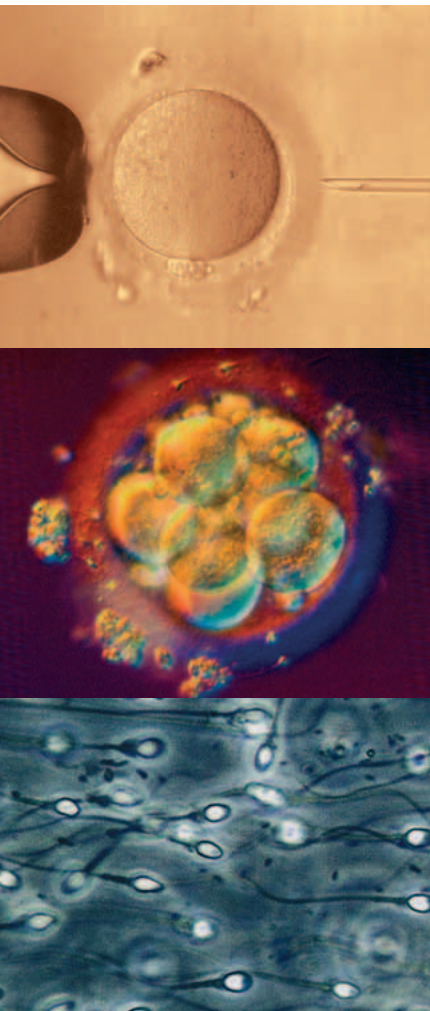


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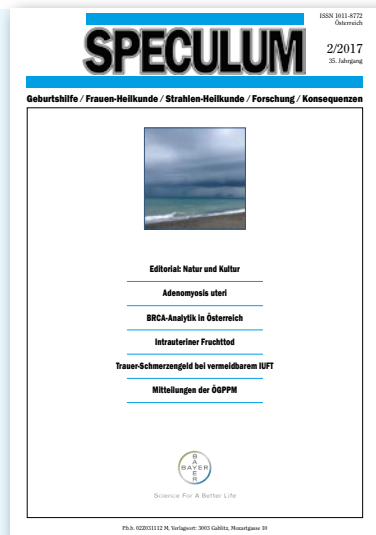
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Adult Stem Cell Manipulation and Possible Clinical Perspectives

N. H. Zech

Growing interest on the potential use of pluripotent stem cells as a source of differentiated cells for repair of degenerating or damaged tissue in humans can be noticed. With the rapid advance of biotechnical research, which was initially started in Europe, stem cell engineering and therapy is becoming more and more centralised in clinical aspects. The definition of stem cells, both of embryonic and adult origin, has been discussed extensively in the last couple of years. Recently adult stem cell plasticity has been further elucidated. Pros and contras of the use of embryonic or adult stem cells are discussed in this review. Animal models are presented to analyse the differences/similarities of embryonic pluripotent cells and tumour stem cells. For clinical application, still many questions have to be solved, e.g. gender specific aspects and age related characteristics of various stem cells. Recent reports on the aspect of fusion of donor stem cells with host cells as an possible explanation for the plasticity of stem cells are outlined.

Key Words: stem cells, cell engineering, therapy, plasticity, fusion, tumour

Adulte Stammzellmanipulation und mögliche klinische Perspektiven. Während der letzten Jahre konnte ein wachsendes Interesse an einem möglichen Einsatz pluripotenter Stammzellen als Ausgangsmaterial für differenzierte Zellen zur Heilung von degenerativem oder geschädigtem Gewebe beim Menschen beobachtet werden. Mit dem raschen Fortschritt der biotechnologischen Forschung, welche ihren Ursprung in Europa nahm, rückt die individuelle Zellmanipulation und Therapie mit Stammzellen immer mehr in klinische Bereiche vor. Die Definition sowohl von embryonalen als auch von adulten Stammzellen wurde in den letzten Jahren intensiv diskutiert. Erst seit kurzem wird die Plastizität von adulten Stammzellen näher untersucht. Pros und Kontras des Einsatzes von embryonalen oder adulten Stammzellen werden in diesem Review beleuchtet. Am Beispiel von Tiermodellen werden die Unterschiede/Ähnlichkeiten von embryonalen pluripotenten Zellen und Tumorstammzellen aufgezeigt. Um Stammzellen in die Klinik einzuführen, sind noch viele Fragen zu klären, wie zum Beispiel die Bedeutung geschlechtsspezifischer Aspekte oder altersbezogener Charakteristika verschiedenster Stammzelltypen. Neueste Publikationen über Aspekte der Fusion von Spenderstammzellen mit Empfängerzellen in bezug auf die Erklärung der Plastizität werden erörtert. **J Reproduktionsmed Endokrinol 2004; 1 (2): 91–9.**

Schlüsselwörter: Stammzellen, Zellmanipulation, Therapie, Plastizität, Fusion, Tumor

Biomedical research on stem cells 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. Diseases and disorders with no therapy or at best partially effective ones are the lure of the pursuit of stem cell research, which will advance the knowledge about how an organism develops from a single cell and how healthy cells replace damaged cells in adult organisms.

Europe has a long tradition in stem cell research and reproductive medicine. From here it was exported to the USA, into the Pacific Area and Middle East, where it has been widely used and improved. Fundamental research in developmental biology and reproductive biology was performed in Europe during the first two thirds of the last century. Stem cells have in fact been part of human *in vitro* fertilization for roundabout three decades. To give credit to the pioneers of stem cell research, I want to briefly outline their enormous contribution to this field.

It all started with Ernst Neumann, who was appointed professor of pathology at Königsberg in 1866 and described in a preliminary communication [1], the presence of nucleated red blood cells in bone marrow saps of rabbits and humans obtained by squeezing bones. He concluded in his subsequent papers, that during postembryonic life, erythropoiesis and leukopoiesis are taking place in the bone marrow. On the basis of his observation, Ernst Neumann was the first to postulate the bone marrow as blood forming organ with a common stem-cell for all hematopoietic cells. Unfortunately, outstanding contemporary investigators were reluctant to accept his novel ideas. Neumann's far sighted findings were finally honoured within the foundation of the Berlin Society of Haematology in 1908 by A. Maximow, F. Weidenreich, C. Bernard and A. Pappenheim.

