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Somatic Plasticity of Neural Stem Cells: Fact or Fancy?

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Abstract Several studies have described the potential for embryonic and adult neural stem cells to differentiate into non-neural cells such as muscle and blood, tissues that are derived from non-neuroectodermal germ layers. This raised the exciting possibility that these cells possessed a broader range of differentiation potential than originally thought and raised interesting prospects for possible transplantation utilization. However, a number of recent reports have raised questions about whether the phenomena observed actually represented true somatic plasticity. In this review, we critically analyze these studies with the aim of providing some criteria by which future studies that address this important problem may be evaluated. J. Cell. Biochem. 88: 51–56, 2003. © 2002 Wiley-Liss, Inc.

Key words: neural stem cells; somatic plasticity; transdifferentiation

A stem cell can be defined as one that has at least two important properties: [Watt and Hogan, 2000] the capacity for unlimited or prolonged self-renewal and [Gage, 2000] the ability to produce at least one type of highly differentiated descendant [Watt and Hogan, 2000]. In mammals, these cells were first characterized in epidermis and blood, tissues that underwent robust renewal during life. However, in recent years, it has become apparent that such cells are also isolable from tissues with a much more restricted regenerative potential, including brain [Bartlett et al., 1998; GarcÚa-Verdugo et al., 1998; Peretto et al., 1999; Gage, 2000]. The characterization of these neural stem cells (NSCs) that persist into adulthood offers potentially exciting applications to the problems of neural recovery and transplantation [Fisher, 1997; Pincus et al., 1998; Svendsen et al., 1999].

Although not explicitly stated, it was assumed until recently that the spectrum of a stem cell's differentiation capacity was restricted to the organ from which it was derived. Several studies from the late 1990's, however, dispelled this notion, indicating that stem cells derived from one organ could be induced to differentiate into cells of another both in vitro and more surprisingly, in vivo [Eglitis and Mezey, 1997; Ferrari et al., 1998; Jackson et al., 1999; Petersen et al., 1999; Brazelton et al., 2000; Mezey et al., 2000]. This process has been referred to by some as *transdifferentiation*, but we prefer the term *somatic plasticity* to distinguish it from the process whereby one differentiated cell (such as lens or exocrine pancreas) can be induced to assume characteristics of another (such as retinal pigment epithelium and endocrine pancreas, respectively) [Okada et al., 1979; Okada, 1980; Agata et al., 1983].

As they are easy to isolate and grow for extended periods in vitro, the EGF- and FGF-responsive NSCs that can be isolated from mammalian subventricular zone (SVZ), including human, could represent an unlimited supply of cells for transplantation. Thus, recent reports indicating that these cells had the ability to differentiate into different non-neural cell types such as muscle and blood, attracted much attention. More recently, however, several other studies suggested that the results may have represented events other than somatic plasticity or if this did occur, it happened with such low frequencies as to make it unlikely to ever have any therapeutic application.

In this review, we will critically analyze these studies in an attempt to evaluate the evidence supporting somatic plasticity of NSCs as well as

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to offer some criteria for adjudging future studies on this potentially important biologic phenomenon.

EVIDENCE FOR SOMATIC PLASTICITY OF NSCs

Three well-described studies reported results that were consistent with the phenomenon of somatic plasticity in NSCs [Clarke et al., 2000] (Table I) [Bjornson et al., 1999; Galli et al., 2000]. Bjornson et al. [1999] described a phenomenon where NSCs differentiated into hematopoietic elements after intravenous infusion into sublethally irradiated donor mice.

Using the ROSA26 transgenic mouse in which the bacterial β-galactosidase (βgal) gene is ubiquitously expressed as the donor, they transplanted 10^6 clonally derived or bulk cultured EGF- and FGF-responsive NSCs isolated from the SVZ of either embryonic or adult mice, often after extensive passaging. Five to 12 months later, bone marrow, spleen, and peripheral blood of recipient mice were screened for the expression of lacZ or various cell surface markers of donor cells (which, since the donors were derived from a different strain, were differentially expressed), using histochemistry, RT-PCR and flow cytometry.

Chimerism was detected in nearly all mice receiving either control ROSA26 bone marrow or embryonic NSCs and in over 60% of bone marrows examined after injection with adult cells when marrow cells were assessed with flow cytometry for the presence of H-$2K^{b+}$ (which are only expressed on ROSA26). Furthermore, clonogenic assays of bone marrow cells isolated from recipients revealed that over 95% of the colonies were labeled with βgal, regardless the origin of the donor cells and that cells isolated from these colonies formed different types of blood cells. The extent of engraftment and somatic plasticity was dramatic; mice transplanted with embryonic NSCs attained a level of chimerism equivalent to bone marrow derived cells, suggesting virtually complete replacement of the hematopoietic systems with donor cells. This therefore represented a striking result that suggested that a host hematopoietic system could be eventually replaced by NSCs after sublethal irradiation.

