DOI 10.1007/s10517-021-05099-2
Cell Techologies in Biology and Medicine, No. 4, February, 2021

CELL TECHOLOGIES IN BIOLOGY AND MEDICINE

Postnatal Pluripotent Cells: Quarter of a Century of Research

N. K. Yarygina[1,2], K. N. Yarygin[1]

Translated from *Kletochnye Tekhnologii v Biologii i Meditsine*, No. 4, pp. 223-230, December, 2020
Original article submitted October 9, 2020

Almost quarter of a century long studies aimed at identification, isolation, culturing, and use of postnatal pluripotent cells for the development of cell-based technologies have not met with success and failed to provide reliable and reproducible protocols of cell isolation, identification, and culturing. At the same time, experimental data in this field suggest that postnatal pluripotent cells are not the copies of embryonic cells and, therefore, the tests routinely used for identification of embryonic pluripotent cells are not fully adequate for characterization of their postnatal analogues. Therefore, cell lineage tracing methods showing the differentiation routes of the studied cells in human or animal body after birth should be developed and used.

Key Words: *pluripotency; postnatal pluripotent cells*

The question of whether postnatal mammalian tissues contain pluripotent cells, *i.e*. stem cells with the maximum potential for differentiation capable of differentiating into any type of somatic cells and into germinative cells, arose immediately after isolation of pluripotent cells from mouse [13] and human [51] embryos and still remains relevant. Several interrelated fundamental and practical aspects are still discussed, such as the concept of pluripotency, natural and artificially created pluripotency, markers of pluripotency, transdifferentiation, mechanisms of tissue regeneration, and the fundamental bases of cell and gene technologies. It should be born in mind that the development of technology for obtaining pluripotent cell cultures from postnatal tissues would be a breakthrough in cell technologies and regenerative medicine, because it would solve the problem of obtaining safe autologous cell material for cell therapy, tissue engineering, personalized testing of the effectiveness and toxicity of drugs, and other medical applications. Postnatal pluripotent cells could successfully compete with potentially unsafe induced pluripotent cells as an object of basic and applied research.

We present a critical review of the most interesting studies in the field of identification of pluripotent cells and cells that presumably are pluripotent in pre- and postnatal mammalian tissues.

Pluripotent cells in ontogeny. As is known, several homoblastic divisions of the egg (*i.e*. not changing the total mass of the embryo) at the very beginning of embryogenesis result in the formation of a morula consisting exclusively of pluripotent cells [46]. Then, a fluid-filled cavity appears in the center of the morula and a blastocyst is formed. At the blastocyst stage, physical segregation of pluripotent cells from the remainder cells occurs, they form a compact clump of cells in the blastocyst cavity, so-called inner cell mass. Then, the inner cell mass gives rise to the epiblast cells that retain pluripotency and the hypoblast that differentiates into the yolk sac and eventually into the chorion. In the embryos of triploblastic animals, including mammals, pluripotent epiblast cells during gastrulation differentiate to form three germ layers, the ectoderm, mesoderm, and endoderm. According to

[1]V. N. Orekhovich Research Institute of Biomedical Chemistry, Moscow, Russia; [2]V. I. Kulakov National Medical Research Centre of Obstetrics, Gynecology, and Perinatology, Ministry of Health of the Russian Federation, Moscow, Russia. ***Address for correspondence:*** kyarygin@yandex.ru. K. N. Yarygin

classical concepts, the cells of each of the germ layer can differentiate only in destined directions characteristic of this germ layer. After gastrulation, the cells of the embryo are subdivided into ectoderm-derived cells, mesoderm-derived cells, and endoderm-derived cells. The question whether all embryonic cells lose pluripotency at the gastrula stage remains open. During further embryo development, combinations of bioactive substances produced by embryonic and maternal cells trigger intracellular signaling cascades, first of all, Wnt and Nodal signaling pathways, leading to further restriction of the differentiation potential of individual cells and formation of specialized cell types each of which belongs to derivatives of one germ layer [4].