W. Heap [2] 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. Further studies in the animal model followed to increase the understanding of mammalian early development and the capacity of isolated blastomeres to develop into viable embryos [3–8] which also included observations on scarce human embryos [9]. Alan Beatty, at the institute of Animal Genetics in Edinburgh was working on producing haploid, triploid and tetraploid embryos by warming fertilized mouse oocytes [10]. Robert G. Edwards, inspired by the findings of A. Maximow, who was manipulating mammalian embryos *in vitro* [11], began his scientific carrier as PhD student 1957 in Alan Beatty's laboratory [12] by starting to produce androgenetic and gynogenetic haploid embryos, triploids, tetraploids and various heteroploids [13].

Krzysztof Tarkowski from Warsaw published a study on regulation in the development of isolated blastomeres of the mouse in 1959 [14] 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 [15]. Subsequently, Beatrice Mintz introduced various technical improvements that enabled chimaeric mice to be produced routinely [16]. 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 stem cell lines. His initial studies were undertaken in collaboration with R. Cole, who was one of the first to perform studies on the outgrowths from rabbit blastocysts and inner cell mass (ICM) [17] and J. Paul, who provided the facility to culture his cells [18]. One of his students, Richard Gardner, could refine the work of Tarkowski and Mintz by obtaining chimaeras after

Aus dem Department für Frauenheilkunde, Universitätsspital, Zürich

Korrespondenzadresse: Dr. med. Nicolas H. Zech, Department für Frauenheilkunde, Klinik für Gynäkologie, Universitätsspital Zürich, Frauenklinikstrasse 10, CH-8091 Zürich; E-Mail: nicolas.zech@fhk.usz.ch

injecting embryonic cells into blastocysts (injection chimaeras) [19] instead of fusing blastomeres or morulae (fusion chimaeras) to produce chimaeras. With this, the value of chimaeras for studying development [20] was enhanced and resulted in being the standard way for obtaining germline transmission of genetically modified embryonic stem cells.

Chimaeras helped in clarifying cell allocations to various tissues [21–24] and widened the scope of analyses on clonal differentiation of tissues [25–27]. For Edwards, it was clear that embryo cells had immense potential *in vitro*, thus having the vision of using stem cells in clinical medicine in mid-1963 (Fig. 1). In 1978 the first baby was born after *in vitro* fertilisation [28]. In 1984, the first human embryo stem cells were reported to be derived from inner cell mass cells of day 5 blastocysts [29] and was „among the primary intentions of introducing human IVF“ [30].

Studies on the grafting potentials of the earliest stages of haematopoietic stem cells were carried out by another 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 bone marrow [31, 32]. He then already postulated

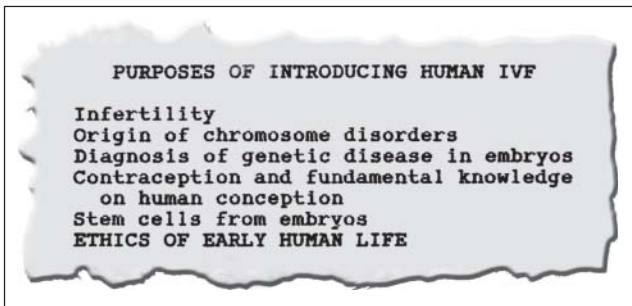


Figure 1: A slide outlining the purpose of introducing human IVF. Reprinted from an article in Reproductive BioMedicine Online by R. G. Edwards (2002) [18], with permission from Reproductive Healthcare Ltd.

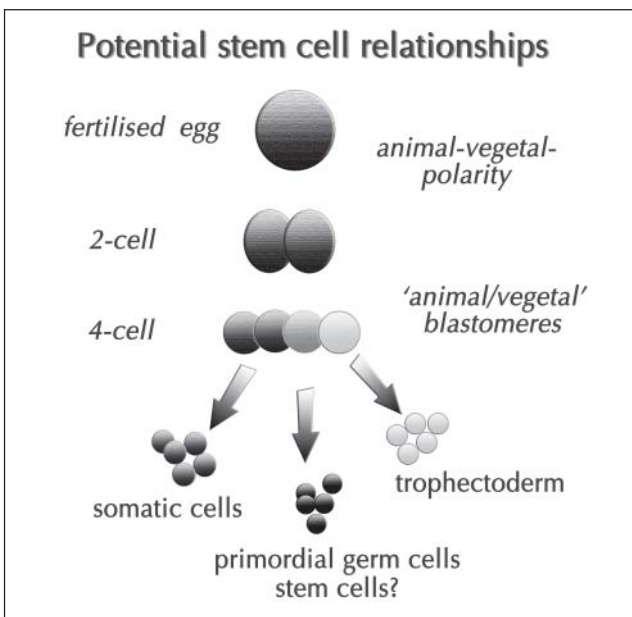


Figure 2: Potential stem cell relationship. Parallels with germ cells may include an origin from one blastomere in 4-cell embryos (Edwards and Beard, 1997). A group of multipotent stem cells might distribute to numerous tissues including gonads. Their failure to colonise trophoblast may be due to its earlier differentiation. Reprinted from an article in Reproductive BioMedicine Online by R. G. Edwards (2002) [18], with permission from Reproductive Healthcare Ltd.

that such cells could also migrate to other sites of the embryo [30]. The circle, starting in the adult setting with the proposal of a common stem cell 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 harbour one blastomere from where stem cells origin and distribute to numerous tissues including gonads [33] (Fig. 2).

