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Haploid Human Embryonic Stem Cells: Half the Genome, Double the Value

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Recent advances in the generation of haploid embryonic stem cells (ESCs), capable of self-renewal and differentiation, have laid the groundwork for numerous biomedical applications in developmental biology and reproductive medicine. When combined with the power of genetic screening, haploid human ESCs could advance cancer research, regenerative medicine, and disease modeling.

The cells in our bodies are diploid, carrying two sets of 23 chromosomes; one set is inherited from the mother and the other from the father. Haploid cells that contain a single set of chromosomes occur only as post-meiotic germ cells, namely egg or sperm. While diploid cells can undergo mitosis and give rise to additional diploid cells, haploid germ cells are terminally differentiated cells incapable of producing progeny by cell division. Nonetheless, haploid mammalian eggs can be artificially induced to divide as early haploid embryos, which remarkably allow the derivation of self-renewing haploid embryonic stem cells (ESCs). Following the initial reports on haploid ESCs from mouse (Leeb and Wutz, 2011; Elling et al., 2011), and later from rat and monkey (reviewed in Wutz, 2014), haploid ESCs have also been recently derived from humans (Sagi et al., 2016a). Surprisingly, and as opposed to their murine counterparts that failed to differentiate into haploid somatic cells, haploid human ESCs readily differentiate into cells of all three embryonic germ layers while remaining haploid (Sagi et al., 2016a). Notably, both the derivation and differentiation potential of haploid human ESCs have been confirmed in a recent report (Zhong et al., 2016). Interestingly, the derivation of haploid cells can be achieved from artificially activated human oocytes (Sagi et al., 2016a), or after removal of the male pronucleus from fertilized oocytes (Zhong et al., 2016). Combining the advantages of haploid genetics and pluripotency, haploid human ESCs now pave the way for a variety of new biomedical applications.

Applications in Developmental Biology and Reproductive Medicine

Haploid human ESCs can be utilized to address basic questions in multiple fields of developmental biology (Figure 1). For example, the ability to potentially obtain any type of haploid human somatic cells (including haploid neurons, cardiomyocytes, and gut cells; Sagi et al., 2016a, Zhong et al., 2016), particularly in contrast to the restricted developmental capacity of haploid mouse cells, may allow investigation into evolutionary differences among mammals regarding the tolerance to haploidy within the context of cellular differentiation. In addition, as haploid human ESCs continuously undergo diploidization in culture at a fairly high rate (estimated as up to 9% of cells per cell cycle) (Sagi et al., 2016a, 2016b), they may also be valuable for delineating the basis of ploidy changes in human development and disease, as well as providing insight as to why haploid cells eventually become diploid while diploid cells do not become tetraploid. Haploid human ESCs can also be useful for studying epigenetic mechanisms such as X chromosome inactivation and parental imprinting during differentiation and development, owing to their uniparental nature and because they are female cells harboring a single X chromosome.

Haploid human ESCs hold a distinctive potential for reproductive medicine (Figure 1). As pluripotent cells, mammalian ESCs can differentiate into any cell type specified from the epiblast, including the germline. However, even when the cells are cultured to differentiate into primordial germ cells, their final maturation into haploid cells necessitates meiosis. With haploid human ESCs the necessity for meiosis is bypassed, and it is possible that the generation of haploid germ cells in vitro may be considerably simplified once the protocols for terminal human germ cell differentiation are refined. Although the diploidization of haploid human ESCs poses a limitation to their use in prolonged and multistep differentiation protocols, this may be overcome by enriching the haploid cells through occasional cell sorting. The ability to obtain haploid germ cells in culture would create new possibilities for reproductive medicine, for instance, by allowing the establishment of egg repositories for both research-oriented and medically oriented purposes. As another source of human pluripotent cells, haploid human ESCs could also be used in regenerative medicine (Figure 1). Notably, haploid cells carry single alleles of the major histocompatibility complex (MHC) genes (rather than two), which significantly reduces their immunological complexity compared with diploid cells. Thus, allogeneic haploid cells may provide a better match in cell therapy procedures. However, since they arise from parthenogenesis and lack paternally expressed genes, the differentiation of haploid human ESCs into certain cell types might be aberrant and not optimal for transplantation (Stelzer et al., 2011).

