Histol Histopathol (2005) 20: 977-986

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Review

Nuclear reprogramming and adult stem cell potential

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Summary. Cell-based therapy may represent a new strategy to treat a vast array of clinical disorders including neurodegenerative diseases. Recent observations indicate that adult somatic stem cells have the capacity to contribute to the regeneration of different tissues, suggesting that differentiative restrictions are not completely irreversible and can be reprogrammed.

Cell fusion might account for some changed phenotype of adult cells but it seems to be biologically irrelevant for its extreme rarity. Other experimental evidences are compatible with the hypothesis of wide multipotency of well-defined stem cell populations, but also with transdifferentiation and/or dedifferentiation. Further studies on nuclear reprogramming mechanisms are necessary to fulfil the promise for developing autologous cellular therapies.

Key words: Stem cells, Therapy, Neurodegenerative disease, Transdifferentiation

Adult stem cells

Stem cells have been defined as cells able to both self-renew through symmetric and asymmetric divisions and generate differentiated progeny. On the basis of their differentiative potential they have been classified as follows: totipotent, for their ability to generate all embryonic and extra-embryonic cell types; pluripotent, for their ability to generate all embryo cells such as Embryonic Stem Cells; and multipotent, giving rise to more than one cell lineage. This progressive restriction in stem cell commitment appears to be a general biological rule of development. Furthermore, this differentiative restriction seems to occur relatively early and with very stable characteristics. In most instances, it is not readily reversible even when cells are transplanted into a permissive environment or with nuclear transfer in egg. In fact, from a classical point of view, stem cells of adult mammals are able to give rise only to cell progeny corresponding to their tissue of origin. Since 1960, the development and regeneration of hematopoietic systems have been interpreted in the light of hierarchical models of progressive cell commitment. Afterwards, this paradigm has been extended to other organs. Cells fulfilling the "stem cell criteria" have been identified as being present virtually in all tissues and most stages of development. In recent years, the classical commitment model seems to have been amplified by the unexpected observations of unusual differentiative phenotypes that can be assumed by the adult mammalian somatic stem cells (Wagers and Weissman, 2004).

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More than one stem cell

An important emerging idea is the observation that more than one stem cell may be present in a tissue. For instance, Bone Marrow contains both hematopoietic stem cells (HSC) and mesenchymal stem cells.

In the CNS, a variety of neural stem cell populations (NSCs), has been identified at different stages of development and at different locations in the brain.

In the early stage of development, FGF-dependent neuroepithelial cells (NEP) are the main stem cell population. Later in neurogenesis, the EGF-FGF dependent stem cells - provided with the ability to generate neurospheres - appear. These two kinds of stem cells have been demonstrated to co-exist in the brain in adulthood too. Furthermore, NSCs generate more restricted precursors that differ in cytokine response, self-renewal ability, and the repertoire of differentiation from NSCs (Pevny and Rao, 2003).

From another point of view, radial glial cells seem to be the stem cells that give rise to all neurons during development (Malatesta et al., 2003) as well as to self-renewing, multipotent neurospheres in the adult lateral ventricular wall (Merkle et al., 2004). Thus, new neurons continue to be produced in adults by precursors ultimately deriving from radial glia.

An explosion of stem cell populations has been described within the skeletal muscle.