During the formation of tissues and organs, most of the cells are differentiated and assembled into specific histological and anatomical structures. Only a relatively small fraction of cells, stem cells (SC), avoids differentiation into working cells. These so-called postnatal SC can undergo asymmetrical division yielding two different daughter cells: one undergoes differentiation, and the other remains identical to the maternal SC. Most of the time, SC are in a resting state in a special tissue compartments, stem cell niches. The concept of stem cell niche was formulated in the 1970s [41]. Under the influence of signals from surrounding cells in the niche, extracellular matrix, blood and tissue fluid, and interstitial nerve elements, SC can undergo activation, enter mitosis, and differentiate [15,25,53]. SC can have different differentiation potencies and be monopotent, *i.e.* capable of differentiating into cells of a single type (*e.g*. SC of the stratified squamous epithelium of the skin) and pluripotent, *i.e*. capable of differentiating into different cell types, derivatives of one germ layer (*e.g*. bone marrow hemopoietic SC, mesoderm derivatives, give rise to all blood cells). The presence of pluripotent cells capable of triploblast differentiation (*i.e*. differentiation into cells of all three germ layers) in adult tissues is not strictly proven, but is actively discussed and is the topic of this review.

In vivo studying of differentiation potency is very difficult even for mouse cells; when studying the potency of human cells, these difficulties multiply many times. Fortunately, the pluripotent inner mass cells isolated from the blastocyst cavity can be maintained and studied *in vitro*. These *in vitro* maintained pluripotent cells were called embryonic stem cells (ESC). At the end of the last century, stable lines of mouse ESC [13] and, after almost two decades, of human ESC [51] were obtained. Later, cell lines similar to ESC were obtained from cells of mouse embryos taken at an even earlier (morula) stage [50]. A necessary condition for pluripotency maintenance is the expression of a number of transcription factors by ESC, the most important of these are the so-called master regulators of stem cell pluripotency Oct4, Sox2, and Nanog [18,56]. The latter combine a network of regulatory pathways, including a large number of transcription factors and epigenetic regulators [8,12,40].

In vitro and *in vivo* studies of mouse embryonic pluripotent cells have demonstrated that these cells in early ontogeny do not remain unchanged, but evolve without losing pluripotency [29]. In mouse embryos, pluripotent cells are present as a compact formation (morula, inner cell mass, or epiblast) up to day 8 of embryonic development (E8). Pluripotent cells of the epiblast in preimplantation and postimplantation embryos differ by the expression profiles, morphology, and behavior in culture and after transplantation to adult animals; they were named naïve and primed pluripotent cells, respectively [30]. Morula cells differ from cells of the inner mass and epiblast even by the potency, because they can differentiate not only into any cells of the embryo, but also into cells of extraembryonic membranes. Providing that pluripotent cells are present in the postnatal mouse tissues, they should significantly differ from embryonic ones. Human embryonic and postnatal pluripotent cells should also differ, because human pluripotent cells, similar to mouse cells, undergo changes during embryogenesis [29].

Markers and criteria of pluripotency. Changes in pluripotent cells occurring during individual development of the body make their identification in tissues challenging. This problem has become particularly important in the context of new data on the possibility of direct transdifferentiation of cells without entering the pluripotency stage [16,44,54]. The pluripotent cell by definition is a SC that can differentiate into any somatic or germinal cell. However, direct application of this criterion of pluripotency in the analysis of tissues for the presence of pluripotent cells is practically impossible. Therefore, the tissue is first examined for the presence of cells expressing embryonic antigens, such as Oct4, Sox2, and Nanog, by immunohistochemical and flow cytometry techniques. In case of detection, these cells are isolated and their expression profiles, DNA methylation profiles, and other characteristics are analyzed. Culturing of these cells is used to characterize the differentiation potential; actually, the propensity to differentiate not into all approximately 230 known cytophenotypes, but only into selected cell types representing different germ layers is analyzed. If the isolated and cultured cells differentiate into ectoderm, mesoderm, and endoderm derivatives, then they are presumably pluripotent. Their similarity to ESC and other known pluripotent cells by morphological characteristics, expression of membrane and intracellular markers, culturing conditions, and other parameters is not critical, though makes these cells even more likely candidates for postnatal pluripotent SC.

However, many researchers believe that these features cannot serve as sufficient basis for recognizing cells as pluripotent and the possibility of generation of all diversity of cells in the body under *in vivo* conditions should be proven. This proof can be obtained by using cell replacement test in a tetraploid embryo [48]. In brief, at the initial stage of fertilized egg division, namely, at the stage of two blastomeres, cell fusion is induced by electrical pulse and a tetraploid cell is formed; further divisions lead to the formation of an embryo and extraembryonic tissues consisting of tetraploid cells. Diploid pluripotent cells being introduced into the blastocyst replace the tetraploid cells, and a normal diploid organism can develop, while the extraembryonic tissues are tetraploid. This was convincingly demonstrated by the example of complementation of the tetraploid inner cell mass of a mouse blastocyst with induced pluripotent cells [21].