Genetic Screening Strategies using Haploid Human ESCs

Aside from the biomedical applications envisioned thus far, the most prominent utility of haploid human ESCs would be through genetic screening. Screening for

loss-of-function (LOF) phenotypes is rather limited in diploid cells, because even if a mutation is introduced into one allele the second intact allele may serve as a backup. Therefore, recessive mutations are likely to be underrepresented among the screened phenotypes in diploid cells. For this reason, many global LOF screens were classically performed in haploid yeast, and only recently has the derivation of mouse haploid ESCs enabled similar screens in non-human mammals (Wutz, 2014). Previous screens in mouse haploid ESCs were aimed at identification of genes that confer resistance to specific drugs or toxins as well as components of biological pathways such as the DNA mismatch repair pathway or the exit from ESC self-renewal (reviewed in Wutz, 2014). Several years ago, a near-haploid leukemic cell line pioneered LOF screening in humans, leading to the identification of host factors exploited by human pathogens (Carette et al., 2009). More recently, these cells have also been utilized for identifying essential genes in the human genome and for studying synthetic lethality between different genes (Blomen et al., 2015; Wang et al., 2015). Now, the availability of haploid ESCs has introduced LOF genetic screenings in human pluripotent cells (Sagi et al., 2016a). By virtue of their normal haploid karyotype and their capacity for multilineage differentiation, these cells provide new opportunities for functional genomics that could advance our comprehension of human biology in health and disease.

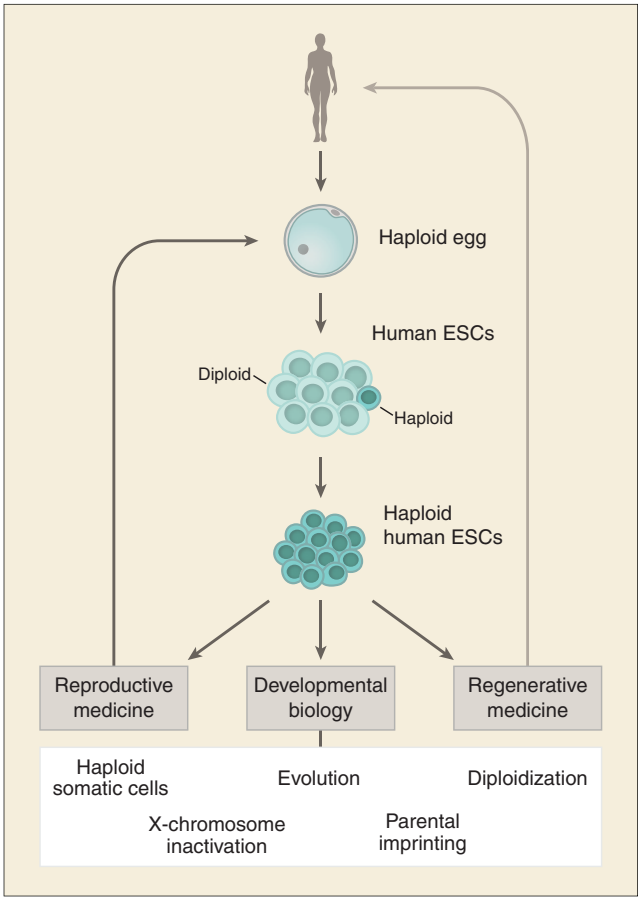

Figure 1. Potential Applications of Haploid Human ESCs in Reproductive Medicine, Developmental Biology, and Regenerative Medicine

Haploid human ESCs can be isolated from mixed populations of haploid and diploid cells within parthenogenetic ESC lines that originate from a haploid egg. These cells could help in the study of various aspects of developmental biology (see text). As haploid pluripotent cells, they might also assist in the derivation of haploid germ cells for reproductive purposes. Their increased immunocompatibility could be utilized for cell-based therapies in the context of regenerative medicine.

