Totipotent of Stem Cell

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Abstract: The definition of stem cell is "an unspecialized cell that gives rise to a specific specialized cell, such as a blood cell". Embryonic stem cells are derived from the inner cell mass of blastocyst stage embryos. Somatic stem cells differentiate into only the cells the specific tissue wherein they reside. Stem Cell is the original of life. All cells come from stem cells. The totipotent of stem cell is the most important property for the stem cell to keep the life continuously. Under the clone principle, all the cells can develop into an entire alive animal body that is same as the baby comes from the fertilized egg – the zygote. It is important to reverse the non-totipotent cell to the totipotent, which is the lifeline reverse that make the animal growth from old to younger.
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Introduction

In the biology field, the totipotency is the ability that a cell can divide and produce a new organism. This means that a single cell has an integrate genes to differentiate to a whole living body (Baud, 2005). Totipotent cells can be any cell in a body (Cantley, 2005). Normally, the differentiation is one direction where an undifferentiated cell (especially stem cell) can differentiate into differentiated cells but differentiated cells cannot reverse to the immatured cells. However, under certain condition, this direction can reverse, especially in plant and low class of the life species. A plant cutting or callus can grow to an entire plant and this appears everywhere in the earth. Many plants reproduce for next generation through schedule and it is widely used in the agriculture. In the animal field, this reverse differentiation normally is not exist, but and only case of reverse differentiation happens in the jellyfish Turritopsis nutricula through the transdifferentiation (Ma, et al, 2011).

The zygote is totipotent, which is the beginning of an animal body. A human begins a zygote that a sperm fertilizes an egg and creates the single totipotent cell. In the beginning hours after fertilization of the human, this cell divides into identical totipotent cells, which can later develop into any of the 3 germ layers of a human (endoderm, mesoderm, or ectoderm) and into cells of the cytotrophoblast layer or syncytiotrophoblast layer of the placenta. After reaching the 16-cell stage, the totipotent cells of the morula differentiate into cells. The differentiated cells will become either the blastocyst's inner cell mass or the outer trophoblasts. Approximately 4 days after the fertilization and a few cell divisions, these totipotent cells will be specialized, then lose the totipotent ability.

Totipotent is the most important point for the stem cells that initiates the whole creature body. In creature development, the egg cell in a lady and the sperm from a man fuses jointly to become a free chamber zygote. The zygote divides many times and forms cells that are the precursors to the trillions of cells as the bricks of the creature body.

Totipotent stem cell can differentiate into all the human body's cells (about 200 types). In most animals, the only true totipotent stem cell is the fertilized egg and its immediate descendants. A totipotent stem cell can potentially generate a complete organism. In some cases, cells can regain totipotency. A plant cutting or callus can be used to grow an entire plant. The plant is different from animal in this point. The totipotency regaining is a potential ability for the life to reverse the lifeline.

Differentiation results from differential gene expression. In order to clone an animal, such as a sheep or a mouse or a rat, udder cells are removed from a ewe and starved for one week to cause G0 arrest. Nuclei from arrested ewe udder cells are fused with enucleated eggs from a ewe, and then stimulated to re-enter the cell cycle. After a few rounds of cell division, the embryo is transplanted into a surrogate sheep mother. The sheep that is born is genetically identical to the ewe from that the nucleus obtains. Clone technique is an important technique in modern biotechnology.

Cellular determination is important in the animal development. Cellular determination results from the asymmetric segregation of cellular determinants. However, normally determination is the

result of inductive signaling between cells. Asymmetric segregation of cellular determinants is caused from the asymmetric localization of cytoplasmic molecules within the cell before dividing. During cell division procedure, one daughter cell receives more localized molecules and the other daughter cell may receive less of these molecules, which results in two different daughter cells taking on different cell fates based on differences in gene expression. The different gen differentiation comes from different gene expression factors that make the genes' differentiate under different condition. The localized cytoplasmic determinants are often transcription factors or mRNAs encoding by the transcription factors.

The field of stem cell biology has undergone tremendous expansion over the past years. Scientific investigation has continued to expand our understanding of these complex cells at a rapidly increasing rate. This understanding has produced a vast array of potential clinical applications (Hemmat, Lieberman et al. 2010).

The direct induction of adventitious buds and somatic embryos from explants is a morphogenetic process that is under the influence of exogenous plant growth regulators and its interactions with endogenous phytohormones (de Almeida, de Almeida et al. 2012).

The ontogeny is also related to de-differentiated mesophyll cells that acquire totipotency and form the majority of embryos (Wang, Nolan et al. 2011).

Somatic cell nuclear transfer (SCNT) is a technically and biologically challenging procedure during which a differentiated committed nucleus undergoes rapid reprogramming into the totipotent state in a few hours (Shufaro and Reubinoff 2011).

Primordial germ cells (PGCs), the precursors of sperm and eggs, are the route to totipotency and require establishment of a unique epigenome in this lineage. The genetic program for PGC specification in the mouse also initiates epigenetic reprogramming that continues when PGCs migrate into the developing gonads. Among these later events is active and genome-wide DNA demethylation, which is linked to extensive chromatin remodeling (Surani and Hajkova 2010).

In many tissues, mammalian aging is associated with a decline in the replicative and functional capacity of somatic stem cells and other self-renewing compartments. Understanding the basis of this decline is a major goal of aging research (Sharpless 2010).

The octamer-binding transcription factor 4 (Oct-4) plays important role in totipotent cells differentiation. Oct-4 expressed in totipotent embryonic cells and germ cells. As totipotent cells differentiate somatic cells, Oct-4 gene is downregulated. Oct-4 expression is maintained after postgastrulation in primordial germ cells. Oct-4 is necessary for embryonic cells to keep the totipotent status keep from their differentiation to somatic cells. (Pesce M, Schöler HR., 2000).

