4 Expression of miR-200c in rat epididymis between Days 7 and 49 obtained from qRT-PCR results.

Time dependence of mRNA expression of TCF8 in juvenile rat epididymis

Figure 5 shows the mRNA expression of TCF8 at different time points in juvenile rat epididymis obtained from qRT-PCR experiment, indicating that the mRNA level of TCF8 peaked at Day 7, then dropped to the lowest point at Day 36, and increased a little thereafter. A comparison of **Figs. 3** and **5** indicated that the levels of TCF8 mRNA expression at different time points were actually inversely related to miR-200c in rat epididymis, implying that the mRNA level of TCF8 during post-natal epididymal development in juvenile rats may be regulated by miR-200c.

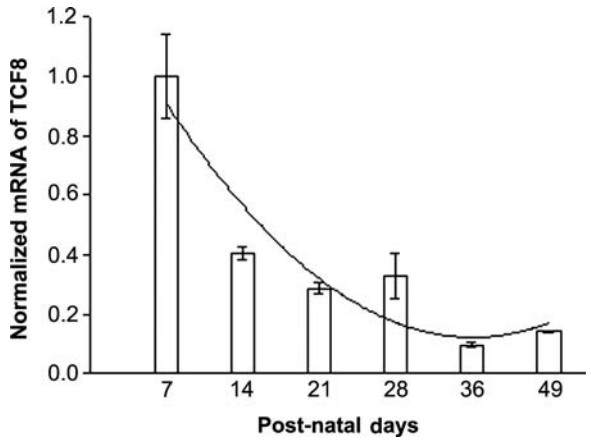

Figure 5 mRNA expression of TCF8 in rat epididymis between Days 7 and 49 obtained from qRT-PCR results.

mRNA expression of E-cadherin and interleukin-2 during post-natal epididymal development in juvenile rats

Results from qRT-PCR, shown in **Fig. 6**, indicated that the mRNA expression of E-cadherin was at the lowest point at Day 7, then increased to the highest level at Day 36 followed by a decrease, which was the same as miR-200c but inversely related to TCF8. This implicates that miR-200c may indirectly affect the mRNA expression of E-cadherin by directly regulating the mRNA expression of TCF8 in rat epididymis.

However, the result of interleukin-2 indicated that its mRNA expression was not totally inversely related to TCF8 (**Fig. 7**). The expression reached the lowest level at Day 7, then increased to the highest point at Day 21, and decreased not dramatically thereafter. **Figure 7** shows that interleukin-2 mRNA maintained a high level after Day 7, suggesting that interleukin-2 may be also regulated by other transcription factors, not only by TCF8 [24].

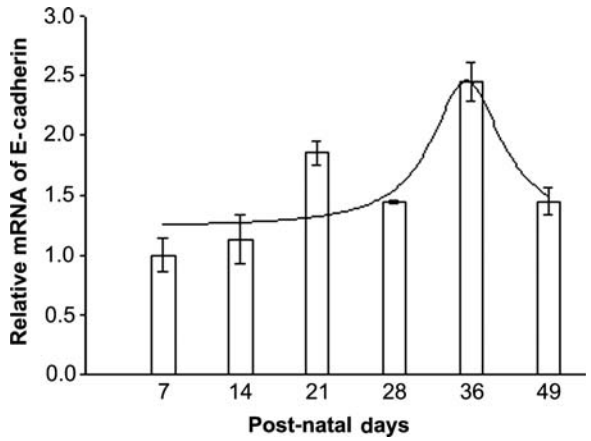

Figure 6 mRNA expression of E-cadherin during post-natal epididymal development in juvenile rats between Days 7 and 49 obtained from qRT-PCR results.

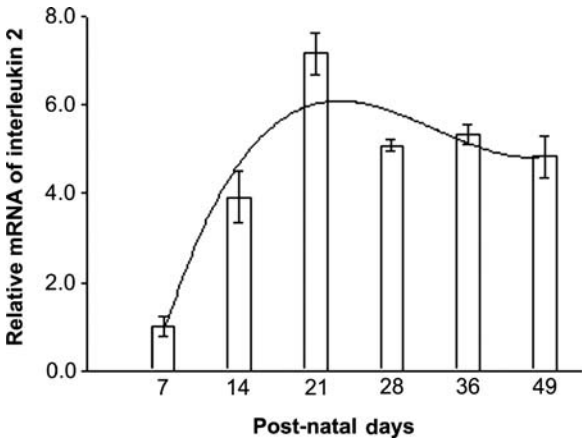

Figure 7 mRNA expression of interleukin-2 during post-natal epididymal development in juvenile rats between Days 7 and 49 obtained from qRT-PCR results.