Since its discovery in 1961, the satellite cell,

previously defined on an anatomical basis as cell resident beneath the muscle basal lamina, has been the almost uncontested candidate as muscle precursor or tissue-specific stem cell. In the past six years, several investigators have demonstrated that cells derived from bone marrow (BM) possess homing properties within injured skeletal muscle and contribute to skeletal muscle regeneration (Ferrari et al., 1998; Gussoni et al., 1999 and LaBarge and Blau, 2002). Indeed, these studies evidenced how damaged skeletal muscles may be repaired either after murine systemic whole BM transplantation in injured or mdx dystrophic mouse models (Gussoni et al., 1999; Ferrari et al., 2001), or by direct injection of BM cells into a chemically damaged muscle (Ferrari et al., 1998). The proportion of detectable BM-derived muscle fibers reported varies considerably from 0.1% to 1%. In 2003 and 2004, some authors (Camargo et al., 2003; Corbel et al., 2003; Sherwood et al., 2004) demonstrated that a single hematopoietic stem cell possessing the capacity to reconstitute the entire blood system (Wagers et al., 2002), can give rise to the expression of donor markers to skeletal myofibers, particularly of injured muscle, even if this phenomenon is very rare. However, the identification of a BM multipotent stem cell and its specific markers remains controversial. For example, Gussoni et al. (1999) reported that a population of stem cells called Side Population (SP) which was isolated from mice BM by purification of Hoechst 33342-stained cells was able to rescue lethally irradiated recipients and differentiate into muscle. Other authors reported that multipotent adult progenitor cells (MAPCs) derived from human adult BM have been shown to express some myogenic markers “in vitro” after 5-azacytidine treatment (Reyes et al., 2001). Other cell populations have been isolated in different tissues for their ability to exclude Hoechst 33342 (Asakura et al., 2002), and in muscle they are called muscle-resident side population (muSP) cells. These cells display only very limited myogenic activity (Asakura et al., 2002) and only in vivo (McKinney-Freeman et al., 2002). Another method based on cell-preplating has been reported in order to isolate fractions of myogenic cells with stem cell properties (Torrente et al., 2001). Novel populations of adult stem cells with high proliferative potential and the ability to differentiate into multiple cell types (long-term proliferating cells), including muscle, neural, endothelial, and hematopoietic lineages, have been isolated from muscle (Qu-Petersen et al., 2002; Cao et al., 2003). For example, McKinney-Freeman et al. (2002) reported that the CD45-positive muscle-HSC may be distinct from the satellite cell and the main muscle progenitor cell compartment. Moreover, CD45+ stem cells resident in muscle, and not circulating stem cells, may play a major role in effecting muscle regeneration or in response to muscle injury or activation of Wnt signaling by LiCl (Polesskaya et al., 2003). It is unclear, however, whether msSP cells with hematopoietic activity are also capable of muscle differentiation or if the SP compartment contains distinct muscle and blood forming cells.

These cells may be part of a different stage of satellite cell maturation and they may be pluripotent. Other authors have questioned whether the progression from adult bone marrow to adult muscle fibers could occur via a tissue-specific stem cell intermediate, the quiescent muscle satellite cell (La Barge and Blau, 2002), and they suggested that these cells do not arise from hematopoietic or other bone marrow or circulating precursors (Sherwood et al., 2004) and that random cell fusion is the mechanism responsible for the “myogenic activity in bone marrow” (Camargo et al., 2003).

The paper by Sherwood et al. (2004) "reenthrones" the satellite cell as the main player in the mechanism of regeneration of skeletal muscle and suggests that BM and blood are not primary nor physiological sources of cell replacement in normal or regenerating muscle. This subset of cells is sorted for $CD34^{+}Sca1^{-}$, a phenotype that well corresponds to the preactivated satellite cell on isolated muscle fibers (Zammit and Beauchamp, 2001).

The study by Sherwood et al. (2004), however, failed to confirm the thesis that $CD45^{+}$ cells residing in muscle act as significant source of myogenic stem cells in mice (Polesskaya et al., 2003). On the other hand, it is necessary to keep in mind the limits present in the passage from one species to another, especially in view of the recent description of what appears to be a robust myogenic activity in vivo among a tiny minority population of circulating cells in human blood (Torrente et al., 2004).

Adult stem cell properties

Stem cells can be classified according to their characteristics, which are strictly linked to their tissue of origin and to their prominent, intrinsic differentiative fate. It is likely that cells in any organism or tissue use similar strategies, unique to stem cell populations, to maintain their stem cell state and to balance self-renewal and commitment to differentiation. It is thus probable that, although deriving from different tissues, stem cells share certain common markers and metabolic pathways.

One of the most frequently used methods of stem cell selection is represented by the identification of their surface antigen pattern, such as CD133. This antigen is a 120 kDa five transmembrane domain glycoprotein expressed on primitive cell populations. CD133 was initially demonstrated to be expressed on hematopoietic stem cells and retinoblastoma (Yin et al., 1997). No natural ligand has been detected for the CD133 molecule and its function is unknown. The expression patterns of human CD133 mimic those of the murine prominin molecule (Weigmann et al., 1997). Recently, CD133 has been shown to be expressed in other stem cell populations such as hemangioblasts (Gehling et al., 2000), neural stem cells (Uchida et al., 2000), and developing epithelium (Corbeil et al., 2000).

A second stem cell selection strategy is based on the

high-level activity of ATP binding cassette (ABC) transporter proteins which appears to be a common, though not exclusive, marker of multiple stem cell populations. This property is responsible for the enhanced efflux of the fluorescent dyes like Hoechst 33342 (the "Side Population," SP, phenotype) (Goodell et al., 1996; Zhou et al., 2001) and rhodamine.