A second report indicated that NSCs were capable of assuming a myogenic phenotype [Galli et al., 2000]. Using NSCs derived from either ROSA26 mice or transgenic mice in which nuclear *lacZ* expression was under the transcriptional control of the mouse myosin promoter, Galli et al. [2000] noted conversion of either freshly isolated or cultured adult mouse NSCs into muscle cells cocultured with C2C12 myoblasts or when directly injected into muscle undergoing regeneration. This property seemed to be unique to NSCs since identical incubations carried out with dissociated striatum or cortex in coculture with C2C12 myoblasts did not undergo myogenic conversion.

This study noted that conversion was most prominent when NSCs were incubated with C2C12 myoblasts, where induction of 7–9% of total plated NSCs was observed as assessed by the number of βgal-positive nuclei. Incubation with fibroblasts or glioblastoma cells was ineffective and only a small percentage of cells converted (>1%) after incubation with normal myoblasts. Direct contact with muscle cells was also necessary; exposure through a porous membrane did not produce conversion. Moreover, mosaic fibers containing both βgal and non-βgal nuclei, as well as fibers containing only βgal nuclei expressed markers characteristic of myogenic cells, suggesting that NSCs had the capacity to fuse as well as to convert into muscle fibers in vitro.

The authors also demonstrated this effect in vivo by injecting labeled NSCs derived both from MLC3F/nLacZ transgenics or human neural stem like cells into anterior tibialis muscle damaged by cardiotoxin. Using a variety of histochemical markers, they were able to demonstrate βgal within cross striated muscles as well

TABLE I. Summary of Evidence for Neural Stem Cell Somatic Plasticity

Neural stem cells	Site of transplant	Chimeric tissues	References
Striatal E14 or adult Rosa26 Passage 12–35	10^6 cells in sublethally irradiated Balb/c	Bone marrow, spleen, peripheral blood	Bjornson et al. [1999]
Periventricular adult Rosa26 or MLC3F/nlacZ	Coculture with C2C12 myoblasts injection into damaged muscles	Myogenic conversion in vitro and in vivo	Galli et al. [2000]
Periventricular adult Rosa26	Stage 4 chick embryos mice blastocysts	Ectodermal, mesodermal, and endodermal tissues	Clarke et al. [2000]

as noting heterokaryocytic fibers containing both types of nuclei. A similar result was noted when human cells were injected. These findings therefore led the authors to conclude that NSCs could also produce skeletal muscle, suggesting their differentiation could be controlled through epigenetic signaling.

Perhaps the most extreme form of NSC plasticity was reported when NSCs were exposed to early embryonic environments, both in vitro and in vivo [Clarke et al., 2000]. When adult NSCs isolated from ROSA26 mice were cocultured with embryoid bodies that were then eliminated by exposure to G418 (since ROSA26 cells have a neomycin resistance gene), βgal containing cells could be detected that expressed desmin and myosin, suggesting myogenic differentiation. Furthermore, when undissociated neurospheres were implanted into stage 18 chick embryos, incorporation of cells was noted in over 20% of embryos; areas of incorporation involved not only in brain but also in mesodermally derived tissues as well as the epithelial cells of the liver and intestine (i.e., endoderm). Finally, after injection into murine blastocysts, incorporation of NSCs occurred in 1% and 12% of E11.5 embryos after injecting dissociated cells and small neurospheres, respectively. Based on immunocytochemical and PCR analyses, the authors also noted a high percentage of chimerism in many organs derived from all germinal layers, although interestingly, no evidence of incorporation into hematopoietic tissues was noted.

CONFOUNDING EVIDENCE ON SOMATIC CELL PLASTICITY OF NSCS

Although these studies have created much excitement among stem cell neurobiologists, a number of more recent ones raise questions not only about both the potential relevance of these findings, but also whether alternative explanations to somatic plasticity might underlie the observations.