Thomson’s group who published the culture of human embryonic stem cells (ES) in 1998 [34] used methods virtually identical to the rabbit models used by Cole RJ et al. in Glasgow, Scotland, 30 years earlier [35], methods, which were also of utmost importance to establish the first pluripotent cell lines from mouse embryos in 1981 [36].

Definition of stem cells

There are four criteria to be fulfilled when defining a cell as a stem cell (Figures 3–5):

- First, such cells have to be able to undergo self-renewing cell divisions, a prerequisite for sustaining the population.
- Second, daughter cells derived from a single stem cell must have the ability to differentiate into multiple cell types. Examples include haematopoietic stem cells (HSC) which give rise to all haematopoietic cells [37–40]; neural stem cells (NSC) which give rise to neurons, astrocytes, and oligodendrocytes [41–44]; germline

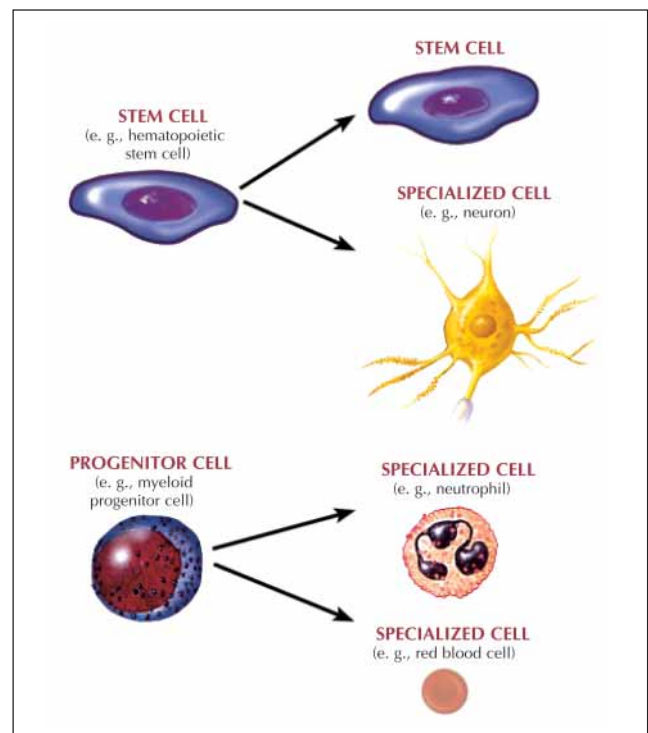


Figure 3: Distinguishing features of progenitor/precursor cells and stem cells. A stem cell is an unspecialised cell that is capable of replicating or self renewing itself and developing into specialised cells of a variety of cell types. The product of a stem cell undergoing division is at least one additional cell that has the same capabilities of the original cell. Shown here is an example of a hematopoietic stem cell producing a second generation stem cell and a neuron. A progenitor cell (also known as a precursor cell) is unspecialised or has partial characteristics of a specialised cell that is capable of undergoing cell division and yielding two specialised cells. Shown here is an example of a myeloid progenitor/precursor undergoing cell division to yield two specialised cells (a neutrophil and a red blood cell). © 2001 Terese Winslow assisted by Lydia Kibiuk and Caitlin Duckwall.

stem cells which produce new oocytes [45] and mesenchymal stem cells (MSC) which differentiate into fibroblasts, osteoblasts, chondroblasts, and adipocytes [46]. Some adult stem cells may give rise to only a single mature cell type, such as the corneal stem cell.

- A third criterion is that such cells should be able to functionally repopulate a damaged tissue when transplanted. This has been shown extensively for HSC and more recently for other cells such as from the liver [47] and from the central nervous system [48, 49].
- Fourth, a less well established criterion is that such cells should have the capacity to contribute differentiated progeny *in vivo* even in the absence of tissue damage.

In vitro cultured inner cell mass cells (ICM) derived from blastocysts – termed embryonic stem cells – which do not equal the original inner cell mass cells because they result from a culture of ICM, fulfil all of the four criteria [34, 50], even though they show intrinsic defects and compromise the development of completely ES-derived mice compared to completely ICM-derived mice [51]. Prolonged *in vitro* culture seems to further negatively affect this initial potency of ES to generate viable offspring as was shown by Nagy A. et al. [52] when mice completely derived from cultured ES-cells with the help of tetraploid embryos had more and more problems to survive to term after prolonged cell-culture.

For adult stem cells, these criteria have been most extensively used to characterize HSC.

During mammalian embryogenesis, a first transient wave of primitive haematopoiesis originates in the extra-embryonic yolk sac. Later, the foetal liver is colonized by HSCs from the aorta gonad mesonephros region, which is regarded as the first site of definitive haematopoiesis. All of these haematopoietic progenitor cells can be found in the foetal circulation and during birth in the umbilical cord blood vessels. They are by definition adult stem cells. Sub-

sequently, HSCs migrate to the bone marrow, which is the haematopoietic active tissue of the adult [53–56]. Single HSC can undergo at least asymmetric self-renewing cell division [Fig. 6], give rise to all blood elements, reconstitute the haematopoietic system when transplanted in lethally irradiated recipients, and engraft and differentiate in animals, even if the recipient is not irradiated [57]. In