A similar, but somewhat less complex test for pluripotency is complementation of a diploid embryo [9] that served as the basis of the above tetraploid embryo complementation test. Candidate cells that differ from cells of the developing embryo by a natural phenotypically manifested mutation (*e.g.* determining the color of the hair), or by an artificially introduced change in the genome (*e.g.* carrying GFP transgene) are transplanted into a normal diploid blastocyst. The appearance of a mottled mouse or a mouse that contains cells responding to laser irradiation with a wavelength of 395 nm by green fluorescence with a peak at 498 nm indicates generation of chimeric animals and hence, the studied cells are pluripotent.

Postnatal cells demonstrating signs of pluripotency. Over the past two decades, detection of pluripotent cells in postnatal tissues was reported not once [5,14]. Of great interest are studies describing the following presumably pluripotent cells: multipotent adult progenitor cells (MAPC), "spore-like" cells, very small embryonic-like stem cells (VSEL), pluripotent non-tumorigenic multilineage differentiating stress enduring cells (Muse cells) and others. The reports about discovery of one more type of postnatal pluripotent cells always aroused great enthusiasm, but the results were often unreproducible; and now, this field of cell biology is still full of contradictions. The situation can be illustrated by the history of studying MAPC, spore-like cells, and VSEL.

In 2001 and 2002, the Catherine Verfaillie team from the University of Minnesota reported detection of postnatal pluripotent cells in mouse bone marrow [19,39], as well as in mouse muscles and brain [20]. These cells had a CD-phenotype similar in many respects to mesenchymal stem cells (MSC), represented a minor subpopulation in bone marrow mononuclear suspension (no more than 1 per 10^6 cells), and were present as an impurity in the MSC culture during early passages. They could be separated from MSC by negative selection of $CD45^+$/$glycophorin^+$ cytophenotype cells. They were named MAPC. MAPC were also found in many other mouse tissues and in the above mentioned and other rat and human tissues [22]. These cells demonstrated signs of pluripotency and functional similarity to ESC, because they were capable of triploblastic differentiation in culture; after injection into the blastocyst cavity, these cells participated in the development of a chimeric organism. The similarity, however, was incomplete, because these cells did not form teratomas after transplantation. The first publications of the group about the discovery of MAPC caused a great resonance not only among specialists, but also in the media, because SC and the future achievements of regenerative medicine were very popular topics at the beginning of this century, and MAPC were considered by some researchers as an alternative to ESC, which were then high hopes. Unfortunately, some of the original results of Catherine Verfaillie's group were not reproduced by other scientific groups, in particular, the results on the participation of MAPC transplanted into the blastocyst in the formation of a chimeric embryo. In addition, a thorough analysis of publications showed that at least one of the co-authors of reports published in 2001-2002, M. Reyes, falsified some of the results. A reputation scandal broke out. C. Verfaillie, who led the group, left the United States and moved to her native Belgium, changed her research profile, but still defends her previous results to this day. Working in Belgium, she published an article on the effects of MAPC on $CD8^+$ cytotoxic lymphocytes [35]. Studies of MAPC continue in some other laboratories in different countries [22].

In parallel with MAPC, another type of presumably pluripotent cells was discovered in postnatal tissues by the Charles Vacanti group from the University of Massachusetts [52]. They were described as small, rounded, spore-like cells with a diameter <5 μ containing a large nucleus, a small amount of the cytoplasm, and few mitochondria. These "spore-like" cells in an inactive (dormant) state and in very small amounts were present in many tissues and were exceptionally resistant to oxygen and nutrient deficit and remained viable in the brain and spinal cord of laboratory animals for several days after death. In a living organism, these dormant cells could be activated in response to injury or disease and replenish the cellular composition of tissues. In a few years, extremely high resistance of these cells was confirmed; it was shown that spore-like cells withstand freezing to -86°C without a cryoprotectant and heating to 85°C for 30 min [10]. Later, Haruko Obokata joined Vacanti's laboratory and

took an active part in working with spore-like cells. She showed that they can differentiate into cells of three germ layers [32]. High resistance of "spore-like" cells to adverse factors and their ability to differentiate in different directions led H. Obokata and co-workers to an assumption that these properties can be related.