Discussion

Rat epididymis experiences a series of morphological changes during its post-natal development, which leads to the establishment of a variety of functions including sperm protection, maturation, concentration, and storage that are important to male fertility. In this study, we isolated small RNAs from juvenile rat epididymis at six time points in its post-natal development: Days 7, 14, 21, 28, 36, and 49, since it has been known that a series of developmental changes occur at these time points. For example, Halo cells first become visible at Day 14, both narrow and columnar cells are first observed at approximately Day 21, columnar cells differentiate into principal cells and basal cells at approximately Day 28, clear cells are observed throughout the epithelium at approximately Day 36, and spermatozoa first enter the epididymis at Day 49 [11,16]. Therefore, it is most possible to get some information about relationships between miRNA and epididymal development in rats by profiling miRNA expression at these six time points. In fact, just as expected, some miRNAs were found to relate epididymal development (**Table 2**). The expression pattern of miR-200c is most interesting in these miRNAs. As mentioned above, the expression of miR-200c shows a unique time dependence: its expression level increases with time and gets the maximum at around Day 36, then has an obvious decrease. The qRT-PCR analysis also gave a consistent expression pattern, indicating that our home-made miRNA microarray is reliable. It is known that miRNA is time and tissue specific. In order to reveal the specificity of miR-200c, we monitored miR-200c expression of the heart and liver in juvenile rats at Days 21, 36, and 49 with qRT-PCR. Results shown in **Fig. 8** indicated that no significant change for miR-200c expression was found in the heart between Days 21 and 49, which is very different from that observed in epididymis. In the liver, although a slight

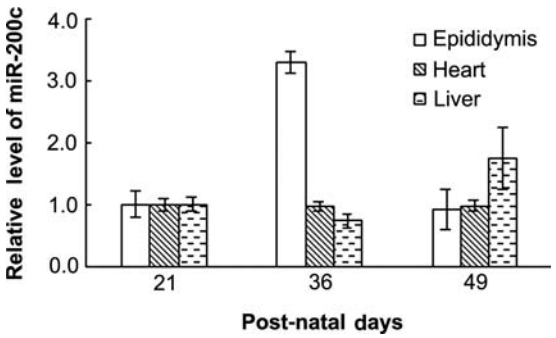

Figure 8 Expression of miR-200c in the rat epididymis, heart, and liver at different time points obtained from qRT-PCR results.

decrease at 36 days and an increase about 0.7 fold at 49 days were found, the extent of changes is much smaller than that in epididymis (about 3 folds in change). This indicates that the expression pattern of miR-200c in epididymis is specific, which implies that miR-200c might play a specific role in post-natal development of rat epididymis.

Since it has been reported that miR-200c can cause degradation of mRNA of TCF8, and TCF8 can regulate transcription of several genes concerning development such as E-cadherin and interleukin-2 [13–15], we studied the relationship between miR-200c and TCF8, E-cadherin, and interleukin-2 in rat epididymis so as to explore the potential roles for miR-200c in rat epididymal development. Our study showed that the temporal expression of TCF8 mRNA was inversely related to miR-200c in juvenile rat epididymis. This indicates that miR-200c can directly regulate the mRNA level of TCF8 during post-natal development of rat epididymis. Having identified such an inverse relation between miR-200c and TCF8 in rat epididymis, we next want to examine the effect of miR-200c on E-cadherin and interleukin-2, which are repressed by TCF8 in their transcription. E-cadherin is a cell surface protein that mediates intercellular adhesion and is involved in adherence, gap, and tight junctions between epithelial cells, which results in the formation and maintenance of blood–epididymal barrier [16]. Interleukin-2 is a cytokine playing a role in activating macrophages that can carry out endocytosis to prevent microbial infection and eliminate senescent cells [17].

Results from our study indicated that the period between Days 7 and 21 witnessed an increase in miR-200c expression and a decrease in TCF8 mRNA level, which resulted in an increase in E-cadherin mRNA between Days 7 and 21. This increase in E-cadherin mRNA is required because during this period, the blood–epididymal barrier is being formed and complete by Day 21 [16]. After Day 21, miR-200c expression peaked at Day 36 so that it could maintain the low level of TCF8 and the high level of E-cadherin. The mRNA level of E-cadherin remained high between Days 21 and 36 and reached its peak at Day 36 perhaps for the accumulation of E-cadherin protein for the following period. Although we monitored a decrease in mRNA expression of E-cadherin after Day 36 as a result of dramatic decrease in miR-200c, we can assume that the level of E-cadherin protein is still high at Day 49 due to the accumulation since Day 36 besides the current expression of E-cadherin protein at Day 49. Such an accumulation is critical for rat epididymal development because the high level of E-cadherin protein is urgently needed at around Day 49. When spermatozoa enter the epididymis at Day 49, the diameter of the lumen of the tubule increases substantially as the pressure of the intraluminal fluid increases [16]. To maintain the integrity of the blood–epididymal barrier as well as intercellular adhesion between cells, high concentration of E-cadherin protein is required. These results of mRNA expression of E-cadherin during rat epididymal development in our study are consistent with those of Cyr's research [16]. However, he did not find the factors leading to such an expression pattern of E-cadherin mRNA. Our study indicates that the fluctuation of miR-200c results in changes in E-cadherin mRNA expression by regulating the mRNA level of TCF8, which provides supplementary explanations for Cyr's study and evidence for roles of miR-200c in regulating rat epididymal development.

