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Review – Mini-Series on Germ Cell Development

Small RNAs in mammalian germline: Tiny for immortal

Fuchou Tang

Wellcome Trust/Cancer Research UK Gurdon Institute, University of Cambridge, Tennis Court Road, Cambridge CB2 1QN, UK

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ABSTRACT

Germ cells are the only immortal cells in a mammalian organism. Here, I review recent progress in the research on the role of small non-coding RNAs – namely microRNAs (miRNAs), endogenous siRNAs (endo-siRNAs), and piwi-interacting RNAs (piRNAs) – in the development of mammalian germ cells. Two key functions of small RNAs in germ cells are to globally regulate the germ cell developmental program and to keep selfish genetic elements under strict surveillance in order to maintain the germline immortality and to keep the species stable and eternal. I propose that many new members of small RNAs and even completely new types of small RNAs acting in the germline, especially in early primordial germ cells (PGCs) will be discovered in the near future.

Contents

1. Germ cell development in mammals

There are more than two hundred different types of cells in an adult mammal. They can be classified into two general types: somatic cells and germ cells (Surani et al., 2008). Somatic cells work synergistically to maintain the homeostatic form and physiological function of an individual organism, whereas germ cells work to faithfully maintain their genetic information and developmental potential to establish the subsequent generation of individuals: eventually re-establishing both the somatic and germ cells in the next generation (Nakamura and Seydoux, 2008). Therefore, the germ cells are the only type of immortal cells in a mammalian organism. To fulfill these roles, germ cells exhibit unique properties not shared by any type of somatic cells. This includes the execution of global epigenetic reprogramming, the strict maintenance of genomic integrity and stability, meiosis to form either haploid sperm or oocytes, and the reversion to diploidy concomitant with resetting the developmental totipotency through fusion of an oocyte with a sperm to give rise to an entire new organism.

In the mouse, germ cell development start during early gastrulation at embryonic day (E)6.5, when about six cells in the proximal posterior epiblast turn on the expression of *Prdm1* (also known as *Blimp1*) (Hayashi et al., 2007). Then *Prdm14* and *Stella* (also known as *Dppa3 and PGC7*) are turned on sequentially and the specification of primordial germ cells (PGCs) is completed at around E7.5 at which time alkaline phosphatase activity becomes high in these cells (Surani et al., 2008). After this, at E8.5, PGCs start migrating through the hindgut and enter the genital ridges, their final destination, at E10.5. During migration, the first round of global epigenetic reprogramming occurs. This includes the reduction of global histone3 lysine9 trimethylation (H3K9me3) levels, upregulation of histone3 lysine27 trimethylation (H3K27me3), and genome wide DNA demethylation (Seki et al., 2005; Sasaki and Matsui, 2008). After their arrival at the genital

E-mail address: f.tang@gurdon.cam.ac.uk

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ridges, through interaction with neighboring somatic cells, the second round of global epigenetic reprogramming, including the erasure of parental imprints, ensues, and is finished by E12.5 (Hajkova et al., 2008). At E13.5, male PGCs enter G1-phase mitotic arrest (now referred to as prospermatogonia) and resume proliferation 2 days after birth (2 dpp) during which the DNA methylation of paternally imprinted genes is established (Lees-Murdock and Walsh, 2008). Subsequently, on 10 dpp, the meiosis of male germ cells starts proceeding sequentially through the leptotene, zygotene, pachytene, and diplotene stages of meiosis after which they differentiate to round spermatids on about 20 dpp and become spermatozoon at around 35 dpp (de Rooij and Grootegoed, 1998). Conversely, at E13.5 female PGCs arrest in meiotic prophase and become primary oocytes (Saga, 2008). The primary oocytes become growing oocytes at around 3 dpp and develop to a mature oocyte arrested at metaphase II of meiosis. It is not until after fertilization by a sperm, that the oocyte meiosis is completed with the second polar body exclusion and the initiation of the development of the next generation.

