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Human Germline: A New Research Frontier

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SUMMARY

We recently elucidated the mechanism of human primordial germ cell (hPGC) specification and resetting of the epigenome for totipotency. The regulators of hPGC specification also initiate resetting of the epigenome, leading to a comprehensive erasure of DNA methylation, erasure of imprints and X reactivation in early hPGCs in vivo. These studies reveal differences with the mouse model, which are probably due to differences in the regulation of human pluripotency, and in postimplantation development at gastrulation, which indicates the importance of non-rodent models for investigations. Within the extreme hypomethylated environment of the early human germline are loci that are resistant to DNA demethylation, with subsequent predominant expression in neural cells. These loci provide a model for studies on the mechanism of transgenerational epigenetic inheritance, and their response to environmental factors. Such epigenetic mechanism of inheritance could potentially provide greater phenotypic plasticity, with significant consequences for human development and disease.

Germline: The Immortal Lineage

A primary role of germline is to generate the totipotent state, which precedes establishment of pluripotency during preimplantation development (Hayashi and Surani, 2009; Leitch et al., 2013). With totipotency, human germline not only gives rise to a new organism, but also theoretically at least, to an endless series of generations. Thus, germ cell lineage is considered "immortal," unlike somatic cells that perish with each individual. Germline transmits genetic as well as epigenetic information to subsequent generations. To accomplish this significant role, the germline epigenome undergoes comprehensive and unprecedented chromatin modifications, and global erasure of DNA methylation (Hackett et al., 2013; Kagiwada et al., 2013; Seisenberger et al., 2012). This process will also ensure erasure of epimutations. Without such erasure, there would be progressive accumulation of epimutations, which would compromise germline functions and survival of the species.

DNA methylation is also a key mechanism for the repression of transposable elements (TEs). The global erasure of DNA methylation therefore creates conditions for the activation of TEs and their transpositions (Zamudio and Bourc'his, 2010; Tang et al., 2015). These repetitive elements make up more than half of the mammalian genome, indicating that neither the invasion of our genome by these foreign elements, nor their expansion once acquired can be completely restrained. The comprehensive erasure of DNA methylation creates a key battleground between TEs and host defense mechanisms, resulting in an arms race to regulate their activity. Transposition events have the potential for inducing mutations; however, not all of these will have deleterious consequences. The TEs have also been crucial for mammalian evolution; some have been co-opted for important functions to regulate mammalian development (Gifford et al., 2013).

Mammalian germline also generates critical epigenetic information for totipotency and development through imprinted genes. Expression of these genes is strictly dependent on their parental origin, which explains why both male and female genomes are essential for mammalian development. Imprints are erased and re-initiated in the germline; following fertilization, they are subsequently detected as robust and heritable parent-of-origin-dependent DNA methylation marks in embryos that persist into adulthood. Imprinted genes provide reciprocal epigenetic information in parental genomes, which results in functional differences between parental genomes during development. Thus, whereas the parental genomes contribute equivalent genetic information to the zygote, the epigenetic information strictly depends on their parental origin. Parental imprints are first erased in primordial germ cells (PGCs) and then re-established appropriately during every germline cycle, and not passed on transgenerationally. Inheritance of epigenetic information through imprinting is a highly regulated process with clearly defined mechanism for erasure and re-initiation.

This epigenetic information transmitted from germline via imprinted genes, differs from the epigenetic information that is apparently acquired in response to diverse environmental factors, and transmitted through the germline. The mechanistic basis for how such epigenetic information might be acquired and transmitted either inter- or transgenerationally is unclear (Radford et al., 2014; Heard and Martienssen, 2014), which remains a major question for mammalian germline biology. The consequences of such epigenetic inheritance in regulating phenotypic traits and any potential role during mammalian evolution also remain to be elucidated.

