REVIEWS

Neural Stem Cell: Biology and Prospects of Neurotransplantation

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Biological science enters the new millennium with achievements that completely transformed some fundamental concepts. New experimental data demonstrating high plasticity of the organism of higher animals were obtained and conceived, in particular, the existence of latent reserves of somatic cell renewal in the postnatal ontogeny due to the pool of stem cells (SC), precursors of various lines of cell differentiation.

Less than half a century ago the concept of the limited regenerative potential of adult species of higher animals was undoubted.

It was believed that adult mammals possess very limited regeneration capacities and regeneration in these animals occurs primarily at the expense of cambial cells present only in tissues requiring permanent regeneration, in particular, hemopoietic tissues, epidermis, epithelium. Regenerative potential of other organs and tissues was believed to be extremely low or absent.

An essential stage in further investigations was to establish the type of cells possessing reparative functions, their functional range, and mechanisms of realization of their potential. Multicellular organisms consisting of many types of specialized cells, tissues, and organs forms as a result of division of one cell (zygote) and subsequent proliferation and differentiation of few SC. Zygote is a totipotent SC giving rise to all cells of the organism, including extraembryonic cells (trophoblast). Embryonic SC (blastocyst core) are pluripotent and give rise to all cells of the organism.

Discovery of hemopoietic SC extended our notions about cells with a high differentiation potential. Clones of self-maintaining cells restoring hemopoiesis in lethally irradiated syngeneic recipients were for the first time discovered in the bone marrow and spleen of adult mammals [1,2,45]. Studies of hemopoietic SC helped to formulate a definition of a multipotent SC as the cell retaining its capacity to self-renewal throughout the whole life of the organism [16]. By its proliferative potential, SC is superior to other proliferating cells and ensures tissue regeneration throughout the life; it contains all necessary information for expression of adequate proliferation and differentiation programs [5] and transfers this information to SC descendants realizing these programs. Evidently, SC occupies an privileged position within tissue, and its proliferation is strictly regulated. This regulatory function is mediated by specific microenvironment containing local elements maintaining viability and proliferation of SC [43]. After discovery of hemopoietic SC, similar cell populations were found in other tissues. Particular attention is now focused on mesenchymal and epidermal SC [5,29].

Detection of neural SC in mammalian brain became a sensation. The status of mitotic silence in the CNS cells of adult mammals was never doubted before. Proliferating cells found in the "subependymal lamina" in 1941 were believed to give rise only to the glia and are responsible for its malignant transformation.

It was recently demonstrated that actively proliferating neural precursors persist in specific zones of the CNS of adult mammals [35,50]. They are located in the subependymal zone, dentate gyrus of the hippocampus, and olfactory bulb of rodent and human brain [44,48]. These cells give rise to neurons and glial cells and maintain local neurogenesis throughout the life (Fig. 1) [9,20]. Later neural precursor cells were detected outside the above-mentioned regenerative zones

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in all CNS areas [49]. The population of "neural precursors" is heterogeneous and includes multipotent SC and their oligo- and unipotent descendants. Development of methods for identification, isolation, maintenance, *in vitro* propagation, and then transplantation of neural SC opens up new prospect in studying the early ontogeny of the nervous system at a cellular molecular level and modeling regenerative and pathological processes in the CNS.

Identification of neural stem cell. Markers of neural differentiation. Due to extremely intricate structure of the CNS in mammals, studies of its development and regeneration remain a difficult problem. Reductionist approach to its solution is obviously unjustified. The only possible way is to study embryonic neurogenesis, and first of all, to identify and isolate multipotent neuroepithelial precursor cells from the CNS. Monoclonal antibodies Rat-401 were obtained, which detect more than 90% cells in the neurotubule of 11-day rat embryos. These antibodies recognize nestin, an intermediate filament protein of neuroepithelial SC, which is not expressed on differentiated descendants [17]. Detection of this cell marker helped to identify neuroepithelial precursor cells and confirmed commonality of neural precursors in various CNS regions and the concept that they are true SC of the nervous system. Neural differentiation can be traced using a panel of phenotypical cell markers. Markers of neural SC and their offspring determining the lineage of neural cell differentiation are presented in Fig. 2. Apart from neural SC, nestin is expressed in early neural precursors, postmitotic neurons [32], and early neuroblasts [46]. Another intermediate filament protein, β-III-tubulin, is a marker of neuronal lineage, while vimentin is expressed in neurons and immature astrocytes. Glial fibrillary acidic protein (GFAP) is the marker of type I astrocytes. Oligodendrocytes selectively express galactocerebroside (GalC) [48].