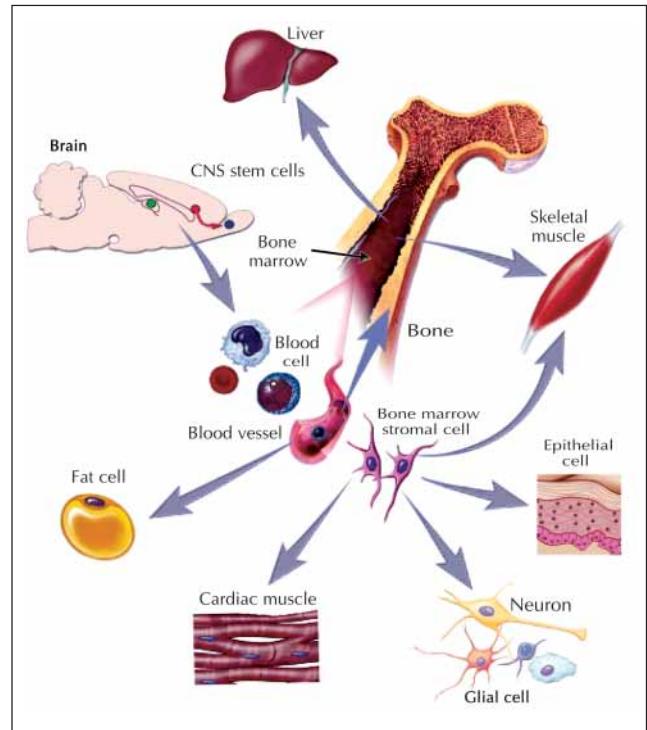


Figure 4: Preliminary evidence of plasticity among nonhuman adult stem cells. © 2001 Terese Winslow assisted by Lydia Kibiuk and Caitlin Duckwall.

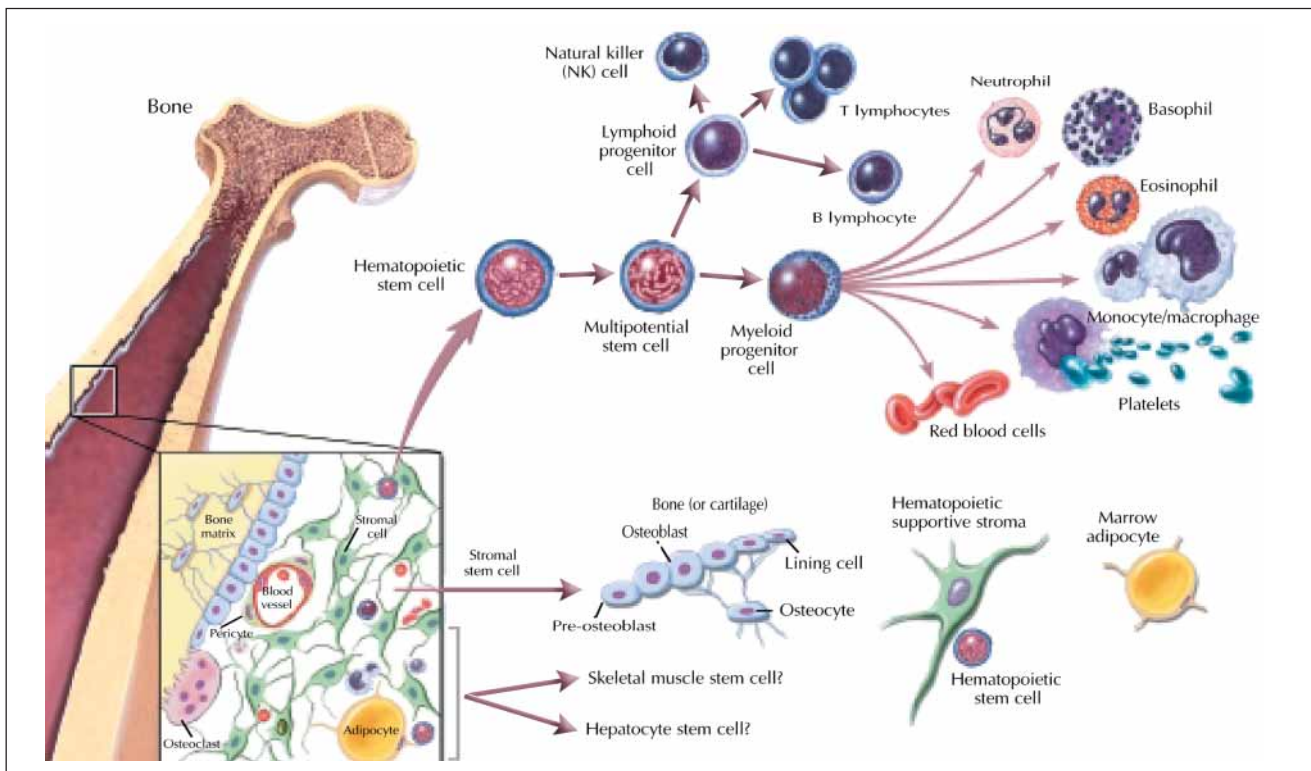


Figure 5: Hematopoietic and stromal stem cell differentiation. © 2001 Terese Winslow assisted by Lydia Kibiuk and Caitlin Duckwall.

mice, HSC have been purified to near-homogeneity based on cell surface characteristics [39].

Whether human HSC have been enriched to near-homogeneity, as has been achieved for murine HSC, is not clear, given that characterization of human HSC depends on transplantation in xenogenic hosts. A minimum of 200–500 cells is still required to achieve human haematopoietic cell progeny engraftment in immunodeficient mice or foetal sheep [58]. Proof that single HSC can differentiate into multiple blood cell lineages comes from transplants of single mouse HSC [40].

Alternatively, retroviral marking has been used. As retroviruses integrate randomly into the host cell DNA, the

DNA sequence flanking the viral integrant is cell specific and can be used to follow progeny of individual cells *in vitro* or *in vivo*. This approach has been used to demonstrate in both mice [59] and humans [60] that single HSC can give rise to multiple progeny cells. In addition, this approach has allowed for the demonstration that single HSC undergo self-renewing cell divisions and that multiple daughter cells have multilineage differentiation potential [59].

Adult stem cell plasticity

True multi- or pluripotent stem cells persist in postnatal life. Over the past couple of years, a series of reports has been published suggesting that the previous dogma of tissue specificity associated with adult stem cells may not be correct. The presumed ability of tissue-specific stem cells to acquire the fate of cell types different from the tissue of origin has been termed adult stem cell plasticity.

