The magic behind stem cells

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Received: 28 January 2007 / Accepted: 27 February 2007 / Published online: 24 March 2007
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Abstract This review article summarizes historical development of stem cell research, presents current knowledge on the plasticity potential of both embryonic and adult stem cells and discusses on the future of stem cell based therapies.

Keywords Embryonic stem cells · Adult stem cells · Therapy · Plasticity

Introduction

Stem cells (SC) are distinguished by their potential for self-renewal and multi-lineage differentiation. Concerning their state of development it is possible to distinguish embryonic stem (ES) cells from adult stem (AS) cells. While AS cells are responsible for maintenance and repair of most adult tissues. ES cells normally exist only in preimplantation embryos (4–8 cell stage to blastocyst) and have the ability to form all the cells of the body. ES cells could be used to set up cell lines *in-vitro*, to explore, for example, the function of genes. Biomedical research on SC is at an early stage, but is advancing rapidly. This promising area of science is very attractive for the investigation of the possibility of cell-based therapies. However, we are at the very beginning of understanding how SC influence the organism to keep its balance.

There are many regulation systems involved, which dictate the replacement of damaged, aged or diseased tissues with new cells.

It could be shown that there are factors secreted by SC, which act from the distance [1] and it was postulated that trophic factors play a role in tissue regeneration [2]. As it could be shown recently [3], systemic factors in an organism also play a crucial role in modulating signalling pathways critical to the activation of tissue-specific SC. It seems that SC retain much of their intrinsic proliferative potential even when old, but that age-related changes in the systemic environment and niche in which SC reside preclude full activation of these cells for productive tissue regeneration. Besides the environment with its systemic factors, it is clear that there are certain genes controlling the action of SC and their aging [4].

History of stem cell research

The history concerning SC research did not follow a straight path. A lot of other scientific disciplines in biomedicine exerted and still exert great influence on this special topic. The history of ES cells is tightly connected with outcomes and methods of studies on early embryonic development of different organism and *in-vitro* fertilization strategies. In contrast, works on AS cells are in close context of aging, cell repair strategies and haematopoiesis.

The beginning of SC research can be dated back to Ernst Neumann, who was appointed professor of pathology at Königsberg in 1866 and described in a preliminary communication [5], the presence of nucleated red blood cells in bone marrow (BM) saps. He concluded in his subsequent papers, that during postembryonic life, erythropoiesis and leukopoiesis are taking place in the BM. On the basis

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of his observation, Ernst Neumann was the first to postulate the BM as blood forming organ with a common SC for all hematopoietic cells. Walter Heap succeeded for the first time in 1890 in generating live born rabbits by culturing embryos to the blastocysts stage and transferring them back into the uterus [6]. Further studies followed to increase the understanding of mammalian early development and the capacity of isolated blastomeres to develop into viable embryos [7–12], which also included observations on scarce human embryos [13]. In 1957, the era of Robert G. Edwards begun. In that year he started his scientific career as a PhD student in Alan Beatty's laboratory (Institute of Animal Genetics, Edinburgh), who worked on producing haploid, triploid and tetraploid embryos by warming fertilized mouse oocytes [14]. His first work, producing androgenetic and gynogenetic haploid embryos, triploids, tetraploids and various heteroploids [15], was influenced by the work of Alexander A. Maximow, who was manipulating mammalian embryos *in vitro* [16]. Krzysztof Tarkowski from Warsaw published a study on regulation in the development of isolated blastomeres of the mouse in 1959 [17] and aggregated mechanically denuded mouse morulae in pairs to obtain giant blastocysts, some of which developed normally to term following transfer to uterine foster-mothers [18]. Subsequently, Beatrice Mintz introduced various technical improvements that enabled chimaeric mice to be produced routinely [19]. These studies and findings of others were among the first hints on the large potency of mammalian blastomeres and led R. G. Edwards to begin work on individual blastomeres in the rabbit model to search for new approaches to establish SC lines. His initial studies were undertaken in collaboration with Robin Cole, who was one of the first to perform studies on the outgrowths from rabbit blastocysts and inner cell mass (ICM) [20] and John Paul, who provided the facility to culture his cells [21]. One of his students, Richard Gardner, could refine the work of K. Tarkowski and B. Mintz by obtaining chimaeras after injecting embryonic cells into blastocysts (injection chimaeras) [22] instead of fusing blastomeres or morulae (fusion chimaeras) to produce chimaeras. With this, the value of chimaeras for studying development was enhanced and resulted in being the standard way for obtaining germline transmission of genetically modified ES cells [23]. Chimaeras helped in clarifying cell allocations to various tissues [24–27] and widened the scope of analyses on clonal differentiation of tissues [28–30]. The next milestone influencing SC research was the birth of the first baby after *in-vitro* fertilisation (IVF) in 1978 [31]. In 1984, the first human ES cells were reported to be derived from ICM cells of day 5 blastocysts [32] and were “among the primary intentions of introducing human IVF” [33]. Thomson's group who published the culture of human ES cells in 1998 [34] used methods virtually identical to the rabbit models used by Cole RJ et al. in Glasgow, Scotland,

30 years earlier [20], methods, which were also of utmost importance to establish the first SC lines from mouse embryos in 1981 [35].