Example of the totipotent protocols - Proliferation of totipotent hematopoietic stem cells in vitro with retention of long-term competitive in vivo reconstituting ability (Christopher, 1992):

Recombinant Tkneol9 virus at a titer of 1 x 10^6 per ml is generated from a T-2 producer line maintained in 10% calf serum/Dulbecco's modified Eagle's medium. Marrow cells from adult male (C57BL/6J x C3H/HeJ) F1 (B6C3F1) mice injected i.v. 4 days earlier with 5-fluorouracil (5-FU, 150 mg/kg of body weight) are infected with Tkneol9 virus using a supernatant infection protocol in which 3-5 x 10^6 marrow cells are cultured for 24 hr in 100-mm Petri dishes in 10 ml of virus supernatant containing Polybrene at 4 jug/ml, 5% pokeweed mitogen-stimulated spleen cell-conditioned medium, and 10% agar-stimulated human leukocyte-conditioned medium as described. Cells are then recovered by gentle agitation and scraping of dishes with a rubber policeman, essential Eagle medium and resuspended in LTC medium [a medium/10% horse serum/10% fetal calf serum/10^{-6} M sodium hydrocortisone hemisuccinate/10^{-4} M 2-mercaptoethanol]. Aliquots of 3 x 10^6 cells are overlaid on previously established 3-wk-old long-term B6C3F1 female marrow adherent layers that had been irradiated with 15 Gy [250 kilovolt peak (kVp) x-rays] to inactivate persisting hematopoietic cells (Fraser, 1990). LTC are maintained by weekly removal of half of the medium and nonadherent cells and restoration of the volume with fresh LTC medium. To assay cells in LTC for repopulating cells, adherent layers are removed with a rubber policeman, suspended by passage through a 21-gauge needle and then combined with the nonadherent cells. Cells are washed once in 2% fetal calf serum/a-minimal essential medium, and aliquots from individual culture flasks are then injected i.v. into irradiated (8 Gy, 250 kVp x-rays) female recipients. In some cases, 2 x 10^5 female B6C3F1 marrow cells that had been previously subjected to two cycles of transplantation and regeneration are also injected to allow quantitative measurements of CRU in the retrovirally marked test cells to be obtained. For cultures that are used to assess recovery of repopulating cells in the nonadherent fraction over time, all of the medium and nonadherent cells are removed weekly and replaced either with fresh medium alone or with LTC medium containing 25 units per ml of recombinant mouse interleukin-3 (IL-3). The nonadherent cells removed

after 3, 5, 6, and 7 weeks of culture are then injected into irradiated recipients. Hematopoietic Tissue Analysis. Recipients are sacrificed either 5 wk or between 5 and 7 months after transplantation of cultured cells. DNA is routinely extracted from marrow, spleen, and thymus. In some cases, DNA is also extracted from lymph nodes and from various subpopulations of marrow and spleen. Highly enriched (>90%) mast cell populations are generated by culturing marrow or spleen cells for 3 wk in WEHI-3B-supplemented medium (as a source of IL-3), and highly enriched (>95% Mac-i-positive) macrophage populations are generated by culturing marrow or spleen cells for 48 hr in medium supplemented with 10% WEHI-3B-conditioned medium and then for 7-10 days in medium supplemented with 35% human leukocyte-conditioned medium. Highly enriched (>90% Thy-i-positive) T-cell populations are obtained by elution of nonadherent cells after loading of a half-spleen equivalent onto a 3-ml nylon wool column and incubating it for 1 hr at 37°C. Nylon wool adherent cells are then released by gentle agitation for 2-3 min in phosphate- buffered saline/10 mM EDTA and a highly enriched (>90% B220-positive) population of B lymphocytes subsequently isolated from this fraction by panning for 1 hr at 37°C in dishes precoated with unpurified rabbit anti-mouse immunoglobulin ($<10^8$ cells per dish) and selective removal of the adherent cells. Southern Blot Analysis. High-molecular-weight DNA is digested with HindIII or EcoRI, which cuts once in the proviral genome and releases a fragment unique to the integration site. Ten micrograms of digested DNA (5 g for male control lanes) is electrophoresed and analyzed by Southern blotting as described with a 32P-labeled probe specific for the neor gene sequence in the provirus from plasmid pMC1. HindIII-digested blots are stripped and reprobed with a Y chromosome-specific fragment from plasmid pY2 (Thomas, 1987).

Life is unique in the known universe, which is in a diversity form from bacteria to human. The life organisms exist in ever where in the earth. Life is a physical and chemical process. From ontology aspect, the world is timeless and the life exists forever as any other body in the nature. The nature of life is that life is a process of negative entropy, evolution, autopoiesis (auto-organizing), adaptation, emergence and living hierarchy. Up to now, there is no scientific evidence to show that life body and non-life body obey the same natural laws. But, all the researches are made by the methods of biology, biochemistry and molecular biology, etc. It is very possible that the life and non-life are essential different in the biophysics, i. e. the quantum level. In the future, it is possible to make artificial life by either biological method or electronic technique (Ma and Cherng, 2005). The characterizations of stem cell will offer important information to answer what life is, the nature of life, if a life body can live forever or not, if a life procedure (lifeline) can be reversed or not (from old condition to younger condition), etc.

As all the gens exist in all the cells of an animal, all the cells have the totipotent ability. Under the clone principle, all the cells can develop into a entire alive animal body that is same as the baby comes from the fertilized egg – the zygote. It is important to reverse the non-totipotent cell to the totipotent, which is the lifeline reverse that make the animal growth from old to younger. Homebox protein Nanog is an important factor in this schedule. Transdifferentiation may play an important role in the life reverse procedure (Ma, et al, 2011).

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