For example, a recent report raised questions as to whether the hematopoietic competence of NSCs is a consistent property [Morshead et al., 2002]. Using a very large number of mice and techniques that could detect very small amount of engraftments and various mouse strains, a contribution to hematopoiesis was never detected despite transplantation of over 128×10^6 neurosphere cells. This led Morshead et al. [2002] to postulate that methodological issues may have been the source of the discrepancy. Indeed, X-gal histochemistry done at a lower pH than 8 (used by the Bjornson group) appears to induce some non-specific β-gal staining in colonies of control animals. An alternative explanation was that there was some type of transformation event that occurred during passaging that resulted in a clone that possessed this property. In fact, with increasing number of passages, NSCs can acquire new properties such as becoming more adherent, having an accelerated rate of proliferation, developing growth factor-independence, and changes in gene expression [Morshead et al., 2002]. Against this view, however, is that no incorporation was noted in Morshead and colleague's studies even when cells passaged many times were used.

In retrospect, the almost total replacement of host marrow after sublethal irradiation in the Bjornsen study was highly unusual in light of other work revealing that after such irradiation, some host hematopoietic cells are expected to survive [Trevisan et al., 1996]. Thus, considering that such a host population would very likely proliferate over time, it seems likely that a substantial mix of host-donor populations would be expected after a prolonged period. Thus, while it remains possible that the hematopoietic plasticity demonstrated in the Bjornsen study represented an unusual alteration that occurred in a clone of cells in vitro, the rate of chimerism that can be achieved with an i.v. infusion of NSCs must be considered exceedingly low.

The finding that NSCs can be induced to become muscle cells can also be interpreted to have occurred via mechanisms other than somatic plasticity because of the inherent properties of muscle cells to fuse. Fusion is part of the normal development of muscle fibers (and a characteristic of the NSC, see below). Moreover, the formation of heterokaryon muscle fibers has been well studied and reveals that mosaic fibers can occur without any nuclear fusion or cell division, but with free diffusion of various factors between the fused cells of different origins (reviewed in [Blau and Blakeley, 1999]). Thus it is possible that βgal nuclear expression in mosaic muscle fibers may have occurred in vitro simply as the result of diffusible factors between cells allowing its expression.

Furthermore, after injury, adult skeletal muscles have a remarkable regenerative capacity due to the presence of satellite precursor cells that migrate, proliferate, and differentiate to form new muscle fibers (reviewed in [Pastoret and Partridge, 1998]). It cannot be determined therefore whether the cells expressing human antigens and myogenic markers represented NSCs that had differentiated rather than those that had fused with surrounding muscle fibers.

The ability of NSCs to incorporate into all germ lines after blastocyst injection can also be interpreted differently based on recent results indicating that spontaneous fusions of NSC with ES cells can occur [Ying et al., 2002]. In these experiments, coculture of NSCs derived from a transgenic mouse line in which the green fluorescent protein and puromycin resistance is under control of the *Oct4* regulatory gene (which is expressed only in pluripotent and germline cells) with ES cells expressing βgal and neomycin resistance allowed selective depletion of one population. When the surviving cells were analyzed, it was found that the NSCs had developed properties of ES cells, including green fluorescent protein expression and the ability to populate organs such as the intestine, kidney, heart, and liver in mice born after blastocyst injections. However, when the surviving cells were examined after selective depletion in vitro, a very high rate of tetraploidy was noted, suggesting that the apparent assumption of ES properties by NSCs (including in vivo engraftment) represented a fusion event rather than somatic plasticity of the NSC.

Although the rate of spontaneous fusion was low (10^{-4} to 10^{-5} of overall brain cells plated), this finding does raise the issue of whether the result of Clarke et al. [2000] reflected a fusion with surrounding cells rather than somatic plasticity, especially considering that the rate of chimerism was low (<1% with dissociated neurosphere cells).

RESOLVING THE QUESTION OF NSC SOMATIC PLASTICITY

Thus, at the moment, the question of whether the NSC is capable of significant somatic plasticity remains unresolved. Because of its potential importance, not only for NSCs but for stem cells in general, further studies are obviously needed to help resolve whether such a concept has biologic relevance. While the issue remains controversial, the experience to date has provided some insight into how one might approach the issue of what constitutes successful plasticity and what obstacles will need to be surmounted before the question can be adequately addressed.

Importance of Cell Selection

NSCs can be isolated from the embryonic and adult mammalian SVZ and maintained for indefinite periods in growth supplemented media [Reynolds et al., 1992; Reynolds and Weiss, 1996]. It is important to note, however, that these isolated cells represent a heterogeneous population in which only a few cells are capable of clonal growth. The identity of these true stem cells within the SVZ population has been intensively studied and at least three candidates have emerged: the ependymal cell [Johansson et al., 1999], a subventricular astrocyte with characteristics of a radial glial cell [Doetsch et al., 1999] and a large peanut agglutinin binding negative cell [Rietze et al., 2001].

