Identification Of Pig Let-7-family MicroRNAs

Li Mingzhou[*], Ma Jideng[*], Nie Jing[*], Zhou Shuling[*], Zhong Zhijun[*], Xia Youlin[†], Gao Xiaolian[†] and Li Xuewei[*]

Introduction

The lethal-7 (let-7) gene was initially discovered as an essential developmental gene in *C. elegans* and, later, as one of the first microRNAs (miRNAs) (Reinhart *et al.*, 2000). It is now known that mature let-7 is highly conserved across animal species (Pasquinelli *et al.*, 2000). Information concerning the pig let-7-family miRNAs has been long overdue. At present, there are nine members of the let-7-family in mammals deposited in the latest miRBase 14.0 (September 2009): let-7a to -7g, let-7i and let-7j, but only three members, ssc-let-7c, ssc-let-7f and ssc-let-7i are from pig (Reddy *et al.*, 2009; Wernersson *et al.*, 2005). Identification of pig let-7-family members is useful for further defines the expression and function of pig let-7-family miRNAs.

Material and methods

Small RNA library sequencing. The ten small RNA libraries used in this study were culled from tissue samples representing ten developmental stages of Rongchang pigs (a white, Chinese indigenous breed): six prenatal and four postnatal stages. Ten libraries were sequenced individually on the Illumina Genome Analyzer GA-I (formerly known as Solexa) following the vendor's recommended protocol for small RNA sequencing. The raw sequence data were submitted to NCBI-GEO database (see accession no. GSE17885 for details). Analysis of the sequencing data was performed primarily by using ACGT101-miR, an in-house program for discovering miRNAs from deep sequencing data (to be published separately).

Real-time quantitative PCR (q-PCR). Total RNA was extracted using the *mir*Vana Kit (Ambion) from 41 normal tissues from three 120 day old female Rongchang pigs, and four additional male tissues were reproductive tissues (epididymis, testis, prostate and seminal vesicle) from three 120 day old male Rongchang pigs. The forward primers of ssc-let-7c and ssc-let-7f were identical in sequence and length to the miRNA itself in miRBase 14.0. The EvaGreen-based q-PCR (Mao *et al.*, 2007) was performed with the High-Specificity miRNA qRT-PCR Detection Kit (Stratagene) on the iQ5 Real-Time PCR Detection System (Bio-

[*] Institute of Animal Genetics & Breeding, College of Animal Science & Technology, Sichuan Agricultural University, Ya'an, 625014 Sichuan, PR China
[†] Department of Biology & Biochemistry, University of Houston, Houston, 77004 TX, USA
Correspondence: Li Xuewei. Email: xuewei.li@sicau.edu.cn
This work was supported by grants from the National Special Foundation for Transgenic Species of China (2009ZX08009-155B, 2008ZX08006-003) and the National Natural Science Foundation of China (30901024, 30771534).

Rad). The ΔΔCt method was used to determine the expression level differences between surveyed samples. All measurements contained a negative control and a no-*E. coli* poly A polymerase (PAP) control, and each sample of each individual was analyzed in triplicate. Normalized factors (NF) of three internal control genes (U6 snRNA, 18S rRNA and Met-tRNA) and relative quantities of objective miRNAs were analyzed using qBase software (Hellemans *et al.*, 2007).

Results and discussion

We used no-error mapping to differentiate the single base difference in mapping mammalian let-7-family miRNAs, and identified eight pig let-7-family miRNAs besides let-7j, which were exhibited high levels of sequence conservation in mammals (Figure 1).