Several mechanisms may underlie this apparent plasticity:

- multiple tissue-specific stem cells (multipotent) are present in different organs and have the capability to de-differentiate to a less lineage-committed state from where the cell can again start the program of differentiation to a more committed state (re-differentiation). This mechanism can also be termed “graded propensity” (Fig. 7).
- true pluripotent (not yet lineage committed) cells persist beyond early embryonic development and, depending on the milieu, convert to cells different than the organ of origin
- plasticity is the result of fusion of the donor cell with resident cells in an organ – although several *in vitro* studies have shown that lineage switch is possible without co-culture of adult cells with ES cells or other cell

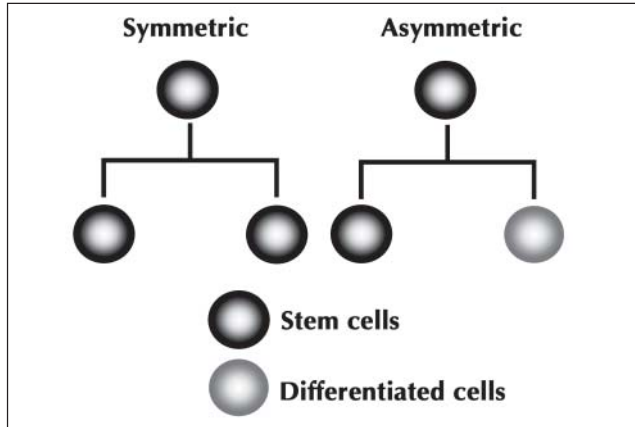


Figure 6: Pattern of segregation of self-renewing stem cells. Symmetric cell division: two identical daughter cells, which resemble the original stem cell, are produced. Asymmetric cell division: a stem cell and a more differentiated (committed) are produced. With permission from Wernig M, Scheffler B, Brüstle O. In: Ganter D, Ruckpaul K (eds). *Grundlagen der Molekularen Medizin*. 2. Auflage. Springer, Berlin, 2003; 680–710. © 2003, Springer-Verlag GmbH.

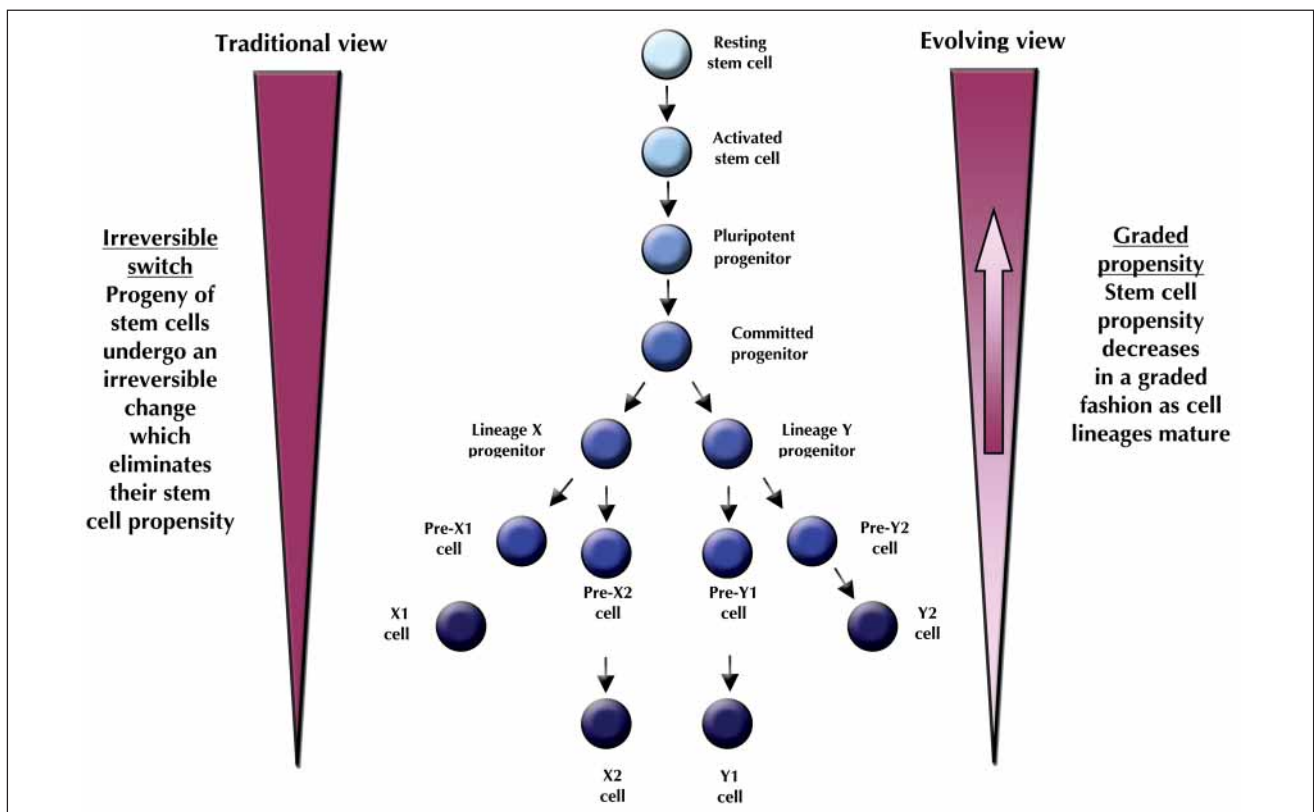


Figure 7: Comparison of two conceptual views of stem cells. Reprinted from Cell, 105, Blau HM, Brazelton TR, Weimann JM. “The evolving concept of a stem cell: entity or function?” 829–41, © 2001, with permission from Elsevier.

types and that cells with novel lineage characteristics are not tetraploid [61–63] new studies suspect cell fusion to be the principal source for *in vivo* plasticity phenomena [64–66] (Fig. 8).

