

From Embryonic Stem Cells to Blastoids - Embryo-Like Structures Derived from Induced Pluripotent Stem Cells

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EMBRYONIC AND ADULT STEM CELLS

Stem cells have entered the center stage of biomedical research for more than two decades and are key to the study of their biology and differentiation, of early human development as well as their therapeutic potential.

Stem cells are somatic cells which are naturally generated by meiosis from diploid cells and conjugation of a haploid egg cell (22 autosomes and 1 sex chromosome X) with a haploid sperm cell (22 autosomes and 1 sex chromosome X or Y) in the process of fertilization. The resulting diploid chromosomal set is made up of 44 autosomes and 2 sex chromosomes XX (female karyotype) or XY (male karyotype). The fertilized egg cell divides to yield a 2-, 4-, 8-, 16-, 32- ... -cell stage to form - through the morula, blastomere and blastocyst (day 6-14) stage- within 84 days the embryo and subsequently the fetus with delivery of the newborn on day 273-281 (**Figure 1A**).

Stem cells are somatic cells with different proliferation and differentiation potential. Embryonic stem cells (ES) are totipotent or pluripotent and are derived prenatally from blastocysts. Adult stem cells (AS) are multipotent and can be isolated postnatally from peripheral blood and many tissues or organs and can be used for different therapeutic applications after *in vitro* differentiation into the desired cell types and tissues ('tissue engineering'). With the development from embryo to fetus the stem cells lose their totipotency (blastomere stage: ability to form all cell types, including oocytes and sperm cells) and become pluripotent (blastocyst stage: ability to form all cell types of the embryo, fetus and newborn/ adult) and can differentiate into several cell types (multipotency). Differentiated cells, such as skin, liver or myocardial cells, are unipotent and can, under physiological conditions, reproduce only the same cell type.

Milestones of stem cell research were the characterization of the first ES with hematopoietic potential in mice (mES) in 1981 [1] for which Sir Martin Evans (UK) was awarded the Nobel Prize for Physiology or Medicine in 2007 together M. Capecchi and O. Smithies (both USA).

CLONING BY NUCLEAR TRANSFER

A further milestone of stem cell research was the cloning of the sheep 'Dolly' by nuclear transfer from an adult sheep skin cell into an enucleated egg cell [2,3] and the generation of human embryonic stem cells (hES) [4] or of primate embryonic stem cells (pES) [5]. From blastocysts of monkey embryos two ES lines were isolated with all characteristics of ES (DNA identical to skin cell 'donor' DNA, mitochondrial DNA of the egg cell, differentiation *in vitro* into myocardial and nerve cells and *in vivo* into teratomas which are derived from ectoderm, endoderm and mesoderm). These data demonstrate that it is possible to reprogram adult somatic cells into pluripotent stem cells by nuclear transfer and to clone primates for therapeutic purposes, a strategy that in principle is also applicable in humans [6]. While this is prohibited by the 2016 Guidelines for Stem Cell Research and Clinical Translation issued by the International Society for Stem Cell Research (ISSCR) this strategy has, for example, been successfully applied to clone a Banteng cow, a relative of the domesticated cattle and an endangered species. Obviously, this strategy could be extended to other endangered species while cloning of pets is at least debatable.

INDUCED PLURIPOTENT STEM CELLS

In 2006 Takahashi and Yamanaka from the University of Kyoto/ Japan reported another milestone in stem cell research (**Figure 1B**): using unipotent fibroblasts from adult mice they could reprogram them *in vitro* to so-called induced pluripotent stem cells (iPSCs) by retroviral transduction and integration of 4 genes into the cellular genome (mOct4, mSox2, mKlf4 and mc-Myc) which were

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almost identical to mES [7]. They extended these findings with human fibroblasts [8] which were independently reported by Yu et al. at the University of Wisconsin-Madison, Madison, USA [9]. The genes' code for transcription factors involved in the control of the 'selfrenewal' of mES and hES, respectively [10]. Subsequently, the generation of IPS has been independently reproduced and optimized [11-13].

The hIPS were practically indistinguishable from hES with respect to morphology, proliferation kinetics, surface markers, the epigenetic make-up specific for pluripotent cells, and telomerase activity. Further, IPS differentiated into cells of ectoderm, endoderm and mesoderm as well as into teratomas. The Kyoto group could further show that IPS can also be generated by transduction of only 3 genes (hOct4, hSox2 and hKlf4, without mc-Myc gene) into adult human or mouse fibroblasts [14]. This strategy was somewhat less efficient than the transduction of 4 genes, but at the same time it was more specific with fewer tumors developing (0% versus 16% after 4-gene transduction).

For the generation of mIPS and hIPS by reprogramming of adult cells S. Yamanaka received the Nobel Prize for Physiology or Medicine in 2012 together with J. Gordon from the UK.