In 1996, Goodell et al. reported on a new method for the isolation of haematopoietic stem cells based on their ability to efflux Hoechst 33342. Similar to P-glycoprotein (encoded by the mdr1 gene), the Hoechst activity is verapamil-sensitive.

Additionally, Rhodamine uptake and Hoechst labelling can be used to select stem cell populations from a diverse range of adult tissues (e.g., skeletal muscle, brain, retina) with highest capability of self-renewal and differentiation, suggesting that ABCG2 expressing cells represent a distinct class of tissue-specific stem cells, perhaps present in all tissues (Gussoni et al., 1999; Bhattacharya et al., 2003; Kim and Morshead, 2003).

Another general metabolic marker expressed at high levels in stem cells compared with more differentiated cells is aldehyde dehydrogenase (ALDH). Determination of ALDH activity using fluorescent substrate has been used to identify hematopoietic stem cells as well as other stem cell populations from several tissues (Cai et al., 2004; Hess et al., 2004).

Furthermore, the telomere function needs to be restored to allow long-term cell survival. Multiple stem cell populations produce high levels and activity of telomerase reverse transcriptase (TERT), while these functions are low or absent in many other cells (Cai et al., 2004).

Even if some markers may be shared among several stem cell populations, they are not interchangeable. For instance, the SP cell population from Bone Marrow differentiates mainly into haematopoietic progenitors (Goodell et al., 1996), SP cells form brain and muscle in neuroglial lineages (Kim and Morshead, 2003) and myoblasts (Gussoni et al., 1999), respectively, which suggests that there is a preferential predefined commitment depending on the tissue of origin.

In the past, several efforts have been made to define the intrinsic cellular and molecular properties of stem cells. On the other hand, in recent times great attention has shifted to the comprehension of the interaction between stem cells and their microenvironment, the so-called stem cell niche (Fuchs et al., 2004). The minimal niche identity is represented by the physical region in which stem cells reside, composed of neighbouring differentiated cell types and an extracellular matrix that must protect stem cell populations from any possible external harmful agents, given their life-long importance. The microenvironment is also critical to establish and maintain stem cell properties by regulating asymmetric cell division and other cell fate decisions through cell adhesion and secreted signals. For instance, muscle satellite cells, normally remain quiescent beneath the basal lamina that ensheathes muscle fibers, and only become reactivated to proliferate and fuse into differentiated myotubes in case of muscle injury (Mauro, 1961).

Furthermore, the niche seems to play an important role in stem cell recruitment known as "homing" (Whetton and Graham, 1999). An empty niche could represent an active center of attraction for stem cells; however, this microenvironment is not sufficient to reprogram somatic cells into bona fide stem cells (Kai and Spradling, 2003).

On the other hand, the niche has to preserve self-renewal properties of resident stem cells. The symmetrical division into two identical cells is a common feature of many cells including stem cells. In addition, many multipotent stem cells possess the ability to undergo asymmetric cell division, giving rise to both committed progenitors and stem cell daughters. The niches could participate in shifting the stem cell divisions in a symmetric or an asymmetric way in order to guarantee a sufficient number of intrinsic stem cells and satisfy the demand for differentiated cells within their surrounding tissues. To maintain stemness, one of the daughter cells should preserve self-renewal ability also by inhibition of differentiation factors, while the other progeny cell should receive very few pro-stemness factors and acquire a number of proliferative/differentiative signals. These features are a consequence of asymmetric cell division, which results after various possible mechanisms such as the generation of cell polarity, the orientation of the mitotic spindle and the segregation of differentiation and/or stem cell determinants.

In *Drosophila melanogaster*, the Numb protein is unequally distributed to one half of the cell membrane in dividing precursor cells, both neural and non-neural, and as a consequence is segregated to only one daughter cell. This asymmetric Numb expression is essential for the two daughter cells to adopt diverse neural cell fates after an asymmetric division (Wai et al., 1999). In murine brain the loss of Numb causes premature progenitor-cell depletion and, consequently, a highly specific malformation of the neocortex and hippocampus. Thus, the role of Numb may explain similar mechanisms of asymmetric cell division also in mammal stem cells (Petersen et al., 2004).