As to interleukin-2, our study showed that its mRNA expression was not completely inversely related to TCF8 in juvenile rat epididymis. This may be explained by the fact that interleukin-2 is also regulated by other transcription factors, not only by TCF8 [24]. However, Hurteau *et al.* [14] have reported that miR-200c can change the mRNA expression of interleukin-2. In our study, we noticed that when miR-200c expression increased between Days 7 and 36 leading to maintenance of the low level of TCF8 mRNA from Day 7, the mRNA level of interleukin-2 remained high after Day 7. Since interleukin-2 can activate macrophages, which can carry out endocytosis, the sustained high level of interleukin-2 mRNA from Day 7 may be related to active endocytosis at around Day 36 in the rat epididymis when miR-200c expression reaches its peak [11]. This implicates that miR-200c may participate in the regulation of interleukin-2 mRNA expression with other regulatory factors so as to affect endocytosis in rat epididymis, which is an indispensable physiological activity in a variety of epididymal cells.

What is worth mentioning is that miR-200c is a member of the miR-200 family including the other four miRNAs, which can be grouped into two subfamilies according to the presence of two types of seed sequences: miR-200a/miR-141 and miR-200b/miR-200c/miR-429 [**Fig. 9(A)**]. miR-200a/miR-141 has no target sites in TCF8 3′UTR, whereas miR-200b/miR-200c/miR-429 has two perfect 7 bp 'seed' matches in TCF8 3′UTR in the rat [**Fig. 9(B)**]. However, results from our miRNA microarray indicated that the expressions of miR-200b and miR-429 did not show

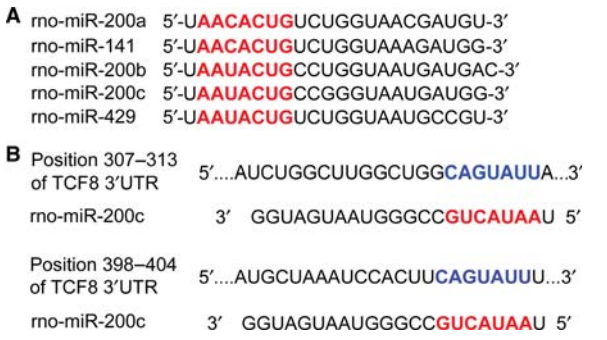

Figure 9 Sequences of some miR-200 subfamilies (A) Sequences of five members of miR-200 family in the rat. The miR-200 family consists of two subfamilies. The corresponding seed sequences are shown in red letters. (B) Two target sites for miR-200c in TCF8 3′UTR at positions 307–313 and 398–404 in the rat. The target sites are shown in blue letters. The target sites for miR-200b and miR-429 are the same as miR-200c.

significant changes between Days 7 and 49 in rat epididymis. This suggests that miR-200c as a single miRNA plays an important role in post-natal development of rat epididymis.

Conclusion

Results from our home-made miRNA microarray and qRT-PCR indicate a specific temporal expression pattern of miR-200c in rat epididymis. Further study shows that the mRNA expression of TCF8 is inversely related to miR-200c, which provides extra evidence for reports made by previous studies that miR-200c targets TCF8 [13–15]. Moreover, we found that miR-200c can affect the mRNA expression of E-cadherin by directly regulating the mRNA level of TCF8 in rat epididymis. Results from our study suggest that interleukin-2 may be regulated by TCF8 along with other factors in rat epididymis.