Epigenetic stimulation of neural SC *in vitro*. Multipotent neural SC were first isolated and transferred into *in vitro* culture from the striatum; specific growth factors stimulating rapid division of these cells were determined [34]. Proliferation of multipotent SC from various CNS areas is stimulated by fibroblast growth factor-2 (FGF-2) alone or in combination with epithelial growth factor (EGF). FGF-2 mediates its effect primarily through FGF-2 receptor 1 (FGF-2-R1). Affinity binding of this factor to the receptor and its mitogenic effect in many cells, including neuroepithelial are potentiated by heparin [8]. In rat telencephalon FGF-2-R1 is present in the early embryogenesis, while at late stages its expression is confined to the ventricular zone. FGF-2 expression peaks after termination of early neurogenesis, predominantly in postmitotic cells. Low level of The expression of EGF receptors at early stages of telencephalon development is low and occurs predominantly in ventral areas, while at later stages it increases and occurs primarily in dorsal structures. In rodent brain EGF binds preferably to transforming growth factor-β receptor. Knockout of

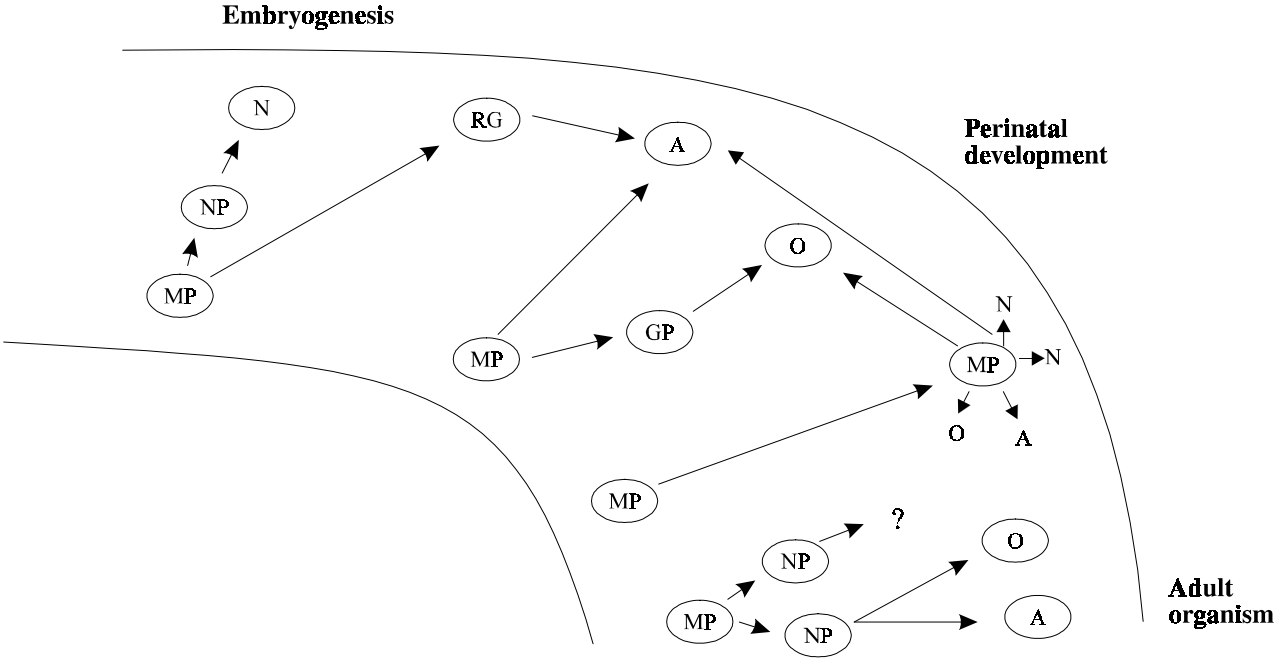

Fig. 1. Role of multipotent neural precursors in the development of mammalian cerebral cortex. MP: multipotent precursors; NP: neural precursors; GP: glial precursors; N: neurons; A: astrocytes; O: oligodendrocytes; RG: radial glia.

EGF receptor gene in mice results in cortical dysgenesis in early embryogenesis and postnatal ontogeny accompanied by impaired forebrain functions, death of cortical cell, and ectopia of the hippocampus. The data obtained on mice with null mutations in mitogenic factor receptors gene suggest that *in vivo* effects of EGF and FGF-2 on the proliferation neural SC in telencephalon are realized at various stages of embryo development. During *in vitro* culturing in a serum-free medium, neuroepithelial cells of 8.5-day mouse embryos proliferate in the presence of FGF-2, while only neural SC isolated from embryos at later stages of development respond to EGF. Neural SC proliferate in response to both mitogens, while in low-density culture the effect of EGF and FGF-2 on proliferation of neural SC is additive. The differences in the proliferative response to EGF and FGF-2 reflect heterogeneity of neural SC population. It was assumed that EGF-reactive neural SC present in 14.5-day mouse embryos are linear descendants from FGF-reactive neural SC first detected in 8.5-day embryos [47].

Proliferation of neural SC in a serum-free medium in the presence of mitogens yields agglomerates of undifferentiated neural precursors, neurospheres. Multipotent clones can symmetrically divide in culture generating new multipotent neural SC. Under natural