A few years later AS cells attracted more and more the intention of scientists. Studies on the grafting potentials of the earliest stages of haematopoietic stem cells (HSC) were carried out by a PhD student of Edwards, Peter Hollands, who could follow the migratory path of such cells from the yolk sac and injected into the tail vein of the mouse to the liver, spleen and BM [36, 37]. He then already postulated that such cells could also migrate to other sites of the embryo [33]. The circle, starting in the adult setting with the proposal of a common SC in the blood by Ernst Neumann, followed by such studies as listed above, first in animal models and later in the human using embryos to study the fate of embryonic cells now closes with the idea, that the 4-cell stage embryo might already harbor one blastomere from where SC origin and distribute to numerous tissues including gonads [38, 39].

Plasticity of stem cells

Injection of ES cells into blastocysts is commonly used to generate chimaeras thus providing the most convincing evidence for stem cell plasticity [22]. Since the publications of Jiang et al. in 2002 [40] and Pochampally et al. in 2004 [41]—where AS cells were either injected into murine blastocyst or otherwise into developing chick embryos and participation of injected cells in tissue development/organ formation was demonstrated—it seems that at least some sort of AS cells have a similar plasticity potential as ES cells. Other reports such as from Geiger et al. [42] and Harder et al. [43, 44] further substantiate such findings. Both groups focused on HSC injected into blastocyst and their contribution to haematopoiesis. More recently, Durr et al. [45] were able to demonstrate erythroid marker expression after injecting acute myeloid leukaemia cells into murine blastocysts. The differentiation potential of microinjected cardiac SC into murine blastocysts was investigated by Oh et al. [46]. However, all these studies, demonstrating the ability of AS cells microinjected into blastocysts to contribute to further development did not include the characterization of such cells from the very beginning, when donor cells start attaching to the ICM and then further follow and characterize their invasion path. In a study intended to establish a system that helps in identifying and further characterizing AS cells in the earliest stages of interaction and integration into a conducive environment, such as a murine blastocyst we could show that after injection of CD34 positive human HSC into mouse blastocysts some of these cells attach to the ICM [47, 48] (Fig. 1).

Taken all those findings on AS cells so far together, the contribution of AS cells to organs/tissues from all these

Fig. 1 Paraffin section (5 μm) of murine blastocyst, cultivated for 1 h after injection of human HSCs. Anti-CD45RB antibody was used for immunohistochemical detection of human cells. Scale bar = 100 μm
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studies was explained as a result of trans-/de-differentiation or plasticity of such cells. Trans-differentiation describes the ability of the AS cells to become differentiated cells of another type than their origin (e.g.; HSC turning into neuronal cells).

Several more recent reports have also challenged the general concept that AS cells are plastic and trans-differentiate into other tissues [49–52]. Studies by Clarke et al. [53] and Galli et al. [52] have shown that myoblast cells or embryoid bodies co-cultured with neuronal SC labelled with β -galactosidase produced β -galactosidase labelled muscle cells. This was interpreted as evidence that SC received signals that caused them to trans-differentiate into muscle cells. The paper by Ying et al. [53] as well as other papers [54, 55] alerted investigators to the possibility that perhaps all instances of trans-differentiation were the result of fusion. In Ying's experiments, a low frequency of fusion was observed when mouse central nerve SC were mixed with ES cells. They could show that the interpretations by Clarke and Galli were wrong, and that there were no signals, but that a fusion had occurred. Another explanation of SC plasticity is based on a possibility of the appearance of epigenetic changes in cells exposed to external stimuli (e.g., organ damage, unphysiological culture conditions or stress) [56]. Both cell fusion and epigenetic changes, however, are extremely rare and randomly occurring events that may not fully account for the previously published positive trans-differentiation data. Furthermore, fusion as a major contributor to the observed donor-derived chimerism was excluded in several recently published studies [59–65]. Besides, ES

cells could reside in adult organs/tissues as was already postulated almost 150 years ago by Rudolf Virchow and Julius Connheim. They hypothesized that adult tissues may contain embryonic remnants that were 'lost' during developmental organogenesis and lie dormant. They also postulated that this population of dormant embryonic cells may give rise to some malignancies [66]. This concept was recently presented by some other investigators [52, 67]. ES cells could in fact reside as a rare and dormant cell population in adult tissues [68, 69]. In support of this, it was demonstrated that adult tissues contain embryonic-like SC [70–75] with the expression of Oct-4 protein — which is a marker of pluripotent ES cells — at least in adult human hair follicles [71] lung epithelium [72], heart tissue and adult testes [73]. However, they were never isolated and characterized at the single-cell level. Furthermore, Kucia et al. [70] provided direct evidence that a homogenous rare population of cells in murine BM, termed very small embryonic-like (VSEL) SC, have the morphology and express several markers of undifferentiated embryonic-like SC such as the transcription factor Oct-4. Cells with a very similar morphology and markers to murine BM-derived VSEs were also recently described by the same group in human umbilical cord blood (UCB) [76].