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References

1 Lee R, Feinbaum RL and Ambros V. The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 1993, 75: 843–854.
2 Lee R and Ambros V. An extensive class of small RNAs in *Caenorhabditis elegans*. Science 2001, 294: 862–864.
3 Ambros V, Lee R, Lavanway A, Williams PT and Jewell D. MicroRNAs and other tiny endogenous RNAs in *C elegans*. Curr Biol 2003, 13: 807–818.
4 Bartel D. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004, 116: 281–297.
5 Bernstein E, Kim SY, Carmell MA, Murchison EP, Alcorn H, Li MZ and Mills AA, *et al*. Dicer is essential for mouse development. Nat Genet 2003, 35: 215–217.
6 Chen CZ, Li L, Lodish HF and Bartel DP. MicroRNAs modulate hematopoietic lineage differentiation. Science 2004, 303: 83–86.
7 Harfe BD, McManus MT, Mansfield JH, Hornstein E and Tabin CJ. The RNaseIII enzyme Dicer is required for morphogenesis but not patterning of the vertebrate limb. Proc Natl Acad Sci USA 2005, 102: 10898–10903.
8 Harris KS, Zhang Z, McManus MT, Harfe BD and Sun X. Dicer function is essential for lung epithelium morphogenesis. Proc Natl Acad Sci USA 2006, 103: 2208–2213.
9 Lakshmipathy U, Love B, Goff LA, Jornsten R, Graichen R, Hart RP and Chesnut JD. MicroRNA expression pattern of undifferentiated and differentiated human embryonic stem cells. Stem Cells Dev 2007, 16: 1003–1016.
10 Liao RM. Development of conditioned place preference induced by intra-accumbens infusion of amphetamine is attenuated by co-infusion of dopamine D1 and D2 receptor antagonists. Pharmacol Biochem Behav 2008, 89: 367–373.
11 Rodriguez CM, Kirby JL and Hinton BT. The development of the epididymis. In: Robaire B and Hinton BT (eds). The Epididymis: From Molecules to Clinical Practice. New York: Kluwer Academic/Prenum Publishers, 2002.
12 Lye RJ and Hinton BT. Technologies for the study of epididymal-specific genes. Mol Cell Endocrinol 2004, 216: 23–30.
13 Hurteau GJ, Spivack SD and Brock GJ. Potential mRNA degradation targets of *has-miR-200c*, identified using informatics and qRT-PCR. Cell Cycle 2006, 5: 1951–1956.
14 Hurteau GJ, Carlson JA, Spivack SD and Brock GJ. Overexpression of the microRNA *has-miR-200c* leads to reduced expression of transcription factor 8 and increased expression of E-cadherin. Cancer Res 2007, 67: 7972–7976.
15 Park SM, Gaur AB, Lengyel E and Peter ME. The miR-200 family determines the epithelial phenotype of cancer cells by targeting the E-cadherin repressors ZEB1 and ZEB2. Genes Dev 2008, 22: 894–907.
16 Cyr DG, Hermo L and Robaire B. Developmental changes in epithelial cadherin messenger ribonucleic acid and immunocytochemical localization of epithelial cadherin during postnatal epididymal development in the rat. Endocrinology 1993, 132: 1115–1124.
17 Zembala M, Siedlar M, Ruggiero I, Wieckiewicz J, Mytar B, Mattei M and Colizzi V. The MHC class-II and CD44 molecules are involved in the induction of tumour necrosis factor (TNF) gene expression by human monocytes stimulated with tumour cells. Int J Cancer 1994, 56: 269–274.
18 Takada S, Berezikov E, Yamashita Y, Lagos-Quintana M, Kloosterman WP, Enomoto M and Hatanaka H, *et al*. Mouse microRNA profiles determined with a new and sensitive cloning method. Nucl Acids Res 2006, 34: e115.
19 Liang RQ, Li W, Li Y, Tan CY, Li JX, Jin YX and Ruan KC. An oligonucleotide microarray for microRNA expression analysis based on labeling RNA with quantum dot and nanogold probe. Nucl Acids Res 2005, 33: e17.
20 Huang B, Li W, Zhao B, Xia C, Liang R, Ruan K and Jing N, *et al*. MicroRNA expression profiling during neural differentiation of mouse embryonic carcinoma P19 cells. Acta Biochim Biophys Sin 2009, 41: 231–236.
21 Li W, Zhao B, Jin Y and Ruan K. Development of a low-cost detection method for miRNA microarray. Acta Biochim Biophys Sin 2010, 42: 296–301.
22 Eisen MB, Spellman PT, Brown PO and Botstein D. Cluster analysis and display of genome-wide expression patterns. Proc Natl Acad Sci USA 1998, 95: 14863–14868.
23 Chen C, Ridzon DA, Broomer AJ, Zhou Z, Lee DH, Nguyen JT and Barbisin M, *et al*. Real-time quantification of microRNAs by stem-loop RT-PCR. Nucl Acids Res 2005, 33: e179.
24 Hughes CC and Pober JS. Transcriptional regulation of the interleukin-2 gene in normal human peripheral blood T cells. Convergence of costimulatory signals and differences from transformed T cells. J Biol Chem 1996, 271: 5369–5377.