Nuclear reprogramming and dedifferentiation

Differentiation may not entirely be a unidirectional process. Cells may revert to an earlier, more primitive phenotype with an extended differentiation potential (dedifferentiation).

Urodele amphibians such as *Ambystoma mexicanum* (i.e., the axolotl) can functionally regenerate complex body structures throughout life such as limbs and tail, including the spinal cord, also through dedifferentiation processes. MSX1 has been hypothesized to be involved in muscle dedifferentiation based on experiments using

cultured myotubes in amphibians and human cells (Odelberg et al., 2000). It has been demonstrated that morpholinos specifically down-regulate protein expression in both cultured urodele cells and in vivo, and MSX1 and PAX7 can be efficiently reduced by morpholinos (Schnapp and Tamaka, 2005). A study by Chen et al. (2004) characterizes the so-called reversine, a substituted purine analog, as a signal molecule for the dedifferentiation of myotubes (formed from a myoblastic cell line) back into progenitor cells that can then differentiate, under appropriate conditions, into osteoblasts or adipocytes. The authors speculate that the process may involve protein kinases and that further work will identify the specific kinase or other molecules to which reversine binds.

During dedifferentiation there is a variation in the gene pattern expression, leading to the reversion towards a more immature phenotype of the cell of interest. For instance, a neuronal precursor could revert to a NSC by retracing the steps followed during normal phenotypic