The future in SC based therapies

There are now more than 1,000 SC based therapies in early human trials around the world. Until now, the vast majority of therapies are conducted with SC from patients' own BM or coming from cord blood banks. There are also intentions of some companies to begin first human trials soon using ES cells. After years of being thought of as science fiction — the domain of animal labs and the distant future-embryonic as well as adult SC-based therapies are increasingly becoming a scientific fact.

Therapies utilizing ES cells

ES cell lines are the *in-vitro* manifestation of cells of the ICM derived from blastocysts. Apart from ethical and moral concerns, ES cells can be expanded and then differentiated into most cell lineages of a living organism — making them a virtually unlimited source for cell replacement therapy. In practice, however, it remains almost an art to coax ES cells into generating specific cell types. It has been suggested that ES cells are likely to be distinct from ICM cells and may be more closely related to epiblast cells or embryonic germ cells (which are the *in-vitro* manifestation of primordial germ cells) [77]. Until now, the tumour-forming potential of ES cells has proved a significant problem for realising therapeutic applications. Numerous papers over the last few years

have shown tumour formation in animals treated with differentiated ES cells derivatives [35, 78–82]. However, there are also more and more publications on strategies to purify differentiating and differentiated derivatives of ES cells with the intention to get rid of those cells, which still harbour the risk of tumour formation [83–89]. Also the site of transplantation [90–92] and the number of transplanted cells [83, 92, 93] have to be taken into consideration. For instance, Koch et al. [83] reported that for observing tumour formation administration of at least $\times 10^5$ cells was required. They postulated that a larger number of cells establish a microenvironment, perhaps an aggregate of cells, into which complement penetrates poorly, conducive to the survival of “protected” cells. *in-vitro* cultivation itself, animal additives in the media and feeder cells also pose risks for the transplantation setting. All these factors bear the risk of being mutagenic and that cells lose their immunologic acceptance, thus becoming useless for clinical application. These methodical problems are all under investigation and will hopefully be solved some time soon. Even if all mentioned problem were solved there is still the fact, that ES cells are not autologous except ES cell lines set up by “therapeutically cloning”—and for that reason they could cause an immunologic reaction.

Therapies utilising AS cells

AS cells, especially HSC were routinely used for transplantation. In 1957, the first BM transplantation with intravenous infusion of HSC was reported [94]. Most of the reports concerning AS cells plasticity were undertaken with HSC. These cells are relatively easily accessible in large amounts from BM or UCB without harming the patient, and are well characterized by several specific surface markers. It had been postulated that some of the AS cells found in the hematopoietic system possess germ-layer unrestricted plasticity and may trans-differentiate into SC from all non-hematopoietic lineages. Nowadays it is well established that UCB-derived cells contribute to multiple tissue-regenerations and recently, multiorgan engraftment [95–99]. Besides HSCs there also exists the ability to isolate mesenchymal stem cells (MSC) [100–102] and monocyte-derived SC [103]. It was also recently possible to generate AS cells expressing ES cell lineage markers after *in-vitro* culture of UCB-derived cells [104] or amniotic fluid cells [105, 106] and sharing properties with human ES cells including differentiation capabilities [107]. One of the biggest advantages to use AS cells is that an autologous transplantation is possible. Donor and recipient could be one person. If the high development potential of AS cells is caused by trans-differentiation or cell fusion is unclear yet, but both events seem to be important for regenerating damaged organs.

Conclusion

Still, much has to be learned on similarities or differences by directly comparing gene expression profiles of differentiated ES cells to isolate AS cells. It is important to know which stages of development of differentiating ES cells correspond to the various differentiation stages of AS cells. This knowledge is crucial for developing optimised culture conditions to generate differentiated or trans-differentiated AS cells as well as ES cells. These studies have to be carried out with cells cultured under GMP-compatible conditions to define comparability, which, until now, does not exist worldwide.