As supposed by Blau HM et al. [67], the fate of a stem cell is dictated by its response to key migration factors and growth factors which leads to the observed cell plasticity by altering the gene expression pattern of the cell. It seems that tissue specific transcription factors are rare. A more plausible explanation is that various cytokines are present in different ratios within a certain tissue which, in their individual concentration, induce explicit patterns of gene expression and thus cause cells to differentiate down pre-determined pathways [67–71].

That way, with a specific constellation of a proposed limited variety of growth and differentiation factors, adult stem cells could be maintained in a non-responsive state until needed, for example for tissue maintenance (skin and blood), in the repair of damage or, when factor constellations are de-regulated in aged/damaged tissue, it may pave the way for malignant transformation, possibly by epigenetic mechanisms with clonal propagation of a stem cell population. This has been shown for instance for the derivation of early primitive ectoderm-like (EPL) cells from ES [72].

The differentiation-state of a cell could also be dictated – maybe in parallel with the specific cytokine mixture of the surrounding tissue – by its direct local environment as has been shown for the dominant role of the niche, where stem cells may reside, for their fate determination [73, 74]. This cell cycle activation by cytokines in an environmental specific concentration mixture is postulated to also dictate the CD34 expression on HSC [75] and may be reversible *in vitro* [75].

One of the most compelling evidence for the observed plasticity of pluripotent cells is the work from Diane Krause and collaborators [77]. After enriching fractioned and lineage depleted bone marrow cells up to 500–1000-fold by recovering cells homing to the bone marrow within 48 hours of transplantation, single bone marrow-derived stem cells showed multi-organ, multi-lineage engraftment. Jiang et al. [65] demonstrated that cells known as multipotent adult progenitor cells (MAPC), co-purifying with mesenchymal cells (MSC) from BM can, at the single cell level, differentiate *in vitro* into cells with characteristics of mesodermal lineages, neuroectodermal lineages, and endodermal lineages. MAPC can also contribute to most, if not all, cells when injected into a blastocyst.

Except for MAPC, which express Oct-4 and Rex-1 – albeit at significantly lower levels than what is seen in ES cells –, no adult cell has been identified which is characterized by these criteria. More recently, cells in the amniotic fluid were also found to express embryonic stem cell markers such as Oct-4 [78]. It still lacks the prove that such cells have similar characteristics as MAPC. As it is not yet known whether MAPC exist as such *in vivo* or are the result of de-differentiation of an MSC-like cell into a cell with greater potential, there is currently no definitive prove that true pluripotent stem cells exist *in vivo* during post-natal life.

Downsides and advantages for stem cells of embryonic and adult origin

An important area that requires substantially more research concerns the immunologic characteristics of ES cells and adult stem cells. If any of these stem cells are to be used as

the basis for therapy, it is critical to understand how the body's immune system will respond to the transplantation of tissue derived from these cells.

Embryonic stem cells from a donor introduced into a patient could cause transplant rejection [79]. However, whether the recipient would reject donor ES has not been determined in human experiments. A primary goal of the study of ES is to identify how undifferentiated stem cells become differentiated for their use in clinical therapy [80–82] and how to be able to control the outgrowth of such cells into teratomas, which is a major limitation for their application [3]. Stem cell-based therapies are a major area of investigation in cancer research [83, 84] because the activity of genes which are essential for the process of cell division and differentiation is abnormal in some medical conditions like for example in cancer [85–87].

The pluripotency of ES is evident by being able to create all tissues in the body, as well as non-trophoblast structures which support the embryo [88]. Thus, such pluripotent cells can be looked at as an optimised system to study the cell-fate of various pluripotent cells (e.g. adult neural stem cells, adult pancreatic stem cells), diseased or damaged cells/tissue in different culture systems *in vitro* as well as *in vivo* using animal models.

Adult stem cells are rare and generally restricted to differentiate into cell types of their tissue of origin [88–90]. Often they are difficult to identify, isolate and purify. Although stem cells from the bone marrow are the most studied type of adult stem cells, the restricted capacity of haematopoietic stem cells to grow in large numbers and re-

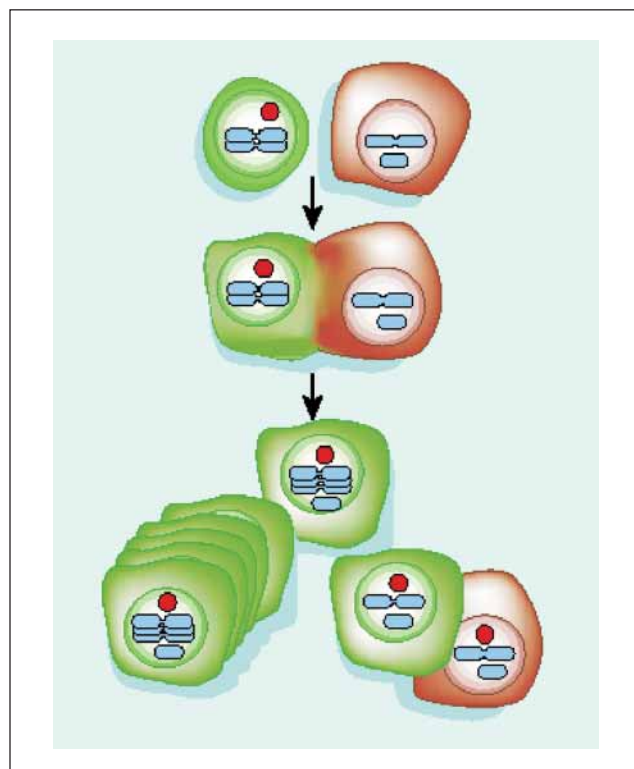


Figure 8: Formation and reduction of hybrids. Cell fusion first produces a cell containing two nuclei; the exchange of specific molecules reprogrammes gene expression. The nuclei then fuse to generate a mononuclear hybrid cell that is polyploid (it has more than the usual chromosomal content). The hybrid divides to give identical polyploid daughters (lower left). Wang et al. [64] present evidence that hybrids can also generate daughters of normal ploidy, presumably through a reduction division (lower right). The expulsion of chromosomes can mask the fusion history of these cells. Blue shading indicates donor chromosomes. Red represents a specific gene product from the donor (in this case, the Fah enzyme). With permission from [111]; © Nature Publishing Group.

main undifferentiated in the culture dish is a major limitation to their broader use for research and transplantation studies. Evidence to date indicates that umbilical cord blood is an abundant source of haematopoietic stem cells. They do not appear to be any qualitative differences between the stem cells obtained from umbilical cord blood and those obtained from bone marrow or peripheral blood. A potential advantage of using stem cells from an adult is that the patient's own cells could be expanded in culture and then reintroduced into the patient, without rejection by the immune system.