After moving to Japan and working at the Riken Institute, H. Obokata continued her research, which also involved the staff of the C. Vacanti laboratory. At the very beginning of 2014, two papers were published in Nature journal [33,34] that presented a new, very simple and effective method for isolation of pluripotent cells from newborn mouse cells. A suspension of splenic of liver cells was exposed to low pH or high pressure; most of the cells died under these conditions, but some cell survived and formed aggregates. A significant portion of survived cells acquired pluripotency. They were capable of triploblastic differentiation *in vitro* and provided chimerization of not only the embryo, but also extraembryonic tissues after injection into the blastocyst. The authors called this phenomenon as stimulus-triggered acquisition of pluripotency (STAP). The co-author of these publications, C. Vacanti, believed that the detected pluripotent cells were "spore-like" cells undergoing stress rather than the result of pluripotency induction. As in case of MAPC, other groups [3,11,49] and even some co-authors [31] failed to reproduce the results reported by H. Obokata; they observed very rare cells expressing embryonic markers, but unable to chimerize the embryo or extraembryonic organs upon injection into the blastocyst. Earlier, almost immediately after publication in Nature, an inspection was carried out at the Riken Institute and a suspicion that some of the results were fabricated turned into confidence. All this led to quick retraction of Obokata's article published in 2011 and two articles in Nature journal published in 2014, suicide of Yashiki Sasai, supervisor of H. Obokata, revoke of doctoral degree, and resignation from the University; C. Vacanti also retired from all his positions after the scandal. However, H. Obokata continues to insist that the results are generally reliable, though somewhat embellished, and C. Vacanti is convinced of the importance of further studying of "spore-like" cells.

The most consistent results of research in the field of postnatal pluripotent cells were obtained in studies initiated by the Janina Ratajczak group at the University of Louisville; most of these results were later confirmed. In the first article of the group on this subject published in 2006, they described small rounded cells that were detected by flow cytometry in organs of adult mice; the cells had a diameter of 2-4 μ, CD phenotype of $LIN^{-}/S\text{-}1^{+}/CD45^{-}$, high volume ratio of nucleus to cytoplasm, predominance of euchromatin in the nucleus, and low number of mitochondria [26]. These cells, VSEL, expressed pluripotency markers Oct-4, SSEA-1, Nanog, and Rex-1 (although not as actively as ESC), almost all cells were in the G0 phase of the cell cycle, and differentiated into cells derived from all three germ layers. Cells with similar properties were later found in human tissues, including cord blood [17,28]. The Ratajczak group optimized the conditions for the expansion of VSEL cells *in vitro* [37]. It turned out, in particular, that addition of valproic acid and nicotinamide promoted expansion. Genome-wide expression analysis of VSEL cells showed that their inactive state can be associated with maternal imprinting at locus H19 [27]. VSEL cells were found to be more resistant to whole-body irradiation than hematopoietic cells [38].

The data of the Ratajczak group were confirmed in independent studies of at least two groups [42,47], but one group failed to reproduce the protocol of flow cytometry [1]. J. Ratajczak and D. Bhartya, Indian researcher who succeeded in isolating VSEL cells, revised and improved the protocol, but the parties did not come to a full agreement. Studies of VSEL cells and the development of the concept of the tissue niche for pluripotent SC were continued by Ratajczak [2] and Bhartya [6,7] groups.

It is clear that VSEL cells are very similar to "spore-like" and STAP cells, as well as to small cells with unknown function detected in the bone marrow at the end of the last century [36,43] and considered a subpopulation or contamination of the population of hematopoietic cells. The resistance of VSEL cells to radiation also attests to their similarity to "spore-like" and STAP cells [38].

The revealed contradictions in the results of different research groups can be explained by small size of VSEL cells and "spore-like" cells, due to which they can simply be lost during standard isolation of cells from tissues. As was shown by the D. Bhartiya group [6,7], they can be lost during centrifugation at 250*g* commonly used for cell washing. To prevent loss of small cells, they should be sedimented at higher speeds (1000*g*). In the original works cited above, data have been obtained indicating that MAPC, STAP cells, and VSEL cells have specific needs in medium composition in comparison with other cells. These two considerations explain many failures in isolating and culturing postnatal pluripotent cells.