References

1. Fraidenaich D, Stillwell E, Romero E, Wilkes D, Manova K, Basson CT, Benezra R. Rescue of cardiac defects in id knock-out embryos by injection of embryonic stem cells. *Science* 2004;306:247–52.
2. Borlongan CV, Hadman M, Sanberg CD, Sanberg PR. Central nervous system entry of peripherally injected umbilical cord blood cells is not required for neuroprotection in stroke. *Stroke* 2004;35:2385–9.
3. Conboy IM, Conboy MJ, Wagers AJ, Girma ER, Weissman IL, Rando TA. Rejuvenation of aged progenitor cells by exposure to a young systemic environment. *Nature* 2005;433:760–4.
4. Geiger H, Rennebeck G, Van Zant G. Regulation of hematopoietic stem cell aging *in vivo* by a distinct genetic element. *Proc Natl Acad Sci USA* 2005;102:5102–7.
5. Neumann E. Über die Bedeutung des Knochenmarks für die Blutbildung. *Zentralblatt für die medizinischen Wissenschaften* 1868;44:122.
6. Heape W. Preliminary note on the implantation and growth of mammalian ova within a foster mother. *Proc Royal Soc London* 1890;B48:457–8.
7. Lewis WH, Gregory PW. Cinematographs of living developing rabbit eggs. *Science* 1933;69:226–9.
8. Lewis WH, Wright ES. On the early development of the mouse egg. *Carnegie Inst Contr Embryol* 1935;25:113–43.
9. Pincus G, Saunders B. The comparative behaviour of mammalian eggs *in vitro* and *in vivo*. VI. The maturation of human ovarian ova. *Anat Rec* 1939;75:537–45.
10. Nicholas JS, Hall BV. Experiments on developing rats. II. The development of isolated blastomeres and fused eggs. *J Exper Zool* 1940;90:441–57.
11. Seidel F. Die Entwicklungspotenzen einer isolierten Blastomere des Zweizellstadiums der Säugetiere. *Naturwissenschaften* 1952;39:355–6.
12. Beatty RA, Fischberg M. Heteroploidy in mammals. 1. Spontaneous heteroploidy in pre-implantation mouse eggs. *J Genet* 1951;50:345–59.
13. Hertig AT, Rock J. Two human ova at the previllous stage, having a developmental age of about eight and nine days respectively. *Carnegie Inst Contr Embryol* 1949;33:169–86.
14. Beatty RA. Parthenogenesis and Polyploidy in Mammalian Development. Cambridge University Press, London, 1957.
15. Edwards RG. The experimental induction of gynogenesis in the mouse. I. Irradiation of the sperm by x-rays. *Proc R Soc Lond B Biol Sci* 1957;146:469–87.