The discovery of Yamanaka and his team at the University of Kyoto [7,8] and of James A. Thomson and his team at the University of Wisconsin-Madison, Madison, USA [9] revolutionized stem cell research by making the generation of hES independent from human embryos. IPS have a tremendous potential for the molecular and biologic study of the pathogenesis of human diseases and their treatment, such as sickle cell anemia in a mouse model [15]. Further, IPS can be considered as autologous cells with the potential to serve as source for cell and organ transplantation ('regenerative medicine'), theoretically without the risk of rejection. On the other hand, the retroviral or lentiviral transfer and chromosomal integration of genes conferring pluripotency to the IPS in principle carries a risk for chromosomal rearrangements, including a malignant transformation. Therefore, problems associated with using retroviruses and oncogenes for reprogramming need to be resolved before IPS cells are considered for use in humans.

FROM IPS TO BLASTOIDS: A NOVEL PLATFORM FOR THE STUDY OF EARLY HUMAN DEVELOPMENT

Recently, a novel milestone in the field of early human development was independently reported by a research team at the University of Texas Southwestern Medical Center, Dallas, TX, USA [16] and scientists at Monash University, Victoria, Australia, respectively [17]. By reprogramming human fibroblasts *in vitro* to IPS and applying a three-dimensional culture strategy, it was possible to generate blastocyst-like structures (Figure 1C), termed 'human

blastoids' and 'iBlastoids', respectively [16,17]. The characterization of the blastocyst-like structures revealed an inner cell mass made up of epiblasts -that develop into the embryo/ fetus- and hypoblasts -that develop into the embryonal egg yolk sac- as well as trophoblasts -that will become part of the placenta- very similar to human blastocysts (Figure 2). They further very much resemble human blastocysts (Figure 1A), among others, in terms of morphology, size, cell number and allocation of different cell lineages. They recapitulate key morphogenetic events that occur during human peri-implantation development. Single-cell transcriptomic analyses confirmed the presence of epiblast-, endoderm- and trophoectoderm-like cells. In addition, iBlastoids give rise to pluripotent and trophoblast stem cells and allow to model several aspects of the early stage of implantation *in vitro* [17].

While stem-cell-derived blastocyst-like structures have initially been generated in mice that allowed the analyses several pre- and peri-implantation developmental processes *in vitro* [18, 19] the novelty of the strategy lies in the extension to human blastoids [16, 17]. Human blastoids were shown to be an alternative to blastocysts for studying early human development. While several limitations still exist (variable derivation efficiency, low efficiency of generating early post-implantation structures and others), human blastoids provide a valuable and versatile model for basic and translational research as an alternative to human blastocysts which require fertilized human eggs (Figure 1A). The 2016 Guidelines for Stem Cell Research and Clinical Translation issued by the International Society for Stem Cell Research (ISSCR) do not permit research on cultures of human embryos or embryo-like structures beyond 14 days post-fertilization. The 2016 Guidelines for Stem Cell Research and Clinical Translation issued by the ISSCR are presently under revision to define the status of blastoids and their use.

SUMMARY AND PERSPECTIVES

Stem cells have entered the center stage of biomedical research for more than the two decades and are key to the study of their biology and differentiation, of early human development as well as their therapeutic potential. Milestones of stem cell research were the discovery of the embryonal stem cells (ES) with hematopoietic potential in mice (mES) [1], the cloning of animals by nuclear transfer [2,3] and the generation of human embryonic stem cells (hES) [4] or of primate embryonic stem cells (pES) by nuclear transfer from a skin cell into an egg cell from which the nucleus had been removed [5]. Another major milestone was the reprogramming of adult cells, such as keratinocytes derived from a skin biopsy, by viral transduction of genes to generate iPS [7-9], that have a tremendous potential in basic as well as translational sciences.