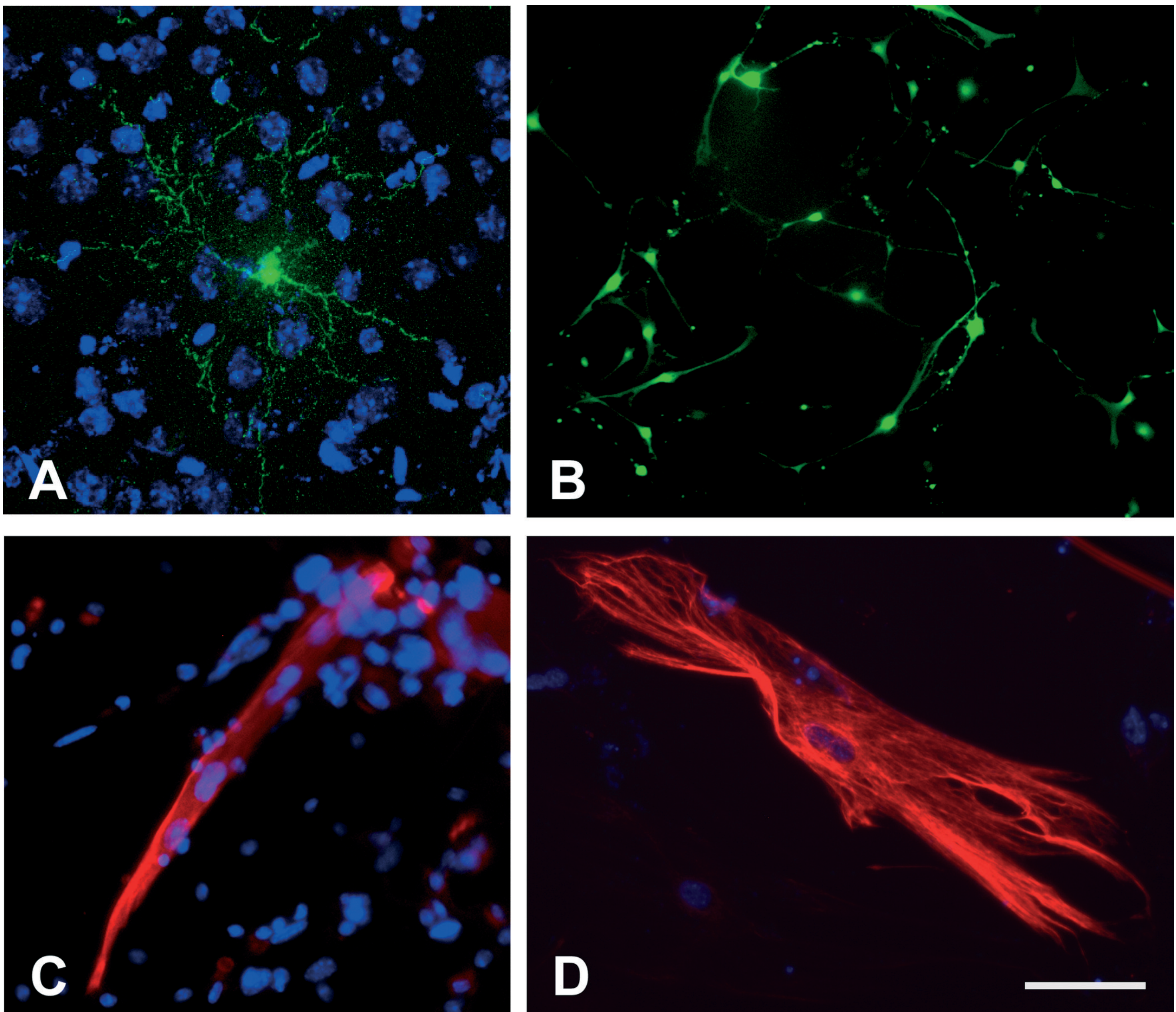

Fig. 1. Somatic stem cells plasticity. **A.** Brain section of mouse brain transplanted with bone marrow cells from transgenic mice expressing yellow fluorescent protein under Thy-1 promoter only in neurons. **B.** Neurons derived from skin of Thy-1-YFP transgenic mice in culture. **C-D.** Neural Stem Cells (NSC) can differentiate into smooth **(C)** and skeletal muscle **(D)** when exposed to appropriate signals. **C.** NSC differentiated into smooth muscle phenotype as shown by the expression of α-smooth muscle actin (SMA). **D.** NSC can fuse in desmin positive multinucleated myotubes. Nuclei are counterstained with DAPI (blue signal). Scale bar: A, 50 μm; B, 100 μm; C, 40 μm; D, 25 μm.

differentiation; and afterwards it may become capable of differentiating into glial cells. In particular, the capacity of dedifferentiation/transdifferentiation of oligodendrocyte precursors and astrocytes into mature neurons has already been demonstrated (Kondo and Raff, 2000; Laywell et al., 2000). This phenomenon also occurs among postmitotic neurons, which re-enter the cell cycle and generate dividing progenitor cells after induction (Brewer, 1999; Alexanian and Nornes, 2001). Purified rat oligodendrocyte precursor cells (OPCs) can be induced by extracellular signals to convert into multipotent neural stem-like cells (NSLCs), which can then generate both neurons and glial cells. This reversion is controlled by the reactivation of the Sox2 gene, which in turn depends on the recruitment of the tumor suppressor protein Brca1 and the chromatin-remodeling protein Brahma (Brm) to an enhancer in the Sox2 promoter. Concurrently, the conversion is associated with the modification of Lys 4 and Lys 9 of histone H3 at the same enhancer. Thus, oligodendrocyte dedifferentiation is based on progressive chromatin remodelling (Kondo and Raf, 2004).

Transdifferentiation

Alternatively, cells can differentiate into novel phenotypes without passing through an immature stage by a process called transdifferentiation rather than dedifferentiation. Cells may transdifferentiate from one cell type to another within the same tissue or into a completely different tissue without acquiring an intermediate recognizable, undifferentiated, progenitor state.

It is possible to find several convincing studies of unambiguous transdifferentiation in which the responsible molecular mechanisms are fully explained.

For instance, the identification of MyoD1 - one of the genes that master myogenic specification - was permitted by the observation of transdifferentiation of C3H10T1/2 into myoblasts after 5-azacytidine treatment, which induces a demethylation in the MyoD gene (Davis, 1987). Furthermore, forced MyoD expression in fibroblasts and in other cells may lead to the generation of a skeletal muscle phenotype (Del Bo et al., 2001).

Transdifferentiation has been achieved also in Drosophila in vivo by 'false' expression of developmental genes; for example, ectopic expression of vestigial gene can convert a Drosophila's leg to wing (Couso et al., 1995). These experiments could indicate the possibility that changes in single genes may be sufficient to alter cell phenotypes in a decisive way.

In recent years, a variety of somatic stem cells that seem to transdifferentiate have been described, thus opening up a new area to explore in order to obtain a possible cell source for therapeutic purposes. Likewise, mesenchymal stem cells (MSCs) and bone marrow cells can generate astrocytes and neurons in the brain (Fig. 1A) (Eglitis and Mezey et al., 1997; Mezey et al., 2000; Brazelton et al., 2000; Corti et al., 2002a,b, 2004), cardiac myocytes in models of infarction (Orlic et al., 2001), skeletal muscle (Ferrari et al., 1998) and hepatocytes (Theise et al., 2000).