If it is these cells that are responsible for somatic plasticity, then it might explain the low rates that have been reported using unseparated cells. In support of this is data from other types of stem cells. For example, chimerism occurs only when purified stem cells derived from either epidermis, hematopoietic or bone marrow stroma are injected into blastocysts [Geiger et al., 1998; Liang and Bickenbach, 2002; Jiang et al., 2002]. In fact, in light of these other studies, it is interesting that *any* chimerism was observed with unseparated NSCs.

Further work is therefore needed in this area that assesses whether somatic plasticity (if it occurs at all) is a property of a particular cell of the NSC population. Using carefully separated freshly isolated NSC populations will help resolve this issue.

Criteria for Assessing Somatic Plasticity

It is also becoming increasingly clear that defined criteria need to be set to experimentally determine the extent of stem cell somatic plasticity, including NSCs [Anderson et al., 2001; Temple, 2001]. We suggest that a stem cell will need to progress through three successive steps before achieving somatic plasticity (Table II).

The first step is *incorporation* into the proper tissue or stem cell niche. The adoption of a new cellular identity rather than mere fusion with host cells calls for a specific mechanism to be

TABLE II. Important Steps in Successful Somatic Plasticity

- **Step 1**. Incorporation into the correct tissue or stem cell compartment
  —The importance of homing mechanisms including integrins, adhesion molecules
- **Step 2**. Differentiation into the appropriate tissue
  —Fusion events ruled out
- **Step 3**. Functionality
  —Appropriate models

in place for NSC to recognize the cues, and to induce early intracellular changes allowing engraftment in the host environment. Such "homing" mechanisms are felt to be important in effecting bone marrow transplantation and represent an important correlate of success [Hardy, 1995]. It would not be unreasonable therefore to suggest that incorporation is a crucial component of somatic plasticity. This may be an effect of expressed adhesion molecules or integrins, two areas that have not been studied in detail in this issue.

Incorporation is necessary but not sufficient, however, for successful plasticity. For this to occur, cells must *survive* and *appropriately differentiate*. This means that properties of the tissue should be demonstrated by donor cells using morphologic studies, usually with marked cells double stained with a differentiation marker. Because many plasticity experiments as well as transplant studies have used donor cell tagged with a variety of markers, it is therefore necessary to ensure that these markers do not get "turned-off," if and when the cells are undergoing major morphologic transformations. For example, some cells will no longer express βgal over time [Morshead et al., 2002] or when used in transplant studies. Perhaps more importantly based on recent studies suggesting that it may occur frequently, fusion phenomenon should be ruled out. A potentially useful way to assess this would be to use female donors into male recipients (instead of the reverse, which is the typical strategy), since new cells that express the characteristics of the tissue but not a Y chromosome would indicate that this effect was not due to fusion.

The final step in successful somatic plasticity is demonstration of physiologic function characteristic of the recipient tissue. Other studies of tissue plasticity have demonstrated that although engraftment of stem cells and partial recovery of a function may occur when donor cells are placed in an environment different from their original sites, recovery of function does not necessarily correlate with adequate morphology and instead results from the responses of neighboring cells [Zhao et al., 2002]. To date, the question of functionality has not been addressed at all with NSCs, but has been elegantly demonstrated to occur after hematopoietic transplants in the fumarylacetoacetate hydrolase (FAH) deficient mouse [Lagasse et al., 2000]. These mice develop progressive liver failure and renal tubular damage unless treated with 2-(2-nitro-4-trifluoro-methylbenzoyl)-1,3-cyclohexanedione, thus allowing mice to be treated while engraftment was taking place, after which functional engraftment could be assessed after drug withdrawal. Discovery and utilization of other such models in which a deficit can be treated while somatic plasticity develops would greatly assist workers in assessing whether this plasticity is associated with functional recovery.

SUMMARY

Until recently, it has been believed that the differentiation profile of stem cells isolated from one organ was restricted to cells of that organ. A number of studies have challenged this concept, however, and suggest a wider range of differentiation capacity, a phenomenon we term somatic plasticity. NSCs isolated from adult brain, for example, have been reported to differentiate into cells derived from germ layers other than ectoderm. In this review, we assess these reports as well as a number of more recent ones that question whether such observations actually represent somatic plasticity. Finally, we propose a sequence of steps that must be analyzed before a firm conclusion about these cells' differentiative capacity can be made.

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