The history of the search for postnatal pluripotent cells is instructive in that it seems to have been conducted in the wrong direction and using suboptimal tests. Indeed, cells similar to embryonic ones were searched in adult tissues. At the same time, the conditions of the adult organism differ radically from cell microenvironment in the blastocyst, where the cells of

the inner mass and epiblast have few neighbors and did not contact with the extracellular matrix and elements of the peripheral nervous system; hormonal and paracrine signals radically differ from those received in the adult tissue. The conditions in blastocyst are similar to those in 2D adhesion culture of pluripotent cells, while in adult tissues, the cell exists in three dimensions.

In this regard, a question arises on adequacy of the tests used. It is unclear, for example, whether the test for complementation of a diploid or tetraploid embryo by the studied adult cells is an adequate test for pluripotency of these cells, because cells taken from adult tissue can simply not find suitable conditions for the manifestation of pluripotency in the blastocyst. The test for triploblastic differentiation under conditions of an adult organism is required. The problem of cell loss during isolation and cultivation is also worthy of note.

It should be admitted that the search for postnatal pluripotent cells, despite the accompanying scandals, led to a considerable progress in understanding of the nature of pluripotency. It became clear that the search for the cells that are copies of ESC in a healthy adult body is unpromising. After all, these cells in case they are present in the body, would initiate the formation of teratomas. Indeed, ESC, being a part of an early embryo, receive very few regulatory signals and develop largely independently; upon transplantation into an adult body, they continue their intrinsic development program and eventually form teratomas. Being injected into the blastocyst in the preimplantation embryo complement test, these cells develop normally and participate in chimerization of the embryo. Instead, these tests, cell lineage tracing methods showing the differentiation routes of the studied cells in human or animal body after birth should be used. Based on the data presented in the review, a population of small dormant cells really exists and can be activated, proliferate, and differentiate in the appropriate direction under the influence of specific stimuli. However, reliable isolation of these cells for study and use in cell technologies requires more methodological work, while convincing evidence of their pluripotency has not yet been provided.

Taking a broader approach, we can consider this is part of a more general problem of reversibility of differentiation and reversibility of inactivation of a part of the cell genome occurring in ontogeny. The dogma of irreversibility of genome differentiation/inactivation, though questioned, has existed in cell biology for a very long time and was refuted relatively recently, after appearance of the technique of preparing pluripotent cells from somatic cells by transferring the nucleus from a somatic cell to an unfertilized haploid egg or by transfecting somatic cells with genes encoding embryonic pluripotency master factors [45]. After transplantation into the blastocyst, these pluripotent cells differentiate into all types of somatic cells, germinal cells, and cells of extraembryonic organs [45,55]. In parallel, methods of direct cell transdifferentiation bypassing the pluripotency stage were developed [16]. However, all these methods are artificial and leave unanswered the question of whether deep dedifferentiation or direct transdifferentiation occurs routinely *in vivo*. Now, there are more and more reasons to answer this question positively. For example, the cell composition of the liver during homeostatic and reparative regeneration, depending on the situation, is replenished from different sources such as temporarily dedifferentiating hepatocytes, local epithelial SC, local MSC, and circulating bone marrow cells similar to hematopoietic SC and MSC [23,24].

All presumably pluripotent postnatal cells discussed in this review were isolated from populations of hematopoietic SC or MSC, or from cell mixtures containing these cell types. Therefore, comprehensive analysis of heterogeneous cell mixtures that are now called hematopoietic SC and MSC is a pressing problem.

In conclusion, the history of the search for postnatal pluripotent cells has taught the scientific community a lot and in this sense is important not only for cell biologists, but also for science in general.

The work was performed within the framework of the Program for Basic Research of State Academies of Sciences for 2013-2020.