Also, other somatic stem cells isolated from skin or muscle may be capable of differentiating into various phenotypes including neurons (Fig. 1B) (Toma et al., 2001; Alessandri et al., 2004).

While some observations can be explained by cell fusion (see below), some transdifferentiation phenomena probably result from a real broad differentiation potential of stem cells that becomes evident in the presence of inductive signals in vitro. The conversion of NSCs to endothelial cells can be accomplished without cell fusion (Wurmser et al., 2004), confirming the fact that stem-cell plasticity is a true characteristic of NSCs. Furthermore, it has been demonstrated that NSCs transplanted into irradiated mice, can generate hematopoietic derivatives (Bjornson et al., 1999) and can give rise to cells of all three germ layers when injected into blastocysts (Clarke and Frisen, 2001). However, these data are not easily reproducible (Morshead et al., 2002).

On the other hand, in some cases, the acquisition of a new phenotype is surprisingly rapid and does not follow a normal sequence of differentiation. In fact, a fast neuronal differentiation (within minutes to hours) of somatic cells (Woodbury et al., 2000) without performing any cell cycle has been reported (Munoz-Elias et al., 2003).

The differentiation of mesenchymal cells in neural cells upon exposure to chemical antigens like dimethylsulfoxide (DMSO) or Beta-Mercaptoethanol can be explained by rapid disruption of cytoskeleton with a retraction of cytoplasm resembling neurites. This change in shape was accompanied by the expression of some neuronal and glial markers. However, the absence of other essential neuronal features may indicate a low level of illegitimate transcriptions, already present before neuronal induction, or an aberrantly induced protein synthesis rather than a sequence of regulated gene expression (such as that occurring in normal neurogenesis) (Lu et al., 2004).

However, other evidence indicates the possibility of mesenchymal stem cell conversion into neurons following developmental rules. A highly efficient and specific induction of post mitotic neuronal cells was achieved in both rat and human MSCs by gene transfer of Notch intracellular domain and subsequent treatment with basic fibroblast growth factor (bFGF), forskolin and ciliary neurotrophic factor (Dezawa et al., 2004).

Fusion and nuclear reprogramming

Cell fusion studies and somatic nuclear transfer have produced new solid data on the reprogramming process which has been known to depend on developmental-stage specific trans-acting factors. This evidence suggests the possibility of modulation of the lineage restriction.

Since 1980, it has been demonstrated that cell

differentiation can be reprogrammed in heterokaryon myotubes regardless of any cell cycle phase and in the absence of detectable DNA synthesis.

When fused with differentiated mouse muscle cells, human somatic stem cells deriving from all three embryonic lineages (hepatocytes, keratinocytes, and fibroblasts) are reprogrammed and start expressing myogenic genes (Blau et al., 1983). These experiments demonstrate that genes previously silenced during development could be reactivated by cytoplasmic factors. However, nuclear transfer experiments with amphibians led to the conclusion that not all cells were equivalent in their capacity to successfully produce animals. In fact, success diminished with increasing developmental progression of the donor nuclei.

Recent somatic nuclear transfer experiments have demonstrated that a somatic nucleus from a differentiated cell could undergo reprogramming to re-establish totipotency, after transplantation into an oocyte (Wilmut et al., 1997; Wakayama and Yanagimachi, 1998). Despite the inefficiency of the phenomena, it seems obvious that epigenetic mechanisms are responsible for the maintenance of the cell differentiation state and that in certain conditions cells can be induced to change their differentiation potential. Similar cloning studies have been performed in different species such as in mice, rats, cows, pigs, and sheep, suggesting that environmental factors and appropriate circumstances influence cell fate reprogramming.

The generation of mice from post-mitotic olfactory neurons has been recently described, demonstrating that even terminally differentiated nuclei can be reprogrammed (Eggan et al., 2004).

Two recent works have elegantly verified that cell coculture may determine spontaneous fusion and the resulting cell hybrid possesses the properties of both parent populations. Ying et al. (2002) co-cultured NSCs with pluripotent ES cells and recovered a stem cell population that exhibited neural and embryonic cell markers respectively, suggesting the occurrence of cell fusion rather than NSC dedifferentiation/trans-differentiation into ES cells. Also, Terada et al. (2002) reported that cell fusion between murine bone marrow and ES led to a novel phenotype. Furthermore, cell fusion derived heterokaryons have also been obtained in vivo. In particular, donor BM cells contribute to adult mouse Purkinje neurons through cell fusion. These heterokaryons are stable and increase in a linear way over time. The dominant Purkinje background also determines the activation of a Purkinje neuron specific gene reporter from the donor nucleus (Weimann et al., 2003). In addition to the Purkinje neurons in the brain, cell fusion events occur between bone marrow derived cells and hepatocytes in liver and cardiac muscle in the heart, as demonstrated using the Cre/lox recombination system (Alvarez-Dolado et al., 2003).