Global LOF screening can be performed by various methodologies. One such methodology is based on random insertions of DNA cassettes into the genome (i.e., transposon-mediated insertional mutagenesis), which usually confers LOF of a gene by inducing an aberrant splicing event and preventing the synthesis of the normal gene product. Another methodology relies on the targeted introduction of a double-stranded DNA break at a specific sequence in the genome (i.e., by nuclease-mediated targeted mutagenesis, such as with the CRISPR-Cas9 system). Repairing such breaks is typically accompanied by nucleotide deletions or insertions, potentially leading to LOF of the specific gene due to frameshift or deleterious alteration of the amino acid sequence. Generating transposon-based genome-wide mutant libraries requires the delivery of a single DNA cassette randomly into the genome of the cells, which makes this methodology readily applicable without being limited to insertions in known target sequences. Yet it is often more difficult to control the repertoire of mutated genes in this system. In contrast, nuclease-mediated mutant libraries are generated through more laborious steps of preparing a collection of sequence-specific targeting molecules, such as small guide RNAs for the CRISPR-Cas9 system, but this methodology is better suited to targeting precise loci in the genome. The advantages and disadvantages of these two methodologies make them complementary, and they can indeed be used in parallel (Wang et al., 2015). Importantly, in both methodologies generating LOF mutations in haploid cells significantly increases the chances of performing a comprehensive functional genetic screen, since obtaining homozygous mutations in diploid cells is considerably less efficient in both systems.

Haploid human ESCs can be used as a platform for both positive and negative selection screens (Figure 2). It is worth noting that although haploid ESCs inevitably become diploid over time, LOF phenotypes are in principle retained in diploids as endoreduplication results in homozygous mutations. Once a library of LOF mutant clones is prepared, the most straightforward application is to identify genes whose loss provides cells with a growth advantage under a given selective pressure. In this case, cells with mutations in specific genes continue to proliferate while the rest die or grow significantly slower (positive selection). An example for this type of screen in haploid human ESCs has already been demonstrated by screening for resistance to the drug 6-thioguanine (Sagi et al., 2016a). Similarly, positive selection screens would be useful for studying resistance to chemotherapy drugs, with potential implications for cancer therapy. As opposed to positive selection, where clones surviving the selective pressure are sought, screening