REFERENCES

1. Abbott A. Doubt cast over tiny stem cells. Nature. 2013; 499:390. doi: 10.1038/499390a
2. Adamiak M, Bujko K, Brzezniakiewicz-Janus K, Kucia M, Ratajczak J, Ratajczak M.Z. The inhibition of CD39 and CD73 cell surface ectonucleotidases by small molecular inhibitors enhances the mobilization of bone marrow residing stem cells by decreasing the extracellular level of adenosine. Stem Cell Rev. Rep. 2019;15(6):892-899.
3. Aizawa S. Results of an attempt to reproduce the STAP phenomenon. F1000Res. 2016;5:1056. doi: 10.12688/f1000research.8731.2
4. Arnold SJ, Robertson EJ. Making a commitment: cell lineage allocation and axis patterning in the early mouse embryo. Nat. Rev. Mol. Cell. Biol. 2009;10(2):91-103.
5. Bhartiya D. Pluripotent stem cells in adult tissues: struggling to be acknowledged over two decades. Stem Cell Rev. Rep. 2017;13(6):713-724.
6. Bhartiya D, Mohammad SA. Which stem cells will eventually translate to the clinics for treatment of diabetes? Stem Cell Res. Ther. 2020;11(1):211. doi: 10.1186/s13287-020-01718-3
7. Bhartiya D, Sharma D. Ovary does harbor stem cells — size of the cells matter! J. Ovarian Res. 2020;13(1):39. doi: 10.1186/s13048-020-00647-2