Although a well defined BM cell phenotype involved in cell fusion processes has not been identified, it is likely that this role could be played by a myelo-monocytic cell, as evidenced by BM transplantation experiments, using CD45-cre (Alvarez-Dolado et al., 2003) or lysozyme M-cre (Ye et al., 2003; Camargo et al., 2003) transgenic mice.

Furthermore, macrophages are able to fuse among themselves and with other cell types (Vignery et al., 2000), suggesting that this "illegitimate" fusion may occur upon recruitment of inflammatory cells to injured tissues.

In this way, cell fusion may represent a physiological repair mechanism; however, its low frequency suggests that this phenomenon is not sufficient to support normal tissue maintenance and regeneration.

Normal differentiative pathways

Some apparent transdifferentiative events may be more correctly explained in the light of previously unrecognized normal differentiative pathways that may remain unexpressed or repressed during normal development and be induced by appropriate signals.

Indeed, neural crest cells migrate from the dorsal neural tube and generate both ectodermal and mesodermal cells. Neural crest cells do not only generate neural components of the peripheral nervous system (PNS) (Ziller et al., 1983; reviewed in Rao, 1999) but also muscle, bone, cartilage, melanocytes, fibroblasts and smooth muscle.

The ability of CNS-derived neural stem cells to differentiate in neural crest can explain some phenotypes observed in the NSC culture like smooth muscle (Fig. 1C,D) and cartilage cells (Mujtaba et al., 1998; Tsai and McKay, 2000).

The presence in the skin of neural crest derivatives may also account for the generation of neuronal cells and glia from this tissue.

Recently, the existence of a multipotent precursor cell population in the adult mammalian dermis has been described, the so-called skin-derived precursors (SKPs) (Toma et al., 2001; Fernandes et al., 2004). These cells were isolated and expanded from rodent and human skin and differentiated into both neural and mesodermal progeny. It has been proposed that SKPs represent multipotent neural-crest-like precursors that arise in embryonic mammalian tissues, and persist also in adulthood.

SKPs express the precursor cell marker Sca-1 and other neural crest markers such as the transcription factor genes slug, snail, twist, Pax3 and Sox9. SKPs differentiate into cell types that are exclusively neural-crest-derived during embryogenesis, such as peripheral catecholaminergic neurons and Schwann cells. Immunocytochemistry, RT-PCR and Western blot analysis revealed a sub-population of differentiated cells with neuronal morphology that co-express the pan-neuronal markers ß-III-tubulin and neurofilament M, and proteins typical of peripheral neurons, including p75NTR, peripherin, NCAM and the catecholaminergic

markers tyrosine hydroxylase and dopamine-ß-hydroxylase. Transplantation of SKPs into the chick neural crest migratory stream in ovo revealed that about half of the transplanted cells had migrated into peripheral neural crest targets, whereas very few had migrated into the neural tube. To test the possible neural crest origin of SKPs, a genetic method to 'tag' neural-crest-derived cells *in vivo* has been used. Specifically, mice expressing a *Wnt1–Cre* transgene were crossed to those expressing a 'floxed' RosaR26R reporter allele, thereby marking the progeny of NSCs with ß-galactosidase. In this way, it has been shown that follicle papillae contain neural-crest-derived cells, and that SKPs themselves are neural-crest-derived.

Alternatively, an additional neural derivative may be present in non-neural tissue, the ventrally emigrating neural tube (VENT) cells, so called on the basis of their site of origin and route of migration (Dickinson et al., 2004).

From a classical point of view, cells from the dorsal part of the developing neural tube emigrate and become neural crest cells, which in turn contribute to the development of the peripheral nervous system and a variety of non-neural structures. The remaining neural tube cells were thought to be restricted to forming the CNS. However, investigations of chick, quail and duck embryos utilizing a variety of different labelling techniques (DiI, LacZ, GFP and quail chimera) demonstrate the existence of a second neural tube-derived emigrating cell population. These cells originate from the ventral part of the cranial neural tube, emigrate at the exit/entry site of the cranial nerves, migrate in association with the nerves and populate their target tissues. VENT cells also differ from neural crest cells for their emigration even after the neural crest cells, and the lack of expression of the neural crest cell antigen HNK-1. VENT cells are multipotent, differentiating into cell types belonging to all four basic tissues in the body: the nerve, muscle, connective and epithelium.

