

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.

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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 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. A human begins a zygote that a sperm fertilizes an egg and creates the single totipotent cell. In the beginning hours after fertilization, 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.

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.

Differentiation results from differential gene expression. In order to clone an animal, such as a sheep, 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.

Cellular determination results from the asymmetric segregation of cellular determinants. However, in most cases, 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, 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 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 two decades. 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):

Virus. Recombinant Tkneo9 virus at a titer of 1×10^6 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 Tkneo9 virus using a supernatant infection protocol in which $3-5 \times 10^6$ marrow cells are cultured for 24 hr in 100-

mm Petri dishes in 10 ml of virus supernatant containing Polybrene at 4 μ g/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 3×10^6 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, 2×10^5 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 (<10⁸ 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).

Discussion

Totipotent stem cells have the potential to develop into any cell in the animal body. In human development, a man sperm goes into the woman's body and fuse with a woman's egg cell together to form a single cell (zygote). The zygote divides to form numerous cells that will eventually constitute the human body. The zygote then begins cell divisions that form the entire human body. These early cells are totipotent.

During the early cell divisions in embryonic development many totipotent cells are produced. Within several days, these totipotent cells divide and create replicas to produce totipotent cells. Normal 4 days after the cells begin to specialize into pluripotent cells, which can go on to specialize further but cannot produce an entire organism as totipotent cells do.

In human, a fertilized egg has totipotent property and this totipotent characterization will keep for about four days. Normally 4 days after fertilization, the totipotent stem cell divides and then matures to cause more specialized stem cells called pluripotent stem cells. Pluripotent stem cells also can self-renew and give rise to trillions of cells in the body.

Totipotent stem cells can develop into any cell type. So that, the totipotent stem cells are the good candidate in the cell or gene therapy or in the tissue engineering for medical transplants and replacement of diseased cells (or tissues or organs). The therapy using totipotent is powerful and efficient.

The best material to be used is the foetal. There are real totipotent foetal stem cells that are healthy, efficiency and real valuable in the scientific studies and medical applications. However, the some ethical

and legal debate could be created. This is a challenge. As my opinion, in the ethical and legal point, we need to restrict the human in an alive person after the birth. Only a person after birth he/she is a real person.

Beside the potential applications in the clinical medicine, totipotent stem cells are important in the studies of nature of life, origin of life and the future of life. The totipotent of stem cell is the most important property for the stem cell to keep the life continuously. According to the scientific knowledge up to now, all the life phenomenon follow the basic physical laws. There are now extra law to rule the life processing. One totipotent stem cell can grow a whole life body. This means that all the information for the later life body is there in the totipotent stem cell and the universe environment.

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References

1. Christopher C. Fraser, Stephen J. Szilvassy, Connie J. Eaves, And R. Keith Humphries. Proliferation of totipotent hematopoietic stem cells in vitro with retention of long-term competitive in vivo reconstituting ability. Proc. Nati. Acad. Sci. USA. Vol. 89, pp. 1968-1972, 1992.
2. Fraser, C. C., Eaves, C. J., Szilvassy, S. J. & Humphries, R. K. (1990). Blood 76, 1071-1076.
3. Thomas, K. R. & Capecchi, M. R. (1987) Cell 51, 503-512.
4. Baud L, Haymann JP, Bellocq A, Fouqueray B. Contribution of stem cells to renal repair after ischemia/reperfusion. Bull Acad Natl Med. 2005;189(4):635-43.
5. Bavister BD, Wolf DP, Brenner CA. Challenges of primate embryonic stem cell research. Cloning Stem Cells 2005;7(2):82-94.
6. Bernard Lo, Patricia Zettler, Marcelle I. Cedars, Elena Gates, Arnold R. Kriegstein, Michelle Oberman, Renee Reijo Pera, Richard M. Wagner, Mary T. Wuerth, Leslie E. Wolf, Keith R. Yamamoto. A New Era in the Ethics of Human Embryonic Stem Cell Research. Stem Cells. <http://www.StemCells.com>. <http://stemcells.alphamedpress.org/cgi/reprint/2005-0324v1.pdf>. 2005.
7. Bhatt RI, Brown MD, Hart CA, Gilmore P, Ramani VAC, George NJ, Clarke NW. Novel method for the isolation and characterisation of the putative prostatic stem cell. Cytometry A. 2003;54(2):89-99.