8. Boyer LA, Lee TI, Cole MF, Johnstone SE, Levine SS, Zucker JP, Guenther MG, Kumar RM, Murray HL, Jenner RG, Gifford DK, Melton DA, Jaenisch R, Young RA. Core transcriptional regulatory circuitry in human embryonic stem cells. Cell. 2005;122(6):947-956.
9. Chen J, Lansford R, Stewart V, Young F, Alt FW. RAG-2-deficient blastocyst complementation: An assay of gene function in lymphocyte development. Proc. Nat. Acad. Sci. USA. 1993;90(10):4528-4532.
10. Cortiella J, Nichols JE, Kojima K, Bonassar LJ, Dargon P, Roy AK, Vacant MP, Niles JA, Vacanti CA. Tissue-engineered lung: an in vivo and in vitro comparison of polyglycolic acid and pluronic F-127 hydrogel/somatic lung progenitor cell constructs to support tissue growth. Tissue Eng. 2006;12(5):1213-1225.
11. De Los Angeles A, Ferrari F, Fujiwara Y, Mathieu R, Lee S, Lee S, Tu HC, Ross S, Chou S, Nguyen M, Wu Z, Theunissen TW, Powell BE, Imsoonthornruksa S, Chen J, Borkent M, Krupalnik V, Lujan E, Wernig M, Hanna JH, Hochedlinger K, Pei D, Jaenisch R, Deng H, Orkin SH, Park PJ, Daley GQ. Failure to replicate the STAP cell phenomenon. Nature. 2015;525:E6-E9.
12. Eguizabal C, Aran B, Chuva de Sousa Lopes SM, Geens M, Heindryckx B, Panula S, Popovic M, Vassena R, Veiga A. Two decades of embryonic stem cells: a historical overview. Hum. Reprod. Open. 2019;2019(1). hoy024. doi: 10.1093/hropen/hoy024
13. Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. Nature. 1981;292:154-156.
14. Gao L, Thilakavathy K, Nordin N. A plethora of human pluripotent stem cells. Cell Biol. Int. 2013;37(9):875-887.
15. Goodell MA, Nguyen H, Shroyer N. Somatic stem cell heterogeneity: diversity in the blood, skin and intestinal stem cell compartments. Nat. Rev. Mol. Cell Biol. 2015;16(5):299-309.
16. Grath A, Dai G. Direct cell reprogramming for tissue engineering and regenerative medicine. J. Biol. Eng. 2019;13:14. doi: 10.1186/s13036-019-0144-9
17. Havens AM, Sun H, Shiozawa Y, Jung Y, Wang J, Mishra A, Jiang Y, O'Neill DW, Krebsbach PH, Rodgerson DO, Taichman RS. Human and murine very small embryonic-like cells represent multipotent tissue progenitors, in vitro and in vivo. Stem Cells Dev. 2014;23(7):689-701.
18. He S, Nakada D, Morrison SJ. Mechanisms of stem cell self-renewal. Annu. Rev. Cell Dev. Biol. 2009;25:377-406.
19. Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, Reyes M, Lenvik T, Lund T, Blackstad M, Du J, Aldrich S, Lisberg A, Low WC, Largaespada DA, Verfaillie CM. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature. 2002;418:41-49.
20. Jiang Y, Vaessen B, Lenvik T, Blackstad M, Reyes M, Verfaillie CM. Multipotent progenitor cells can be isolated from postnatal murine bone marrow, muscle, and brain. Exp. Hematol. 2002;30(8):896-904.
21. Kang L, Wang J, Zhang Y, Kou Z, Gao S. iPS cells can support full-term development of tetraploid blastocyst-complemented embryos. Cell Stem Cell. 2009;5(2):135-138.
22. Khan RS, Newsome PN. A comparison of phenotypic and functional properties of mesenchymal stromal cells and multipotent adult progenitor cells. Front. Immunol. 2019;10:1952. doi: 10.3389/fimmu.2019.01952
23. Kholodenko IV, Kurbatov LK, Kholodenko RV, Manukyan GV, Yarygin KN. Mesenchymal stem cells in the adult human liver: hype or hope? Cells. 2019;8(10):1127. doi: 10.3390/cells8101127
24. Kholodenko IV, Yarygin KN. Cellular mechanisms of liver regeneration and cell-based therapies of liver diseases. Biomed. Res. Int. 2017;2017:8910821. doi: 10.1155/2017/8910821
25. Krieger T, Simons BD. Dynamic stem cell heterogeneity. Development. 2015;142(8):1396-1406.
26. Kucia M, Reca R, Campbell FR, Zuba-Surma E, Majka M, Ratajczak J, Ratajczak MZ. A population of very small embryonic-like (VSEL) CXCR4 (+) SSEA-1(+) Oct-4+ stem cells identified in adult bone marrow. Leukemia. 2006;20(5):857-869.
27. Mierzejewska K, Heo J, Kang JW, Kang H, Ratajczak J, Ratajczak MZ, Kucia M, Shin DM. Genome-wide analysis of murine bone marrow-derived very small embryonic-like stem cells reveals that mitogenic growth factor signaling pathways play a crucial role in the quiescence and ageing of these cells. Int. J. Mol. Med. 2013;32(2):281-290.
28. Monti M, Imberti B, Bianchi N, Pezzotta A, Morigi M, Del Fante C, Redi CA, Perotti C. A novel method for isolation of pluripotent stem cells from human umbilical cord blood. Stem Cells Dev. 2017;26(17):1258-1269.
29. Morgani S, Nichols J, Hadjantonakis AK. The many faces of pluripotency: in vitro adaptations of a continuum of in vivo states. BMC Dev. Biol. 2017;17:7. doi: 10.1186/s12861-017-0150-4
30. Nichols J, Smith A. Naive and primed pluripotent states. Cell Stem Cell. 2009;4(6):487-492.
31. Niwa H. Investigation of the cellular reprogramming phenomenon referred to as stimulus-triggered acquisition of pluripotency (STAP). Sci. Rep. 2016;6:28003. doi: 10.1038/srep28003
32. Obokata H, Kojima K, Westerman K, Yamato M, Okano T, Tsuneda S, Vacanti CA. The potential of stem cells in adult tissues representative of the three germ layers. Tissue Eng. Part A. 2011;17(5-6):607-615. Retraction.
33. Obokata H, Sasai Y, Niwa H, Kadota M, Andrabi M, Takata N, Tokoro M, Terashita Y, Yonemura S, Vacanti CA, Wakayama T. Bidirectional developmental potential in reprogrammed cells with acquired pluripotency. Nature. 2014;505:676-680. Retraction.
34. Obokata H, Wakayama T, Sasai Y, Kojima K, Vacanti M.P, Niwa H, Yamato M, Vacanti CA. Stimulus-triggered fate conversion of somatic cells into pluripotency. Nature. 2014; 505:641-647. Retraction.
35. Plessers J, Dekimpe E, Van Woensel M, Roobrouck VD, Bullens DM, Pinxteren J, Verfaillie CM, Van Gool SW. Clinical-grade human multipotent adult progenitor cells block CD8+ cytotoxic T lymphocytes. Stem Cells Transl. Med. 2016;5(12):1607-1619.
36. Randall TD, Weissman IL. Characterization of a population of cells in the bone marrow that phenotypically mimics hematopoietic stem cells: resting stem cells or mystery population? Stem Cells. 1998;16(1):38-48.
37. Ratajczak MZ, Ratajczak J, Suszynska M, Miller DM, Kucia M, Shin DM. A novel view of the adult stem cell compartment from the perspective of a quiescent population of very small embryonic-like stem cells. Circ. Res. 2017;120(1):166-178.
38. Ratajczak J, Wysoczynski M, Zuba-Surma E, Wan W, Kucia M, Yoder MC, Ratajczak MZ. Adult murine bone marrow-derived very small embryonic-like stem cells differentiate into the hematopoietic lineage after coculture over OP9 stromal cells. Exp. Hematol. 2011;39(2):225-237.