Whether neural crest cells acquire a CNS neural phenotype with appropriate inductive signals remains a relevant question to be explored.

An appropriate comparison of the physiologically developmental fate of a stem cell population with the apparently unexpected phenotype and investigation of markers characteristic of specific precursor cells must be made so as not to overestimate the transdifferentiation phenomena.

Adult primitive pluripotent stem cell population

In addition to transdifferentiation and cell fusion, a third mechanism may explain the acquisition of an unexpected phenotype: the presence of a rare population of pluripotent primitive stem cells.

The existence of a population called multipotent adult progenitor cell (MAPC) has been reported, that is co-purified with BM mesenchymal stem cells but manifests a more immature feature. These cells do not only differentiate into mesoderm, but also into neuroectoderm, endothelium and endoderm (Jiang et al., 2002).

Mouse MAPCs injected into mouse blastocyst contribute to most, if not all somatic cell lineages including brain. These cells seem to express low levels of REX and Oct-3/4 which have been considered hallmarks of ES cells.

In addition to MAPC, a new intrinsically pluripotent, CD45-negative population from human cord blood has been described, so-called unrestricted somatic stem cells (USSC) (Kogler et al., 2004).

Several data indicate that lineage-specific genes are active in a totipotent stem cell prior to lineage commitment and strongly support the concept that stem cells express a multilineage transcriptosome.

Most genes (including tissue specific genes) are maintained in an open state with low but detectable levels of transcription in multipotent stem cells. Maintenance of an open transcriptosome likely requires both the presence of positive factors and the absence of negative regulators. The former include as yet unidentified factors such as demethylases, reprogramming molecules present in blastocyst cytoplasm, and regulators of heterochromatin modelling that are segregated during asymmetric cell division.

Conclusions

Transdifferentiation represents an exciting mechanism responsible for cell phenotype modification, which still needs to be proven and distinguished from other possible processes.

It is likely easier to change the fate of embryonic/fetal cells rather than adult or postmitotic cells as well as produce events within a germ layer or within the tissue rather than across germ layers.

Manipulation of cell fate regulators in a precise and controlled way at various stages of development and on specific cell types may shift cell fate. The study of various regulator factors involved in progressive cell differentiation, such as methylation/demethylation, heterochromatin remodelling, global activators, and repressors, etc, could contribute to the understanding of the transdifferentiation event.

Nuclear transfer results in the reprogramming of nuclei from a restricted somatic cell programme to the totipotent pattern by the resetting of the epigenetic status of the donor nucleus. During nuclear transfer, several components of somatic cell chromatin could affect reprogramming. Non-histone chromosomal proteins and methylation are major determinants of transcriptional activity.

The molecular events regulating nuclear reprogramming are accompanied by morphological remodelling, with breakdown of nuclear structure. Transplanted nuclei undergo a marked swelling concurrent with an influx of protein and a loss of nuclear proteins. In addition to this protein exchange, transferred

nuclei rapidly initiate DNA replication (Fujita and Wade, 2004). Further investigations of cytoplasmic factors that mediate this event are needed.

Transdifferentiation is a strictly regulated event. Thus, it is unlikely that we can manage to alterate it in a well-defined and permanent way with simple culture manipulations without generating harmful uncontrolled conditions. In addition, the novel cells could undergo various divisions; however, the new phenotype might not remain intact through subsequent generations. Another question concerns the rate of proliferative potential of transdifferentiated cells in the perspective of a significant contribution to the regeneration of degenerated tissues. Finally, the factors responsible for regulation of transdifferentiation will not necessarily share the same characteristics between different species. A large number of studies on the possible developmental fate of cells and their possibility to gain a new phenotype as well as their progressive fate restriction have been published. In basal conditions cells may be in a fluctuating or stable state, therefore transdifferentiation could represent either a spontaneous process or the result of forced molecular modifications. Stemness may be interpreted as a transient cell "state" characterized by self-renewal and the differentiation ability. The study of the level of stability and the consequent possible regime for cell fate modification could be very important in terms of future therapeutic cell replacing strategies.

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Accepted March 17, 2005