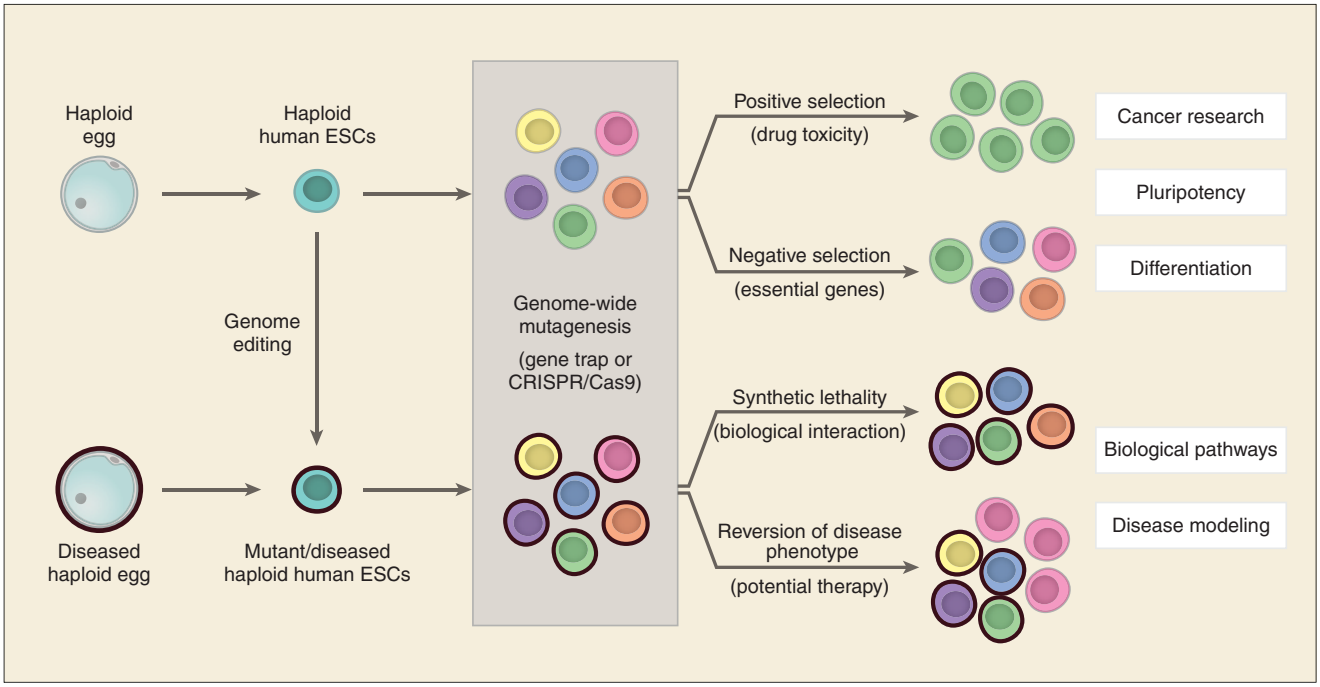

Figure 2. Strategies for Utilizing Haploid Human ESCs for Genetic Screening

Genome-wide mutations can be introduced into human haploid ESCs by different methodologies including insertional mutagenesis and targeted mutagenesis (i.e., using gene trap or CRISPR-Cas9 technologies, respectively). Libraries of mutant cells can then be subjected to different treatments followed by analysis of surviving clones, aiming to identify genes whose loss affords a growth advantage (positive selection) or disadvantage (negative selection). A similar approach is also applicable for haploid ESCs already carrying specific mutations, which can be isolated either from diseased eggs or generated by genome editing. In such cells, genome-wide mutagenesis can be performed to identify synthetic lethality interactions between the pre-existing mutation and a novel mutation within the library, based on mutual exclusivity. Alternatively, using diseased haploid ESCs can reveal genes whose loss leads to reversion of the disease phenotype (the black outline denotes cells with a disease phenotype, and the light gray outline denotes cells with a reverted phenotype).

can also entail negative selection, aiming to identify mutant clones whose growth is restricted or completely abolished. For instance, LOF mutations in essential genes are expected to result in cell death. Thus, in saturated libraries targeting mutations in all genes, those that are underrepresented or absent from the library can be identified as essential. Searching for missing clones in haploid human ESCs or their differentiated derivatives could potentially point to genes that are absolutely necessary for pluripotency or differentiation. In turn, this could enhance our understanding of cell state transitions and their maintenance and allow improvement of differentiation procedures for cell-based therapies in regenerative medicine.

Another screening strategy involves the isolation of haploid ESCs using donor eggs carrying a genetic defect or the introduction of a specific genetic alteration into normal haploid ESCs by genome editing, prior to constructing a genome-wide library of mutant clones (Figure 2). Such libraries could serve in a synthetic lethality assay, where single mutations in either one of two specific genes are viable, but are lethal if co-occurring in the same cell. This type of assay may uncover functional interactions between the mutated gene of interest and any other gene in the genome, as the latter would be depleted from the library in case of synthetic lethality. A related strategy may be beneficial for modeling genetic diseases. Here, the library is generated in haploid ESCs carrying mutations associated with a specific genetic disorder (again, either by deriving them directly from diseased eggs or by genome editing). Correction of the disease phenotype can then be screened for after genome-wide mutagenesis, and genes whose LOF reverts or ameliorates this phenotype can be identified. Taken together, genetic screening in haploid human ESCs or their differentiated progeny may be highly relevant for cancer research; for the study of pluripotency, cellular differentiation, and biological interactions; and for pinpointing genes involved in disease phenotypes and therapy.

Challenges Ahead

Although haploid human ESCs are valuable in studying human development and germ cell differentiation, and especially as a tool in LOF genetic screening, there are various obstacles in using them. Haploid ESCs constantly convert into diploid cells at a rather high rate, and their maintenance in culture requires repeated purification (Sagi et al., 2016a, 2016b). In addition, these cells are uniparental and therefore lack the ability to express a subset of imprinted genes, which might affect their phenotype as undifferentiated cells as well as their differentiation capacity (Stelzer et al., 2011). Nonetheless, we expect that haploid human ESCs will be employed for multiple purposes as outlined above and in Figures 1 and 2. The ability to mutate the haploid human genome and perform large-scale genetic screens should enhance our ability to study genotype-phenotype interactions in the context of human development and disease.

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