Differences/similarities of embryonic pluripotent stem cells and tumour stem cells: data from animal models

Basic research is fundamental for all aspects of stem cell biology, as well as cancer biology as there is emerging evidence of similarities between stem cell biology and cancer biology. The most compelling evidence for the close relationship between tumour stem cells and normal embryonic pluripotent cells is the fact that stem cells taken either from teratocarcinoma or from embryonic carcinoma cell cultures can participate in the development of completely normal adult mice when combined with embryonic cells by the technique of blastocyst injection [91–94].

There are two hypotheses for this observation:

Tumour stem cells could arise as a consequence of a stable but reversible epigenetic change in normal pluripotent embryonic cells. Such "transformed" cells presumably continue proliferation in the undifferentiated state because neoplastic conversion has reduced their efficiency of response to the normal signals for differentiation.

Otherwise, an explanation could be that embryonic carcinoma cells are not transformed but rather represent a selected population of completely normal embryonic cells that are programmed to divide until they receive the appropriate signals for differentiation. When the signals are in an extra-embryonic site, the signals they receive are apparently not conducive to completely normal embryogenesis.

Observations from G. R. Martin in the early 1980's [50] demonstrate that inner cell mass cells isolated from normal mouse blastocysts and cultured in medium conditioned by an established embryonic carcinoma cell line can give rise to cultures of cells with the characteristics of mouse teratocarcinoma stem cells.

These experiments were undertaken on the premise that medium conditioned by teratocarcinoma cells might contain a factor, perhaps identical to a normal endogenous embryonic growth factor, capable of stimulating the proliferation of a small population of pluripotent cells in the normal embryo. A relationship between stem cells and tumour cells seems to be reasonably proposed because there are similar pathways for the regulation of self-renewal of normal stem cells and cancer cells [95–97]. Furthermore, tumour cells could arise from normal stem cells. It could also be possible that tumours harbour "rare cancer stem cells" which proliferate indefinitely and thus drive the formation and growth of tumours.

Understanding the regulation of normal stem cell self-renewal is fundamental to understand the regulation of cancer cell proliferation, because cancer can be considered to be a disease of unregulated self-renewal. Newly arising cancer cells seem to be appropriate to the machinery for self-renewing cell division that is normally expressed in stem cells. It is possible that heterogeneity in tumours arise as a result of continuing mutagenesis but the

other way round it could equally be possible that such cells arise through the aberrant differentiation of cancer cells driven by or rather as a result of environmental differences within the tumour or its surrounding [98]. Our understanding of the cellular biology of cancer has lagged behind our focus on the molecular biology of cancer. We might have gained some insight into the effects of particular mutations on the proliferation and survival of model cells, still the effects of such mutations on the actual cells involved in particular cancers is often only a guessing.

Clinical application

To replace destroyed tissue, up to now, donated organs and tissues are mainly used, but the need for transplantable tissues and organs far outweighs the available supply. Stem cells, directed to differentiate into specific cell types, offer the possibility of a renewable source for replacing cells and tissues to treat diseases including Parkinson's and Alzheimer's diseases, spinal cord injury, blood vessel- and heart diseases, diabetes, osteoarthritis etc. Recent studies in animals generated some promising results towards this application [99–102]. New studies indicate that it may be possible to direct the differentiation of human embryonic stem cells in cell culture to form insulin-producing cells that eventually could be used in transplantation therapy for diabetics [103–105]. Another very promising area for future treatment options with stem cells are age related diseases with their unbalanced immunosystem [106].

To realise the promise of novel cell-based therapies, scientists look for possibilities to manipulate stem cells easily and reproducibly to show the necessary characteristics for successful differentiation, transplantation and engraftment [107]. Gender specific aspects in the use and analysis of cells and tissues have to be taken into consideration from the beginning. At present almost no data are available concerning this aspect of stem cell biology. The study of neonatal (cord blood), juvenile and adult stem cells should also be taken into consideration because this could lead to understand age related characteristics of various stem cells.

Fusion of donor stem cells with host cells as a possible explanation of the plasticity of stem cells: clinical perspectives

Experimentally fusing adult cells with embryonic cells can produce adult cells resembling in some way embryonic cells [108, 109]. This experimental manipulation of adult cells and forcing them to take on characteristics of an embryonic cell is a powerful tool to study the factors necessary for reprogramming the cell nucleus of an adult cell to adopt a less committed state. With this approach it could well be possible to create personalized ES cells in the future by adding reprogramming factors – identified in such experiments – to adult cells. This could help circumvent therapeutic cloning, where an embryo has to be sacrificed after being created by introducing an adult nucleus into an enucleated oocyte [109].

Only recently, in April 2003, two groups have published in "Nature" [64, 65], that all this "pluripotency of adult stem cells" – this time not speaking of *in vitro* manipulated adult cells which are forced to adopt characteristics of ES cells as outlined above, but of adult pluripotent cells studied in a natural environment mainly after transplanting them back into model animals – could mostly be ex-

plained by simple “cell fusion” rather than true plasticity of adult cells (Fig. 8).