16. Maximov A. Tissue cultures of young mammalian embryos. *Carnegie Inst Contr Embryol* 1925;16:47–113.
17. Tarkowski AK. Experimental studies on regulation in the development of isolated blastomeres of mouse eggs. *Acta Theriol* 1959;3:191–267.
18. Tarkowski AK. Experiments on the development of isolated blastomeres of mouse eggs. *Nature* 1961;190:857–60.
19. Mintz B. Formation of genetically mosaic mouse embryos, and early development of “lethal (T12/T12)-normal” mosaics. *J Exp Zool* 1964;157:273–92.
20. Cole R, Edwards RG, Paul J. Cytodifferentiation in cell colonies and cell strains derived from cleaving ova and blastocysts of the rabbit. *Exp Cell Res* 1964;37:501–4.
21. Edwards RG. Personal pathways to embryonic stem cells. *Reprod Biomed Online* 2002;4:263–78.
22. Gardner RL. Mouse chimeras obtained by the injection of cells into the blastocyst. *Nature* 1968;220:596–7.
23. Gardner RL. Contributions of blastocyst micromanipulation to the study of mammalian development. *Bioessays* 1998;20:168–80.
24. Tarkowski AK. Mouse chimeras developed from fused eggs. *Nature* 1961;190:857–60.
25. Gandini E, Gartler SM, Angioni G, Argiolas N, Dell’Acqua G. Developmental implications of multiple tissue studies in G-6-PD deficient heterozygotes. *Proc US Natl Acad Sci* 1968;61:945–8.
26. Gearhart JO, Mintz B. Clonal origins of somites and their muscle derivatives: evidence from allophenic mice. *Develop Biol* 1972;29:27–37.
27. Mintz B. Gene control of mammalian differentiation. *Ann Rev Genetics* 1974;8:411–740.
28. Mintz B. Gene control of mammalian pigmentary deposition. I. Clonal origin of melanocytes. *Proc US Natl Acad Sci* 1967;58:344–51.
29. Tarkowski AK, Wroblewska J. Development of blastomeres of mouse eggs isolated at the 4- and 8-cell stage. *J Embryol Exp Morphol* 1967;18:155–80.
30. McLaren A. Mammalian Chimeras. Cambridge University Press, Cambridge, 1976.
31. Steptoe PC, Edwards RG. Birth after the reimplantation of a human embryo. *Lancet* 1978;2:366.
32. Fishel S, Edwards RG, Evans CJ. Human chorionic gonadotrophin secreted by preimplantation embryos cultured *in vitro*. *Science* 1984;223:816–8.
33. Edwards RG. Stem cells today: A. Origin and potential of embryo stem cells. *Reprod Biomed Online* 2004;8:275–306.
34. 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;282:1145–7.
35. Evans MJ, Kaufman MH. Establishment in culture of pluripotent cells from mouse embryos. *Nature* 1981;292:154–6.
36. Hollands P. Differentiation and grafting of haemopoietic stem cells from early postimplantation mouse embryos. *Development* 1987;99:69–76.
37. Hollands P. Embryonic haemopoietic stem cell grafts in the treatment of murine genetic anaemia. *Br J Haematol* 1988;70:157–63.
38. Edwards RG, Beard HK. Oocyte polarity and cell determination in early mammalian embryos. *Mol Hum Reprod* 1997;3:863–905.
39. Torres-Padilla ME, Parfitt DE, Kouzarides T, Zernicka-Goetz M. Histone arginine methylation regulates pluripotency in the early mouse embryo. *Nature*. 2007;445:214–8.
40. 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;418:41–9.
41. Pochampally RR, Neville BT, Schwarz EJ, Li MM, Prockop DJ. Rat adult stem cells (marrow stromal cells) engraft and differentiate in chick embryos without evidence of cell fusion. *Proc Natl Acad Sci USA* 2004;101:9282–5.
42. Geiger H, Sick S, Bonifer C, Muller AM. Globin gene expression is reprogrammed in chimeras generated by injecting adult hematopoietic stem cells into mouse blastocysts. *Cell* 1998;93:1055–65.
43. Harder F, Henschler R, Junghahn I, Lamers MC, Muller AM. Human hematopoiesis in murine embryos after injecting human cord blood-derived hematopoietic stem cells into murine blastocysts. *Blood* 2002;99:719–21.
44. Harder F, Kirchhof N, Petrovic S, Wiese S, Muller AM. Erythroid-like cells from neural stem cells injected into blastocysts. *Exp Hematol* 2004;32:673–82.
45. Durr M, Harder F, Merkel A, Bug G, Henschler R, Muller AM. Chimaerism and erythroid marker expression after microinjection of human acute myeloid leukaemia cells into murine blastocysts. *Oncogene* 2003;22:9185–91.
46. Oh H, Chi X, Bradfute SB, Mishina Y, Pocius J, Michael LH, Behringer RR, Schwartz RJ, Entman ML, Schneider MD. Cardiac muscle plasticity in adult and embryo by heart-derived progenitor cells. *Ann NY Acad Sci* 2004;1015:182–9.
47. Zech N, Koestenbauer S, Vanderzwalmen P, Schoonjans L, Danloy S, Zech H, Blaschitz A, Dohr G. Paraffin-embedded manipulated blastocysts: a tool to demonstrate stem cell plasticity? *Reprod Biomed Online* 2005;10:406–14.
48. Koestenbauer S, Vanderzwalmen P, Hammer A, Schoonjans L, Danloy S, Zech H, Dohr G, Zech NH. Apoptosis affects integration frequency: adult stem cells injected in blastocysts show high caspase-3 activity. *Cell Biol Int* 2006;doi:10.1016/j.cellbi.2006.11.023.
49. Castro RF, Jackson KA, Goodell MA, Robertson CS, Liu H, Shine HD. Failure of bone marrow cells to transdifferentiate into neural cells *in vivo*. *Science* 2002;297:1299.
50. Nygren JM, Jovinge S, Breitbach M, Sawen P, Roll W, Hescheler J, Taneera J, Fleischmann BK, Jacobsen SE. Bone marrow-derived hematopoietic cells generate cardiomyocytes at a low frequency through cell fusion, but not transdifferentiation. *Nat Med* 2004;10:494–501.
51. Murry CE, Soonpaa MH, Reinecke H, Nakajima H, Nakajima HO, Rubart M, Pasumarthi KB, Virag JJ, Bartelmez SH, Poppa V, Bradford G, Dowell JD, Williams DA, Field LJ. Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. *Nature* 2004;428:664–8.
52. Orkin SH, Zon LI. Hematopoiesis and stem cells: plasticity versus developmental heterogeneity. *Nat Immunol* 2002;3:323–8.
53. Clarke DL, Johansson CB, Wilbertz J, Veress B, Nilsson E, Karlstrom H, Lendahl U, Frisen J. Generalized potential of adult neural stem cells. *Science* 2000;288:1660–3.
54. Galli R, Borello U, Gritti A, Minasi MG, Bjornson C, Coletta M, Mora M, DeAngelis MG, Fiocco R, Cossu G, Vescovi AL. Skeletal myogenic potential of human and mouse neural stem cells. *Nat Neurosci* 2000;3:986–91.
55. Ying QL, Nichols J, Evans EP, Smith AG. Changing potency by spontaneous fusion. *Nature* 2002;416:545–8.
56. Terada N, Hamazaki T, Oka M, Hoki M, Mastalerz DM, Nakano Y, Meyer EM, Morel L, Petersen BE, Scott EW. Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion. *Nature* 2002;416:542–5.
57. Alvarez-Dolado M, Pardal R, Garcia-Verdugo JM, Fike JR, Lee HO, Pfeffer K, Lois C, Morrison SJ, Alvarez-Buylla A. Fusion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes. *Nature* 2003;425:968–73.
58. Morshead CM, Benveniste P, Iscove NN, Van Der Kooy D. Hematopoietic competence is a rare property of neural stem cells