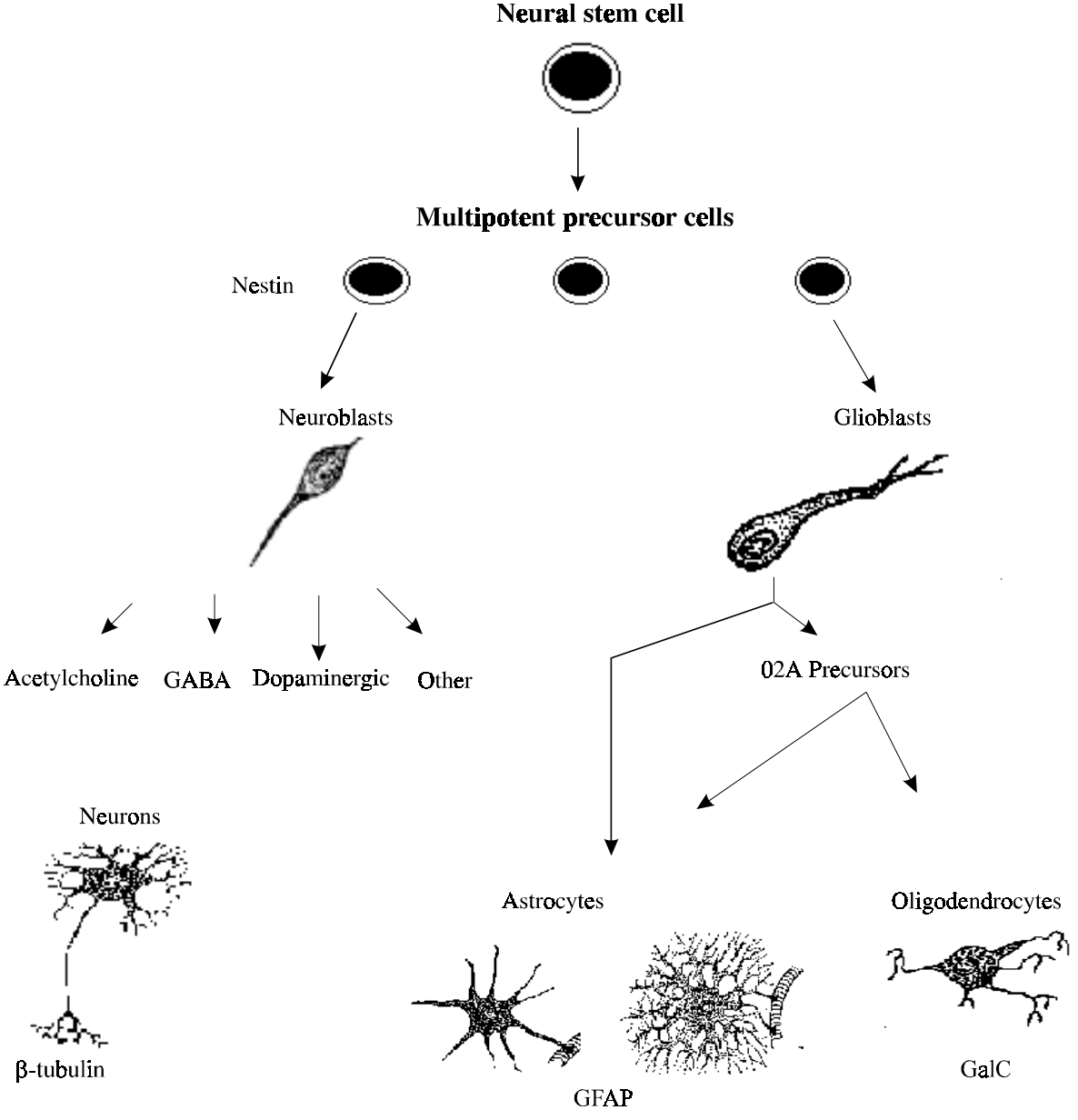

Fig. 2. Neural differentiation. GFAP: glial fibrillary acidic protein; GalC: galactocerebroside C.

conditions symmetrical and asymmetrical division can proceed in parallel yielding a heterogeneous culture containing neural SC, its descendants, and even terminally differentiated cells. The relative content of neurons, oligodendrocytes, and astrocytes in a clone greatly varies during *in vitro* culturing [14,48]. Selective cell death can lead to elimination of a specific class of cells generated from the clone, which creates an erroneous impression of unipotentiality of these cells, while actually they are multipotent. The phenotypical potential of neural SC and other precursor cells depends on complex interactions determined by their origin and environment. Immunophenotyping of neural CNS cells from human 8-12-week embryos and 17-20-week fetuses (periventricular and hippocampal zones) by flow cytofluorometry showed significant variability depending on gestation terms and individual constitution of donors. Neurospheres are forming during culturing of these neural precursors in selective serum-free medium with EGF, FGF-2, and neural cell viability factor (NSF1). The rate of neural SC proliferation varies depending on the term of gestation.