What is more, cells that fused – in these studies with liver cells – were probably not even stem cells *per se* but rather differentiated cells such as macrophages or B and T lymphocytes! Furthermore the frequency of hepatocyte replacement was not increased by liver injury, indicating that the fusion process occurs stochastically and not as a tissue repair response. In October 2003 M. Alvarez-Dolado et al. [66] provided the first *in vivo* evidence for cell fusion of bone-marrow derived cells with neurons and cardiomyocytes, raising the possibility that cell fusion may contribute to the development or maintenance of these key cell types.

The phenomenon of fusion could also be true for other cells derived from cell culture such as for MAPCs as stated by Medvinsky and Smith in the same issue of “Nature” in April 2003 [111]. One could now argue: What is so bad about cell fusion after transplanting adult stem cells back into a recipient, as long as we can have the same results as with real stem cell plasticity and cure diseases? For answering this question, Huntington Potter who works on Alzheimer in the Department of Biochemistry and Molecular Biology, Suncoast Gerontology Centre, University of South Florida stated: “I think mosaicism has been neglected as an underlying cause of disease.”

It is interesting to note that some Alzheimer’s patients have three copies of chromosome 21 in certain cells and researchers theorize that hidden patches of brain cells with an extra copy of chromosome 21 could be what predisposes some people to Alzheimer’s disease. For example in patients with Down’s syndrome, Alzheimer is an often observed disease [112, 113]. In recent years, tantalizing hints have emerged that pockets of genetically mismatched cells may contribute to conditions as common as infertility, autism and it is suspected that mosaicism could be involved in more common diseases [114, 115]. It can further be speculated that the extra copies/missing copies of genes in mosaic cells and organs such as the liver produce proteins/enzymes in such a ratio that it could harm the body or could have an impact on how patients react to e.g. medication [114, 116]. Fusion products – which are genetically mismatched cells – are nothing more or less than simple mosaicism and thus we should be aware of this situation if we want to bring stem cell treatment into the clinic. What if we treat a patient for his Parkinson disease by stem cell transplantation, but he would have an increased chance to develop Alzheimer’s disease instead?

Conclusion

Various strategies have to be applied to broaden the knowledge about stem cell biology. Many innovative technologies for the analysis and characterisation of stem cells, for their *in vitro* culture and manipulation have to be used.

The efforts have to be focussed on all essential events in stem cell differentiation and regulation. A better understanding of normal cell differentiation processes will allow to further delineate the fundamental errors which cause many deadly illnesses or cancer. Human embryonic stem cell research is controversial. Because of this and the intrinsic advantages of accessing adult tissues, intensive research is necessary to also better understand the potential ability of tissue-restricted adult stem cells to be redirected into other cell lineages.

Questions to be answered about adult stem cells

- How many kinds of adult stem cells exist, and in which tissues do they reside? To date, published papers indicate that adult stem cells have been identified in brain [117], bone marrow [63], peripheral blood [118], skeletal muscle [119], connective tissue [120], adipose tissue [121], liver [122], inner ear [88], heart [123] and pancreas [124].
- What are the sources of adult stem cells in the body? Are adult stem cells “leftover” embryonic stem cells, or do they arise in some other way?
- Why do they remain in an undifferentiated state when all the cells around them have differentiated?
- Do adult stem cells normally exhibit plasticity, or do they only trans-differentiate when scientists manipulate them experimentally?
- Do we see this trans-differentiation potential/plasticity which is mainly observed *in vitro* conditions [63] also *in vivo*, or is it all simply a fusion of host cells with donor cells [125]?
- What are the signals which regulate the proliferation and differentiation of stem cells that demonstrate potential plasticity?
- Is it possible to manipulate adult stem cells to enhance their proliferation so that sufficient tissue for transplants can be produced?
- Does a single type of stem cell exist – possibly in the bone marrow or circulating in the blood – that can generate the cells of any organ or tissue?
- What are the factors that stimulate stem cells to relocate to sites of injury or damage?

Box 1: Questions to be answered about adult stem cells

To learn more about stem cell biology and in order to bring such innovative cell-based therapies to the clinic the following questions have to be solved:

- What are the intrinsic controls that keep stem cells from differentiating? Is there a universal stem cell? That is, could a kind of stem cell exist (possibly circulating in the blood) that can generate the cells of any organ or tissue?
- Do adult stem cells exhibit plasticity as a normal event in the body or is it an artefact of culture conditions? If plasticity occurs normally, is it a characteristic of all adult stem cells?
- What are the signals that regulate the proliferation and differentiation of stem cells that demonstrate plasticity?
- What are the factors responsible for stem cells to “home” to sites of injury or damage?
- What are the intrinsic controls that direct stem cells along a particular differentiation pathway to form one specialised cell over another? How are intrinsic regulators, in turn, influenced by the environment or niche where the stem cells normally reside?
- Is it possible to manipulate stem cells to increase their ability to proliferate in a culture dish so that adult stem cells can be used as a sufficient source of tissue for transplants?
- Does the genetic programming status of stem cells play a significant role in maintaining the cells, directing their differentiation, or determining their suitability for transplant?
- What are the mechanisms that allow human embryonic stem cells to proliferate *in vitro* without differentiating?
- Are the embryonic stem cells that appear to be homogeneous and undifferentiated in culture, in fact, homogeneous and undifferentiated? Or are they heterogeneous and/ or “partially” differentiated?
- What are the cellular and molecular signals that are important in activating a human pluripotent stem cell to begin differentiating into a specialised cell type? Will analysis of genes from human pluripotent stem cells reveal a common mechanism that maintains cells in an undifferentiated state?
- Do all pluripotent stem cells pass through a progenitor/precursor cell stage while becoming specialised? If so, can a precursor or progenitor cell stage be maintained and expanded for therapeutic transplantation?
- What stage of differentiation of stem cells will be best for transplantation?
- Will the knowledge about the genetic mechanisms regulating the specialisation of embryonic cells into cells from all embryonic germ layers during development enable the scientists to engineer adult stem cells to do the same?

Box 2: Questions to be solved before bringing stem cell-based therapies into the clinic

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