- that may depend on genetic and epigenetic alterations. *Nat Med* 2002;8:268–73.
59. Jang YY, Sharkis SJ. Metamorphosis from bone marrow derived primitive stem cells to functional liver cells. *Cell Cycle* 2004;3:980–98.
 60. Harris RG, Herzog EL, Bruscia EM, Grove JE, Van Arnem JS, Krause DS. Lack of a fusion requirement for development of bone marrow-derived epithelia. *Science* 2004;305:90–3.
 61. Almeida-Porada G, Porada CD, Chamberlain J, Torabi A, Zanjani ED. Formation of human hepatocytes by human hematopoietic stem cells in sheep. *Blood* 2004;104:2582–90.
 62. Shefer G, Wlekinski-Lee M, Yablonska-Reuveni Z. Skeletal muscle satellite cells can spontaneously enter an alternative mesenchymal pathway. *J Cell Sci* 2004;117:5393–404.
 63. Wurmser AE, Nakashima K, Summers RG, Toni N, D'Amour KA, Lie DC, Gage FH. Cell fusion-independent differentiation of neural stem cells to the endothelial lineage. *Nature* 2004;430:350–6.
 64. Quesenberry PJ, Dooner G, Dooner M, Abedi M. Developmental biology: Ignoratio elenchi: red herrings in stem cell research. *Science* 2005;308:1121–2.
 65. Bonde J, Hess DA, Nolte JA. Recent advances in hematopoietic stem cell biology. *Curr Opin Hematol* 2004;11:392–8.
 66. Virchow R. Editorial Archive fuer pathologische. *Anat Physiol Klin Med* 1855;8:23–54.
 67. Jordan HE. The History of the Primordial Germ Cells in the Loggerhead Turtle Embryo. *Proc Natl Acad Sci USA* 1917;3:271–5.
 68. Kucia M, Reza R, Jala VR, Dawn B, Ratajczak J, Ratajczak MZ. Bone marrow as a home of heterogeneous populations of nonhematopoietic stem cells. *Leukemia* 2005;19:1118–27.
 69. Kucia M, Ratajczak J, Ratajczak MZ. Bone marrow as a source of circulating CXCR4⁺ tissue-committed stem cells. *Biol Cell* 2005;97:133–46.
 70. Kucia M, Reza 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;20:857–69.
 71. Yu H, Fang D, Kumar SM, Li L, Nguyen TK, Acs G, Herlyn M, Xu X. Isolation of a novel population of multipotent adult stem cells from human hair follicles. *Am J Pathol* 2006;168:1879–88.
 72. Ling TY, Kuo MD, Li CL, Yu AL, Huang YH, Wu TJ, Lin YC, Chen SH, Yu J. Identification of pulmonary Oct-4⁺ stem/progenitor cells and demonstration of their susceptibility to SARS coronavirus (SARS-CoV) infection *in vitro*. *Proc Natl Acad Sci USA* 2006;103:9530–5.
 73. Guan K, Nayernia K, Maier LS, Wagner S, Dressel R, Lee JH, Nolte J, Wolf F, Li M, Engel W, Hasenfuss G. Pluripotency of spermatogonial stem cells from adult mouse testis. *Nature* 2006;440:1199–203.
 74. Dyce PW, Wen L, Li J. *In vitro* germline potential of stem cells derived from fetal porcine skin. *Nat Cell Biol* 2006;8:384–90.
 75. Nayernia K, Lee JH, Drusenheimer N, Nolte J, Wulf G, Dressel R, Gromoll J, Engel W. Derivation of male germ cells from bone marrow stem cells. *Lab Invest* 2006;86:654–63.
 76. Kucia M, Halasa M, Wysoczynski M, Baskiewicz-Masiuk M, Moldenhawer S, Zuba-Surma E, Czajka R, Wojakowski W, Machalinski B, Ratajczak MZ. Morphological and molecular characterization of novel population of CXCR4(+) SSEA-4(+) Oct-4(+) very small embryonic-like cells purified from human cord blood – preliminary report. *Leukemia* 2006; doi:10.1038/sj.leu.2404470.
 77. Clark AT, Bodnar MS, Fox M, Rodriguez RT, Abeyta MJ, Firpo MT, Pera RA. Spontaneous differentiation of germ cells from human embryonic stem cells *in vitro*. *Hum Mol Genet* 2004;13:727–39.
 78. Fujikawa T, Oh SH, Pi L, Hatch HM, Shupe T, Petersen BE. Teratoma formation leads to failure of treatment for type I diabetes using embryonic stem cell-derived insulin-producing cells. *Am J Pathol* 2005;166:1781–91.