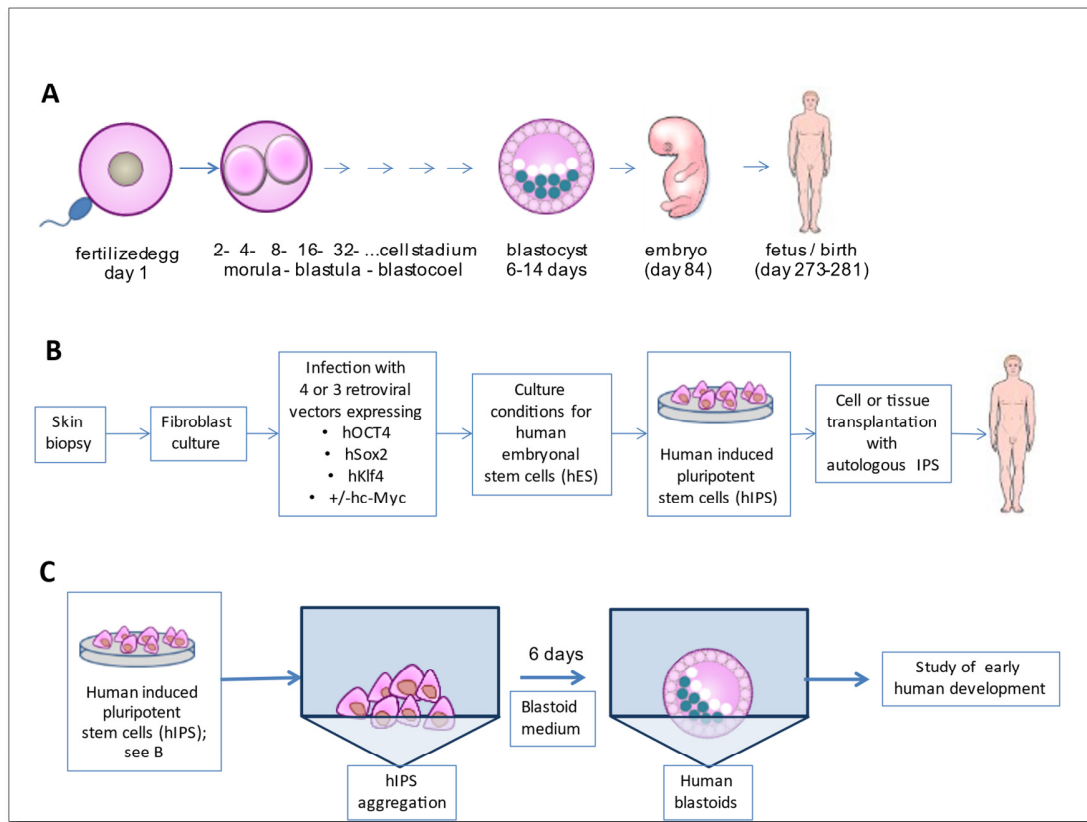


Figure 1A. Human development *in utero* through the following stages: fertilized egg (day 1) - morula - blastomere - blastocyst (day 6-14) - embryo (day 84) - fetus/ delivery of newborn (day 273-281). **Figure 1B.** Principle of *in vitro* reprogramming of fibroblasts (adult cells) to yield induced pluripotent stem cells (IPS). Skin biopsy and *in vitro* culture of fibroblasts, and retroviral transduction of 4 or 3 genes and culture under conditions for human embryonal stem cells (hES) results in reprogramming of fibroblasts to IPS. **Figure 1C.** Generation of blastocyst-like structures from human pluripotent stem cells (IPS). Continued from IPS in **Figure 1B**. When aggregated, IPS form within about 6 days -through differentiation and self-organization- cellular structures that resemble human blastocysts and are termed blastoids or iblastoids.

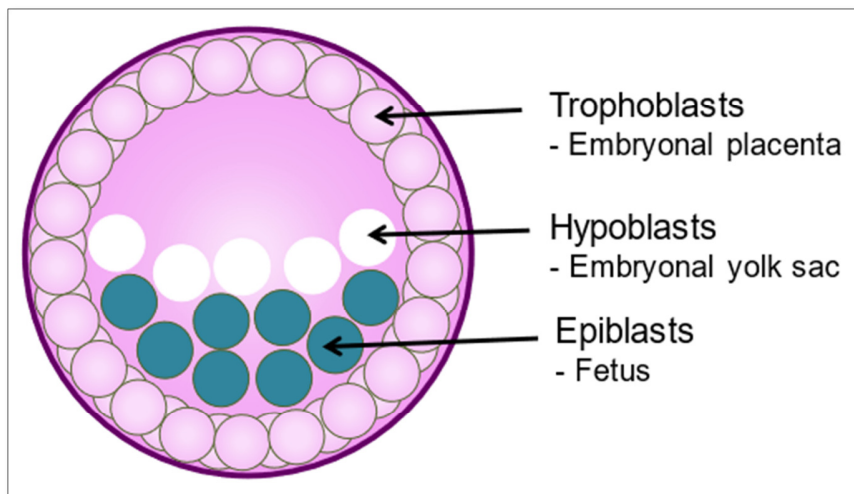


Figure 2. Blastocyst biology.

Blastoids are the most recent milestone in stem cell research documented by two independent seminal publications [16,17]. As lab-grown structures human blastoids mimic human embryo's earliest stage of development [20]. They are *in vitro* models for embryo development and can be studied as an alternative to human embryos. Human blastoids provide a readily accessible and versatile alternative to blastocysts and are expected to give insights into different aspects of early human development, including early pregnancy loss, early developmental defects, effects of gene mutations and toxins during early embryogenesis, as well as the development of new therapies in the context of *in vitro* fertilization.

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