8. Cantley LG. Adult stem cells in the repair of the injured renal tubule. *Nat Clin Pract Nephrol*. 2005;1(1):22-32.
9. Condorelli G, Peschle C. Stem cells for cardiac repair: state of the art. *Front Biosci* 2005;10:3143-50.
10. Daar AS, Sheremeta L. The science of stem cells: ethical, legal and social issues. *Exp Clin Transplant*. 2003;1(2):139-46.
11. Duffield JS, Bonventre JV. Kidney tubular epithelium is restored without replacement with bone marrow-derived cells during repair after ischemic injury. *Kidney Int*. 2005;68(5):1956-61.
12. Duffield JS, Park KM, Hsiao LL, Kelley VR, Scadden DT, Ichimura T, Bonventre JV. Restoration of tubular epithelial cells during repair of the postischemic kidney occurs independently of bone marrow-derived stem cells. *J Clin Invest*. 2005;115(7):1743-55.
13. Herrera MB, Bussolati B, Bruno S, Fonsato V, Romanazzi GM, Camussi G. Mesenchymal stem cells contribute to the renal repair of acute tubular epithelial injury. *Int J Mol Med*. 2004;14(6):1035-41.
14. Hishikawa K, Fujita T. Stem cells and kidney disease. *Hypertens Res*. 2006;29(10):745-9.
15. http://stemcells.nih.gov/staticresources/research/protocols/BresaGen_hESC_manual_2.1.pdf
16. Humphreys BD, Duffield JD, Bonventre JV. Renal stem cells in recovery from acute kidney injury. *Minerva Urol Nefrol*. 2006;58(1):13-21.
17. Kashofer K, Bonnet D. Gene Therapy Progress and Prospects: Stem cell plasticity. *Gene Ther*. 2005 (Epub ahead of print).
18. Kimberly Kasow. OPBMT2 Protocol: Allogeneic Hematopoietic Stem Cell Transplantation for Children Affected with Malignant Osteopetrosis - A Pilot Study. http://www.stjude.org/protocols/0,2881,450_2331_17072,00.html. 2007.
19. Kwang-Soo Kim. Stem cell research continues in Korea beyond the Hwang scandal. *Stem Cells*. <http://www.StemCells.com>. <http://stemcells.alphamedpress.org/cgi/reprint/2007-0089v1.pdf>. 2007.
20. Lin F, Cordes K, Li L, Hood L, Couser WG, Shankland SJ, Igarashi P. Hematopoietic stem cells contribute to the regeneration of renal tubules after renal ischemia-reperfusion injury in mice. *J Am Soc Nephrol*. 2003;14(5):1188-99.
21. Lin F. Stem cells in kidney regeneration following acute renal injury. *Pediatr Res*. 2006;59(4 Pt 2):74R-8R.
22. Ma H. Technique of Animal Clone. *Nature and Science* 2004;2(1):29-35.
23. Mitalipova et al. *Stem Cells*. 2003;21(5):521-6.
24. Morigi M, Benigni A, Remuzzi G, Imberti B. The regenerative potential of stem cells in acute renal failure. *Cell Transplant*. 2006;15 Suppl 1:S111-7.
25. Nigel Hawkes. Scientists find the secret of eternal life for stem cells. <http://www.timesonline.co.uk/tol/news/uk/article137674.ece>
26. Oliver JA. Adult renal stem cells and renal repair. *Curr Opin Nephrol Hypertens*. 2004;13(1):17-22.
27. Paul Woodard. SCDHAP Protocol: ematopoietic Stem Cell Transplantation (HSCT) for Patients with Sickle Cell Disease and Prior Stroke or Abnormal Transcranial Doppler Ultrasound (TCD) using Reduced Conditioning and T-Cell-Depleted Hematopoietic Stem Cells from Partially Matched Family Donors - Phase I Study. http://www.stjude.org/protocols/0,2081,450_2327_18472,00.html. 2007.
28. Renee Madden. SCT521 (COG # ASCT0521) Protocol: Soluble Tumor Necrosis Factor Receptor: Enbrel (Etanercept) for the Treatment of Acute Non-Infectious Pulmonary Dysfunction (Idiopathic Pneumonia Syndrome) Following Allogeneic Stem Cell Transplantation. http://www.stjude.org/protocols/0,2881,450_2333_5873,00.html. 2007.
29. Stedman's Medical Dictionary. The American Heritage®. Houghton Mifflin Company. <http://dictionary.reference.com/search?q=stem%20cell>. 2002.
30. Williams D. Stem cells in medical technology. *Med Device Technol* 2005;16(3):9-11.
31. Wing Leung. INFT2 Protocol: HLA - Nonidentical Stem Cell and Natural Killer Cell Transplantation for Children Less than 2 Years of Age with Hematologic Malignancies. http://www.stjude.org/protocols/0,2881,450_2330_11129,00.html. 2007.
32. Yamashita S, Maeshima A, Nojima Y. Involvement of renal progenitor tubular cells in epithelial-to-mesenchymal transition in fibrotic rat kidneys. *J Am Soc Nephrol*. 2005;16(7):2044-51.
33. de Almeida, M., C. V. de Almeida, E. M. Graner, G. E. Brondani and M. F. de Abreu-Tarazi (2012). "Pre-procambial cells are niches for pluripotent and totipotent stem-like cells for organogenesis and somatic embryogenesis in the peach palm: a histological study." *Plant Cell Rep* 31(8): 1495-1515.
34. Hemmat, S., D. M. Lieberman and S. P. Most (2010). "An introduction to stem cell biology." *Facial Plast Surg* 26(5): 343-349.
35. Sharpless, N. E. (2010). "Hot topics in stem cells and self-renewal: 2010." *Aging Cell* 9(4): 457-461.

36. Shufaro, Y. and B. E. Reubinoff (2011). "Cell cycle synchronization for the purpose of somatic cell nuclear transfer (SCNT)." Methods Mol Biol **761**: 239-247.
37. Surani, M. A. and P. Hajkova (2010). "Epigenetic reprogramming of mouse germ cells toward totipotency." Cold Spring Harb Symp Quant Biol **75**: 211-218.
38. Wang, X. D., K. E. Nolan, R. R. Irwanto, M. B. Sheahan and R. J. Rose (2011). "Ontogeny of embryogenic callus in *Medicago truncatula*: the fate of the pluripotent and totipotent stem cells." Ann Bot **107**(4): 599-609.
39. [Pesce M](#), [Schöler HR](#). Oct-4: control of totipotency and germline determination. Mol Reprod Dev. 2000 Apr;55(4):452-7.

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