 79. Wakitani S, Takaoka K, Hattori T, Miyazawa N, Iwanaga T, Takeda S, Watanabe TK, Tanigami A. Embryonic stem cells injected into the mouse knee joint form teratomas and subsequently destroy the joint. *Rheumatology (Oxford)* 2003;42:162–5.
 80. Asano T, Sasaki K, Kitano Y, Terao K, Hanazono Y. *In vivo* tumor formation from primate embryonic stem cells. *Methods Mol Biol* 2006;329:459–67.
 81. Thinyane K, Baier PC, Schindehutte J, Mansouri A, Paulus W, Trenkwalder C, Flugge G, Fuchs E. Fate of pre-differentiated mouse embryonic stem cells transplanted in unilaterally 6-hydroxydopamine lesioned rats: histological characterization of the grafted cells. *Brain Res* 2005;1045:80–7.
 82. Arnhold S, Klein H, Semkova I, Addicks K, Schraermeyer U. Neurally selected embryonic stem cells induce tumor formation after long-term survival following engraftment into the subretinal space. *Invest Ophthalmol Vis Sci* 2004;45:4251–5.
 83. Koch CA, Jordan CE, Platt JL. Complement-dependent control of teratoma formation by embryonic stem cells. *J Immunol* 2006;177:4803–9.
 84. Chung S, Shin BS, Hedlund E, Pruszk J, Ferree A, Kang UJ, Isacson O, Kim KS. Genetic selection of sox1GFP-expressing neural precursors removes residual tumorigenic pluripotent stem cells and attenuates tumor formation after transplantation. *J Neurochem* 2006;97:1467–80.
 85. Dihne M, Bernreuther C, Hagel C, Wesche KO, Schachner M. Embryonic stem cell-derived neuronally committed precursor cells with reduced teratoma formation after transplantation into the lesioned adult mouse brain. *Stem Cells* 2006;24:1458–66.
 86. Fukuda H, Takahashi J, Watanabe K, Hayashi H, Morizane A, Koyanagi M, Sasai Y, Hashimoto N. Fluorescence-activated cell sorting-based purification of embryonic stem cell-derived neural precursors averts tumor formation after transplantation. *Stem Cells* 2006;24:763–71.
 87. Bieberich E, Silva J, Wang G, Krishnamurthy K, Condie BG. Selective apoptosis of pluripotent mouse and human stem cells by novel ceramide analogues prevents teratoma formation and enriches for neural precursors in ES cell-derived neural transplants. *J Cell Biol* 2004;167:723–34.
 88. Kumashiro Y, Asahina K, Ozeki R, Shimizu-Saito K, Tanaka Y, Kida Y, Inoue K, Kaneko M, Sato T, Teramoto K, Arai S, Teraoka H. Enrichment of hepatocytes differentiated from mouse embryonic stem cells as a transplantable source. *Transplantation* 2005;79:550–7.
 89. Shibata H, Ageyama N, Tanaka Y, Kishi Y, Sasaki K, Nakamura S, Muramatsu S, Hayashi S, Kitano Y, Terao K, Hanazono Y. Improved safety of hematopoietic transplantation with monkey embryonic stem cells in the allogeneic setting. *Stem Cells* 2006;24:1450–7.
 90. Cooke MJ, Stojkovic M, Przyborski SA. Growth of teratomas derived from human pluripotent stem cells is influenced by the graft site. *Stem Cells Dev* 2006;15:254–9.
 91. Wakitani S, Aoki H, Harada Y, Sonobe M, Morita Y, Mu Y, Tomita N, Nakamura Y, Takeda S, Watanabe TK, Tanigami A. Embryonic stem cells form articular cartilage, not teratomas, in osteochondral defects of rat joints. *Cell Transplant* 2004;13:331–6.
 92. Nelson TJ, Ge ZD, Van Orman J, Barron M, Rudy-Reil D, Hacker TA, Misra R, Duncan SA, Auchampach JA, Lough JW. Improved cardiac function in infarcted mice after treatment with pluripotent embryonic stem cells. *Anat Rec A Discov Mol Cell Evol Biol* 2006;288:1216–24.
 93. Behfar A, Hodgson DM, Zingman LV, Perez-Terzic C, Yamada S, Kane GC, Alekseev AE, Puceat M, Terzic A. Administration of