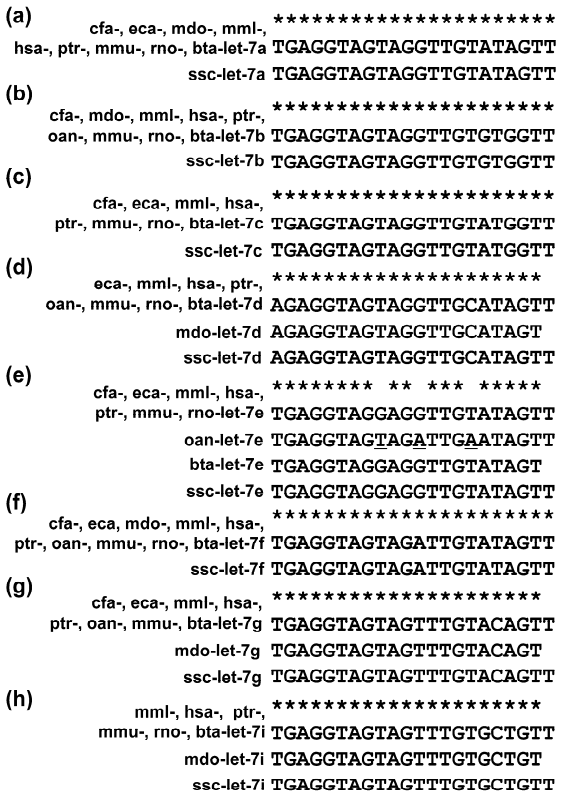

Figure 1: The homologous mammalian let-7 family miRNAs. (a) let-7a, (b) let-7b, (c) let-7c, (d) let-7d, (e) let-7e, (f) let-7f, (g) let-7g and (h) let-7i. bta: *Bos taurus*, cfa: *Canis familiaris*, eca: *Equus caballus,* hsa: *Homo sapiens*, mdo: *Monodelphis domestica*, mml: *Macaca mulatta*, mmu: *Mus musculus*, oan: *Ornithorhynchus anatinus*, ptr: *Pan troglodytes*, rno: *Rattus norvegicus*, ssc: *Sus scrofa.*

Our sequencing detected in pig the presence of the miRBase ssc-let-7 sequences, which are ssc-let-7c (648K), ssc-let-7f (600K) and ssc-let-7i (59K) as well as the previously unidentified ssc-let-7a (1M), ssc-let-7b (520K), ssc-let-7d (35K), ssc-let-7e (50K) and ssc-let-7g (302K) (Figure 2). Obviously, there is a broad range of sequence reads, which varied from millions of sequence reads for the most abundant to tens of thousands. Since these miRNAs can play a wide range of important cellular functions (Roush and Slack, 2008), the clear account of these sequence closely related let-7 miRNAs would have general implications for understanding of pig biology. The region of 2^{nd}-7^{th} of the 5' end ("seed" or "nucleus") in the various miRNAs, has been reported to play a key determinant in base pairing with the 3' untranslated region (UTR) of its target mRNAs (Bartel, 2009). miRNAs that contain identical seed sequences are likely to have similar functions. We found that eight pig let-7-family miRNAs share identical seed sequence (5' -GAGGTA- 3') (Figure 2).

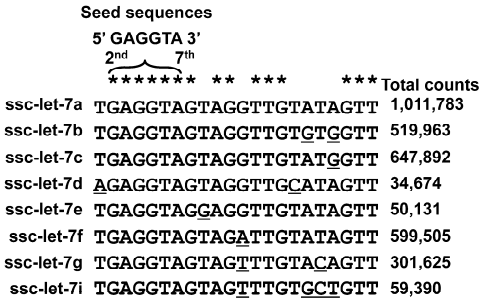

Figure 2: Sequence alignments of pig let-7-family miRNAs. "Total count" is the sum for the sequenced reads in all ten libraries.

Similarly to the previous reports of other mammals (Hausser *et al.*, 2009; Landgraf *et al.*, 2007), we found that ssc-let-7c and ssc-let-7f were ubiquitously expressed in almost all tissues analyzed (Figure 3). It has been hypothesized that the let-7-family miRNAs are responsible for control of the basic cellular and developmental pathways common to most eukaryotes (e.g., cell proliferation and differentiation).

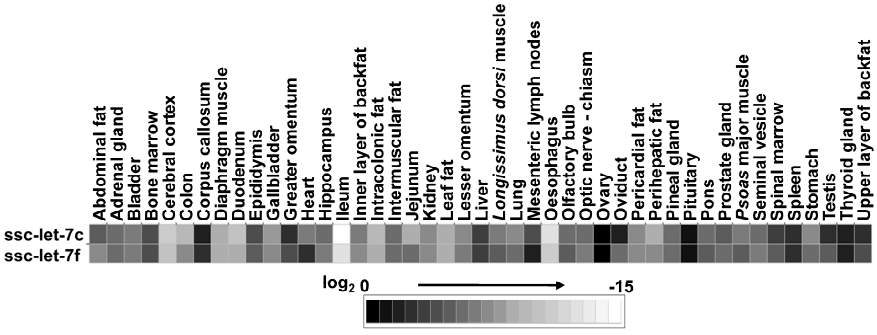

Figure 3: Q-PCR analysis of expression of ssc-let-7c and ssc-let-7f across 45 specific tissues. Data shown are log_2-transformed of the relative expression amount.

Conclusion

These results provide a reliable resource of pig let-7-family miRNA sequences. Further wider spread and in-depth studies for pig let-7-family miRNAs will be useful for pig biology as well the use of pigs as model organism for human biological and biomedical studies.

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