MOLECULAR BIOLOGY & GENETICS

Special Topic: Stem Cell Research in China

New stem cell types for versatile applications

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Haploids, being widespread in lower organisms like bacteria and fungi, can efficiently reveal the recessive traits due to the lack of second compensation allele, and thus have been widely used in biological research [1]. However, haploidy only exists in highly specialized gametes in mammals, posing huge challenges for its application in mammalian biology. Recently, the haploid cell lines, in the form of different types of stem cells, have been successfully generated in multiple mammalian species including mouse, rat, monkey and human [2–9]. These new cell types include the parthenogenetic and androgenetic haploid embryonic stem cells (phESCs and ahESCs) that are derived from the haploid blastocyst containing a single oocyte or sperm genome, respectively; and the interspecies allodiploid embryonic stem cells (AdESCs) that are generated by fusing haploid ESCs from two distantly related mammalian species [10]. All these new types of ESCs are pluripotent and capable of differentiating into cells of all three germ layers, and thus can further produce many haploid or allodiploid somatic cell types. Here, we briefly discuss the applications of these new types of stem cells with such a unique genome composition.

FUNCTIONAL GENOMICS STUDIES AT BOTH CELLULAR AND ANIMAL LEVELS

Haploids are valuable resources for functional genomics studies as their genome complexity is much reduced [11]. The mammalian haploid ESCs combine two unique advantages—rapid proliferation rate and haploidy—which enable the rapid generation of a genome-wide mutant cell library via gene-trapping technology, and therefore can provide a convenient approach for large-scale genetic screening. In comparison to other high-throughput genetic-screening approaches, such as RNA interference (RNAi) and the CRISPR/Cas system, haploid ESC-base screening is more easy to handle, as it only needs a universal gene-trapping vector to produce the mutant library, bypassing the preparation of a collection of sequences to specifically target each gene of the library using the RNAi or CRISPR system. Moreover, the RNAi and CRISPR approaches are not applicable for screening the phenotypic

mutations caused by single-nucleotide variants (SNVs). However, the haploid ESCs are also able to produce global SNV mutant libraries using chemical induction for further screening [12]. Despite these advantages, there is still a challenge for genetic screening with haploid ESCs, namely the spontaneous diploidization of haploid ESCs during culture and differentiation. Currently, why the haploid ESCs undergo diplodization remains unknownand needs to be studied in the future. However, some haploid ESC lines tend to adapt more to the haploid state after repeated purification by fluorescence-activated cell sorting, which can be selected out for screening. Besides global genetic screening and specific gene loss-of-function studies at the cellular level, the haploid ESCs can also extend the functional genomics approach to the animal level through rapid generation of genome-modified animals [4,5,13]. The ahESCs can functionally take the place of sperms to produce live offspring after injection into the oocytes, which allows the direct transmission of genome modifications into the organism level without further time-consuming steps such as the conventional germline transmission. This capacity is especially important for large animals and non-human primates, as efficient technologies for their genome modification are still largely needed. Now monkey phESCs have been derived, however, ahESCs have been derived, as the efficiency of generating androgenetic haploid blastocysts is only half that of generating parthenogenetic haploid blastocysts due to the lethality of embryos without the X chromosome. Hence, in future, the generation of monkey ahESCs and analysis of their capacity to take the place of sperms are worth trying for monkey genome engineering.

STUDIES ON GENOME REGULATION AND EVOLUTION

The interspecific hybrids with heterozygous genetic background and unique traits have been widely used for the mechanistic studies of speciation, evolution, gene expression regulation and X-chromosome inactivation. However, naturally occurring interspecific hybrids are very rare in mammals. The generation of mammalian AdESCs provides a new approach for generating mammalian interspecific allodiploid cells by directly fusing the haploid ESCs from the two species, which bypasses the reproductive isolations between gametes of evolutionarily distant related species [10]. Such AdESCs can maintain a stable allodiploid genome, self-renew indefinitely and differentiate into all three germ layers. They may have applications in the following biological research areas. First, they provide new models for the studies of gene-regulation differences across species, which have been proven to contribute to evolution. A common approach for such studies uses comparative gene expression analysis across species, which can hardly control the environmental issues that affect gene expression. The AdESCs have the different genomes in the same nuclear environment, thereby eliminating the uncontrolled issues. Moreover, they can directly reveal the interactions of divergent genomes and the allelic expression levels, which are informative for determining the *cis*- and *trans*-effects on the evolution of gene-regulation differences. Second, the AdESCs and their derivatives can be utilized to identify the genetic basis of phenotypic differences across species. Heterosis studies have revealed that the non-additively expressed genes in the hybrids can contribute to new phenotypes such as hybrid vigor. Based on this, the phenotypic differences between the two species are more correlated with and potentially determined by the non-additively expressed genes in allodiploid cells. Third, the AdESCs and their derivatives have a large number of distinguishable alleles and show species-specific X-chromosome inactivation (Xi), so they can be used to study the X-inactivation mechanisms and to identify the Xi-escaping genes.

EPIGENETICS AND DEVELOPMENTAL BIOLOGY

The ahESCs and phESCs can take the place of sperms and oocyte-genomes to produce fertile animals, respectively. Compared with the mature gametes, they are easily engineered and can proliferate *in vitro* indefinitely, thus providing a convenient platform to study the effects of genetic and epigenetic issues on animal development, such as to study genomic imprinting—an epigenetic phenomenon that results in parent-origin-specific expression of the imprinted genes and functional nonequivalence of parental genomes [14,15]. The imprinted expression pattern can be re-established in haploid ESCs by genetic deletion of a specific imprinting control region (ICR), such as to 'writie' genomic imprints on haploid ESC genomes. The functions of the genome imprints thus can be directly reflected by the developmental potential of reconstructed embryos between oocytes and haploid ESCs carrying these imprints. Indeed, after 'writing' the paternal imprints and erasing the maternal imprints on phESCs, the modified phESCs can efficiently produce bi-maternal pups after injection into oocytes, demonstrating that the major barrier of mammalian parthenogenesis is genomic imprinting. The other interesting potential application of haploid ESCs is to study the epigenetic features of the mature sperm. Although the ahESCs can take the place of the sperm to support fetal development, the developmental efficiency of ahESC-derived embryos is much lower than that of normal fertilized embryos [4,5]. It suggests that, besides haploidy and genomic imprinting, some epigenetic features carried by the mature sperm, which are still largely unknown, are important for the establishment of authentic totipotency during fertilization. Comparative studies between the ahESCs and mature sperms may shed light on these unknown determinants. Moreover, the functions of such determinants can also be experimentally examined by 'writing' them on ahESCs to improve the developmental efficiency of embryos constructed via injection of an ahESC into an oocyte.

OUTLOOK

The mammalian haploid ESCs and AdESCs have versatile applications in a wide range of biological research fields. With the rapid development of genome

sequencing and engineering technologies, these unique cell types may deepen our understanding of the evolution and function of the mammalian genome. Moreover, the tolerance of haploidy and allodiploidy of mammalian cells points to the mammalian genome being able to actually tolerate more modifications while still maintaining functions, which may boost inspiration to construct and synthesize a minimal mammalian genome.

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