- allogenic stem cells dosed to secure cardiogenesis and sustained infarct repair. *Ann NY Acad Sci* 2005;1049:189–98.
94. Thomas ED, Lochte HL Jr, Lu WC, Ferrebee JW. Intravenous infusion of bone marrow in patients receiving radiation and chemotherapy. *N Engl J Med* 1957;257:491–6.
 95. Shyu WC, Lin SZ, Yang HI, Tzeng YS, Pang CY, Yen PS, Li H. Functional recovery of stroke rats induced by granulocyte colony-stimulating factor-stimulated stem cells. *Circulation* 2004;110:1847–54.
 96. Buzanska L, Machaj EK, Zablocka B, Pojda Z, Domanska-Janik K. Human cord blood-derived cells attain neuronal and glial features *in vitro*. *J Cell Sci* 2002;115:2131–8.
 97. Pesce M, Orlandi A, Iachininoto MG, Straino S, Torella AR, Rizzuti V, Pompilio G, Bonanno G, Scambia G, Capogrossi MC. Myoendothelial differentiation of human umbilical cord blood-derived stem cells in ischemic limb tissues. *Circ Res* 2003;93:e51–e62.
 98. Di Campli C, Piscaglia AC, Pierelli L, Rutella S, Bonanno G, Alison MR, Mariotti A, Vecchio FM, Nestola M, Monego G, Michetti F, Mancuso S, Pola P, Leone G, Gasbarrini G, Gasbarrini A. A human umbilical cord stem cell rescue therapy in a murine model of toxic liver injury. *Dig Liver Dis* 2004;36:603–13.
 99. Newsome PN, Johannessen I, Boyle S, Dalakas E, McAulay KA, Samuel K, Rae F, Forrester L, Turner ML, Hayes PC, Harrison DJ, Bickmore WA, Plevris JN. Human cord blood-derived cells can differentiate into hepatocytes in the mouse liver with no evidence of cellular fusion. *Gastroenterology* 2003;124:1891–900.
 100. Bieback K, Kern S, Kluter H, Eichler H. Critical parameters for the isolation of mesenchymal stem cells from umbilical cord blood. *Stem Cells* 2004;226:625–34.
 101. Gang EJ, Hong SH, Jeong JA, Hwang SH, Kim SW, Yang IH, Ahn C, Han H, Kim H. *In vitro* mesengenic potential of human umbilical cord blood-derived mesenchymal stem cells. *Biochem Biophys Res Commun* 2004;321:102–8.
 102. Kogler G, Sensken S, Airey JA, Trapp T, Muschen M, Feldhahn N, Liedtke S, Sorg RV, Fischer J, Rosenbaum C, Greschat S, Knipper A, Bender J, Degistirici O, Gao J, Caplan AI, Colletti EJ, Almeida-Porada G, Muller HW, Zanjani E, Wernet P. A new human somatic stem cell from placental cord blood with intrinsic pluripotent differentiation potential. *J Exp Med* 2004;200:123–35.
 103. Zhao Y, Mazzone T. Human umbilical cord blood-derived macrophages retain pluripotentiality after thrombopoietin expansion. *Exp Cell Res* 2005;310:311–8.
 104. McGuckin CP, Forraz N, Baradez MO, Navran S, Zhao J, Urban R, Tilton R, Denner L. Production of stem cells with embryonic characteristics from human umbilical cord blood. *Cell Prolif* 2005;38:245–55.
 105. Karlmark KR, Freilinger A, Marton E, Rosner M, Lubec G, Hengstschlager M. Activation of ectopic Oct-4 and Rex-1 promoters in human amniotic fluid cells. *Int J Mol Med* 2005;16:987–92.
 106. Prusa AR, Marton E, Rosner M, Bernaschek G, Hengstschlager M. Oct-4-expressing cells in human amniotic fluid: a new source for stem cell research? *Hum Reprod* 2003;18:1489–93.
 107. Zhao Y, Wang H, Mazzone T. Identification of stem cells from human umbilical cord blood with embryonic and hematopoietic characteristics. *Exp Cell Res* 2006;312:2454–64.
 108. De Coppi P, Bartsch G Jr, Siddiqui MM, Xu T, Santos CC, Perin L, Mostoslavsky G, Serre AC, Snyder EY, Yoo JJ, Furth ME, Soker S, Atala A. Isolation of amniotic stem cell lines with potential for therapy. *Nat Biotechnol* 2007;25:100–6.