Human embryonic brain cells (5-15 weeks gestation) isolated from different cerebral zones were cultured for a short time with FGF-2 in a monolayer culture on Matrigel substrate, containing laminin and trace amounts of growth factors. Cell proliferation in these cultures was maintained for 6 weeks. Most cells expressed nestin. Cells expressing markers of all three lines of neural differentiation were also detected [14]. Cells isolated from human fetal mesencephalon (older than 13 weeks) proliferated in the presence of EGF and formed neurospheres. A combination of EGF and FGF-2 produced a synergistic effect. Intensive proliferation of neural SC accompanied by the formation of neurospheres was observed during culturing of cerebrocortical tissue of human 6-8-week embryos with EGF, insulin-like growth factor-1 (IGF-1), and 5% equine serum. FGF-2-sensitive neural SC isolated from 8-week human fetus cultured in fibronectin-coated flasks formed neurospheres. Short-term exposure with serum is essential for cell adhesion to the substrate. FGF-2 induces detachment of cells and formation of neurospheres. Removal of the growth factor provoked cell differentiation in neurospheres with the predominant development of neurons [41]. Addition of the serum stimulated neuronal and glial differentiation of neural SC. Glial differentiation with the formation of oligodendrocytes can be induced by triiodothyronine (T_3). Brain-derived neurotropic factor (BDNF) induces the appearance of neuronal processes on neurons derived from FGF-2-reactive neural SC.