Pangenesis, Gemmules, Germ Plasm, and Mobile RNAs

Darwin proposed pangenesis in 1868 as a "provisional" hypothesis of heredity. He proposed that organs produce "gemmules," which contain information on the performance of each organ in the body (Darwin, 1868). These gemmules are than passed on to sperm and eggs, and in this way, information from somatic tissues is gathered and transmitted to the next generation. Some recent reports on environmentally induced epigenetic changes and their apparent transgenerational inheritance conform to the idea of pangenesis, which has overtones of Lamarckian inheritance of acquired characters (reviewed by Heard and Martienssen, 2014). Although this is unlikely, it does not entirely negate a degree of phenotypic plasticity that could be induced by environmental factors, albeit the mechanistic basis for the inheritance of such information through the mammalian germline is difficult to envisage. Non-coding RNAs, might be thought of as gemmules, in particular, mobile RNAs in plants and nematodes have been proposed as agents for transmission of information from cell to cell, and potentially through the germline (Sarkies and Miska, 2014). However, in mammals, the germline is set aside during early postimplantation development, which poses additional barriers to be overcome for such transmission from soma to germline.

The idea of a barrier separating germline from soma was proposed by August Weissmann, who in 1889 proposed the concept of germ plasm. Accordingly, only cells that inherit germ plasm acquire germ cell fate, and the remaining cells acquire somatic fates. Furthermore, only the cells inheriting germ plasm during each generation transmit genetic information to the next generation, excluding somatic cells from any such role. A strict interpretation of this idea is that germ cells do not carry information from somatic cells as far as inheritance is concerned. This is sometimes referred to as Weissman's Barrier, which challenges the Lamarckian idea of inheritance of acquired characters. With the advent of induced pluripotent stem cells (iPSC) however, it is possible to generate human primordial germ cells from adult somatic cells via iPSC (Irie et al., 2015), which to some extent breaks the Weissman's Barrier. It is clearly important to resolve the issue of environmentally induced transmission of epigenetic information through the human germline, which apparently has phenotypic consequences. To address this question, it is first essential to know how the human germ cell lineage is established, and gain knowledge of how the germline epigenome is reset. Our recent work has been directed at addressing some of these fundamental questions concerning the human germline (Irie et al., 2015; Tang et al., 2015).

Specification of Human Primordial Germ Cells

First, it is important to elucidate the mechanism of human PGC specification, the precursors of sperm and eggs. PGC specification in mammals does not depend on the inheritance of germ plasm, but is induced by signaling molecules during early postimplantation development (De Fellici, 2013). Indeed, some evidence indicates that all pluripotent cells in blastocysts and all pluripotent embryonic stem cells (ESCs) are potential PGCs. Unlike in some organisms, mammalian germ cells are not allocated early in development. Lawson and Hage (1994) studied the origin of PGCs in mouse embryos and observed them through early postimplantation development to the establishment of founder population of PGCs in mice, which are induced by BMP4 (Lawson et al., 1999). Importantly, genetic studies identified key transcription factors that are induced by BMP4, which play an essential role in germ cell fate determination. These factors are also important for initiating a program for resetting the germline epigenome (Hayashi and Surani, 2009).

We first established the genetic basis of mammalian PGC specification in mice using a single cell transcriptome analysis, which led to the identification of *Prdm1* (encoding BLIMP1) as a key regulator of PGCs (Saitou et al., 2002; Ohinata et al., 2005; Hayashi et al., 2007). A key role of BLIMP1 is to repress somatic fate in the postimplantation epiblast cells from which PGCs are recruited. BLIMP1 mutant cells fail to undergo specification as PGCs and show expression of somatic genes. The use of BLIMP1 mutant cells also led to the identification of PRDM14, which has a significant role in regulating pluripotency and during specification of PGCs (Magnúsdóttir et al., 2013; Nakaki et al., 2013). A third critical gene *Tfap2c* (encoding AP2G), is a direct target of BLIMP1 (Magnúsdóttir et al., 2013). These regulators constitute a tripartite genetic network for mouse PGC specification, which are necessary and sufficient for mouse PGC specification. They act combinatorially by binding to targets to regulate three key functions: suppression of somatic fate, regulation of germ cell program, and the epigenetic program. Genetic studies confirmed that a mutation in BLIMP1 or PRDM14 abrogates PGC fate in vivo. An in vitro method allows development of PGC-like cells from naive pluripotent stem cells (ESCs), with a potential to develop into viable gametes (Hayashi et al., 2011). PGCs can be induced by cytokines or directly by the three transcription factors in vitro without cytokines (Magnúsdóttir et al., 2013; Nakaki et al., 2013).

Our recent work has focused on the mechanism of human PGC specification, which occurs during week 2 of gestation, and therefore cannot be directly investigated in early human embryos. Based on mouse studies, hESCs could be used to examine induction of PGC-like fate in vitro although the mouse model does not work with