39. Reyes M, Verfaillie CM. Characterization of multipotent adult progenitor cells, a subpopulation of mesenchymal stem cells. Ann. N.Y. Acad. Sci. 2001;938(1):231-233. discussion 233-235.
40. Rizzino A. Concise review: The Sox2-Oct4 connection: critical players in a much larger interdependent network integrated at multiple levels. Stem Cells. 2013;31(6):1033-1039.
41. Schofield R. The relationship between the spleen colony-forming cell and the haemopoietic stem cell. Blood Cells. 1978;4(1-2):7-25.
42. Shaikh A, Bhartiya D, Kapoor S, Nimkar H. Delineating the effects of 5-fluorouracil and follicle-stimulating hormone on mouse bone marrow stem/progenitor cells. Stem Cell Res. Ther. 2016;7(1):59. doi: 10.1186/s13287-016-0311-6
43. Sharkis SJ, Collector MI, Barber JP, Vala MS, Jones RJ. Phenotypic and functional characterization of the hematopoietic stem cell. Stem Cells. 1997;15(Suppl. 1):41-44.
44. Shen CN, Burke ZD, Tosh D. Transdifferentiation, metaplasia and tissue regeneration. Organogenesis. 2004;1(2):36-44.
45. Shi Y, Inoue H, Wu JC, Yamanaka S. Induced pluripotent stem cell tecnology: a decade of progress. Nat. Rev. Drug Discov. 2017;16(2):115-130.
46. Srinivas S, Watanabe T. Early embryogenesis. Textbook of Clinical Embryology. Coward K, Wells D, eds. Cambridge, 2013. P. 110-117.
47. Taichman RS, Wang Z, Shiozawa Y, Jung Y, Song J, Balduino A, Wang J, Patel LR, Havens AM, Kucia M, Ratajczak MZ, Krebsbach PH. Prospective identification and skeletal localization of cells capable of multilineage differentiation in vivo. Stem Cells Dev. 2010;19(10):1557-1570.
48. Tam PP, Rossant J. Mouse embryonic chimeras: tools for studying mammalian development. Development. 2003; 130(25):6155-6163.
49. Tang MK, Lo LM, Shi WT, Yao Y, Lee HS, Lee KK. Transient acid treatment cannot induce neonatal somatic cells to become pluripotent stem cells. F1000Res. 2014;3:102. doi: 10.12688/f1000research.4092.1
50. Tesar PJ. Derivation of germ-line-competent embryonic stem cell lines from preblastocyst mouse embryos. Proc. Natl Acad. Sci. USA. 2005;102(23):8239-8244.
51. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. Science. 1998;282:1145-1147.
52. Vacanti MP, Roy A, Cortiella J, Bonassar L, Vacanti CA. Identification and initial characterization of spore-like cells in adult mammals. J. Cell. Biochem. 2001;80(3):455-460.
53. Xin T, Greco V, Myung P. Hardwiring stem cell communication through tissue structure. Cell. 2016;164(6):1212-1225.
54. Xu L, Zhang K, Wang J. Exploring the mechanisms of differentiation, dedifferentiation, reprogramming and transdifferentiation. PLoS One. 2014;9(8):e105216. doi: 10.1371/journal.pone.0105216
55. Yang Y, Liu B, Xu J, Wang J, Wu J, Shi C, Xu Y, Dong J, Wang C, Lai W, Zhu J, Xiong L, Zhu D, Li X, Yang W, Yamauchi T, Sugawara A, Li Z, Sun F, Li X, Li C, He A, Du Y, Wang T, Zhao C, Li H, Chi X, Zhang H, Liu Y, Li C, Duo S, Yin M, Shen H, Belmonte JCI, Deng H. Derivation of pluripotent stem cells with in vivo embryonic and extraembryonic potency. Cell. 2017;169(2):243-257.e25.
56. Young RA. Control of the embryonic stem cell state. Cell. 2011;144(6):940-954.