2. MicroRNAs and endo-siRNAs for germ cell development

MicroRNAs (miRNAs) are a class of 21 nt small non-coding RNAs, which repress their target gene expression post-transcriptionally through sequence complementation to mainly the 3'UTR region of target mRNAs (Alvarez-Garcia and Miska, 2005; Bartel, 2009). In some cases miRNAs can also target the coding region of their target mRNAs (Tay et al., 2008). Recently, several cases were also found where specific miRNAs activate/derepress rather than repress the expression of some of their targets (Vasudevan et al., 2007; Cordes et al., 2009). However, the repression of target genes is still the main effect performed by miRNAs (Mayr and Bartel, 2009; Bartel, 2009). The number of miRNA genes could be about 1–5% of the number of protein-coding genes and there are about 600 miRNAs already being identified in mouse genome. Approximately 50% of those are clustered together in the mouse genome. In addition, about 40% of miRNA genes are located in the introns of protein-coding genes. The remaining 60% have their own transcription units independent of protein-coding genes. For the general mechanism of miRNA biogenesis and processing and their mechanism of action, recently published review by Kim et al. is an excellent source of information (Kim et al., 2009). In general, miRNA genes in the genome are transcribed by RNA polymerase II into several kilobase long primary transcripts with ploy(A) tails. These transcripts are referred to as pri-miRNAs. The miRNAs of a single cluster in the genome are usually transcribed into one single primary transcript. In some cases, miRNA genes can also be transcribed by RNA polymerase III (Borchert et al., 2006). These pri-miRNAs are subsequently processed in the nucleus by the Drosha/DGCR8 complex (Drosha is an RNase III-type endonuclease) into 60–100 nt long Stem-loop hairpin structured miRNA precursors called pre-miRNAs. After this, the pre-miRNAs are transported from the nucleus to the cytoplasm by Exportin5. In the cytoplasm, another RNase III-type endonuclease, Dicer cuts pre-miRNAs into double-stranded miRNAs. One strand of this duplex, the passenger strand, is then removed and degraded. The remaining strand, the guide strand, is then loaded into the RNA-induced silencing complex (RISC) where it becomes single stranded mature miRNA. A recently discovered class of miRNAs are called mirtrons, which reside at the ends of short introns (60–100 nt) of protein-coding genes (Ruby et al., 2007;Okamura et al., 2007; Berezikov et al., 2007). These introns, which structurally mimic pre-miRNAs, are first spliced and debranched to be released from primary transcripts in the nucleus. After that they are treated as pre-miRNAs by the cell and transported to the cytoplasm by Exportin5 and processed into mature miRNAs by the canonical Dicer pathway. The processing of mirtrons is independent of Drosha/DGCR8 complex, which is different from canonical miRNAs. However, due to the size restriction of the mammalian introns acting as pre-miRNAs, only dozens of mirtrons have been found in the genome of mammals.

The Argonaute proteins, namely Ago1–Ago4 serve as the core component of the RISC complex, with Ago2 conferring 'slicer' activity to the complex. This activity directly cuts the target mRNAs into two halves in those cases where the miRNA has a perfect or nearly perfect complementation to the target (Yekta et al., 2004). The mature miRNAs in the RISC complex will guide the repression of a target gene through either the repression of its translation or degradation of the target mRNAs or both. The recognition of target mRNAs is mainly through complementation of the 6–8 nucleotides at the 5′-end of the miRNA called the 'seed' region (Bartel, 2009). In general, every miRNA has several hundreds of targets and up to 60% of the protein-coding genes in the mammalian genome are estimated to be direct targets of miRNAs (Friedman et al., 2009). In some cases, these targets have been validated at whole genome scale through either proteomics approach called SILAC, or a biochemical approach called HITS-CLIP (Selbach et al., 2008; Baek et al., 2008; Chi et al., 2009). However, to date there is no whole genome scale tool available for the validation of the functional contribution of each individual target of miRNAs. Most of the published data confirming the functional contribution of miRNA targets involves cases where just one or several targets are involved in the miRNA's main physiological function. These are probably the exceptions rather than the general rules of miRNA function. In the future, some conceptually new methods need to be developed to validate the functional contribution of all potential targets of a miRNA in parallel and in a high-throughput fashion. Recently, it was found that essentially every step of the miRNA processing including the Drosha processing activity, the exporting activity, Dicing activity, and degradation of pri-miRNAs, pre-miRNAs, and mature miRNAs can be regulated to control the function of miRNAs. The most well dissected post-transcriptional regulation is polyuridylation of specific pre-miRNAs, which results in the pre-miRNA degradation and blocks the production of corresponding mature miRNAs (Heo et al., 2009; Lehrbach et al., 2009). However, transcriptional control still remains the major type of regulation of miRNA biogenesis.

During early PGC development, miRNAs are highly dynamically expressed (Fig. 1) (Hayashi et al., 2008). The miR-17–92 and miR-290–295 clusters of miRNAs are abundantly expressed during early PGC development. This constitutes as high as 69% and 14%, respectively, of all expressed miRNAs in PGCs. The miR-17–92 cluster of miRNAs has been shown to promote cell division (Ventura et al., 2008). It is gradually downregulated from E9.5 to E11.5 and then is further downregulated in female PGCs, while in male PGCs, it is upregulated again after E11.5 and persist in spermatogonia until 3 days after birth (3 dpp) (Fig. 1). The miR-290–295 cluster of miRNAs is associated with pluripotency in mouse embryonic stem (ES) cells. Their abundant expression in PGCs, which persist in spermatogonia of male mice to at least 3 days after birth, may contribute to the pluripotency potential of PGCs. The miR-200c-141 miRNA cluster is strongly downregulated from E9.5 to E13.5 during PGC development. This cluster of miRNAs has been shown to repress the ability of mammary stem cells to self-renew (Shimono et al., 2009). In contrast, Let-7 family miRNAs, which have been correlated with differentiation of ES cells, are continuously upregulated in PGCs during the same period (Fig. 1). MiRNAs are functionally important for the proliferation of early PGCs since Dicer conditional knockout mice have significantly reduced number of