It is essential for practice to know whether sufficient amounts of cells maturing into functioning neurons, astrocytes, and oligodendrocytes can be obtained. The hypothesis about an infinitely long propagation of these cells in a culture is incorrect, because nontransformed somatic cells are not immortal. After 30-50 division cycles the cells in the majority of tissues stop dividing because of gradual shortening of terminal parts of chromosomes (telomeres). In rats, cells in neurospheres undergo no more than 8 division cycles, in humans 4-6 divisions during standard passages. Addition of heparin sulfate is needed for attaining the optimal effect of FGF-2 on free floating neurospheres, but this modification does not prevent cell aging at late stages of growth. Enlargement of spheres impairs diffusion of nutrients and deceases the growth rate. At this stage dissociation of neurospheres to single cells with their transfer into new flasks are recommended. Trypsin sharply inhibited cell growth after a series of passages, probably due to the loss of vitally important receptors. Trypsin treatment induces spontaneous differentiation of many cells with subsequent deceleration of growth rate. Without trypsin new spheres form slowly and up to 50% cells die. Cells in clusters of freshly subcultured neural precursors retain their mitotic activity, while isolated cells in the same cultures only occasionally divide. This observation and the fact that membrane-associated factors stimulate division of neural SC suggest that cell-cell contacts and extracellular matrix are vitally important for effective division of neural SC *in vitro.* A new method of passages was proposed: neurospheres derived from neural SC from the archicortex of 8- or 12-week human fetuses are maintained with EGF, FGF-2, and heparin and then divided into 4 fragments. This procedure preserves cell-cell contacts each fragment rapidly acquires a spherical shape and reaches the size of parent neurosphere in 14 days. Exponential growth goes on for up to 200 days and the resultant neurospheres contained many dividing cells. The period of the population doubling was 4-5 days. All cultures stopped dividing after 250-300 days and grew old, remaining undifferentiated or spontaneously differentiating into neurons or astrocytes. At this stage, the majority of cultures underwent up to 30 population divisions. The cells are believed to reach the Highflick limit. Discontinuation of cell division, *i. e.* the absence of oncogenic transformation in the culture, is worthy of note. These neural SC differentiated into neurons, if differentiation was induced on day 50 or 150 of culturing.

Human neural SC are isolated mainly from fetal telencephalon, which develops much later than caudal structures of the brain stem [6]. Neural SC were obtained from the spinal cord of 43-137-day human fetuses. These cells form neurospheres in the presence of EGF and FGF-2; during the early passages they are multipotent and differentiate into neurons and astro-

cytes. Two cultures of neural precursors were maintained for more than 1 year, but in such a case they were unipotent (differentiated only in astrocytes) [30].

Neural SC were first isolated from human olfactory bulb. In culture they differentiate into neurons if incubated with leukemia-inhibiting factor (LIF). In patients with neurodegenerative diseases these neural SC can be obtained by partial bulbectomy and then transplanted to other CNS compartments [48].

Transplantation of neural SC. Detection of neural SC opens vistas for cell therapy of various pathological processes in the CNS, from metabolic and genetic neurodegenerative diseases to demyelinating diseases and posttraumatic disorders. Replacement cell transplantation in CNS diseases requires selection and *ex vivo* expansion of appropriate neural precursors with their subsequent implantation into damaged brain region where they replace absent cells or locally release "therapeutic" molecules. This purpose can be attained only by transplantation of sufficiently large amounts of cells with the appropriate functional characteristics. The efficiency of therapy is determined by high viability of donor cells after transplantation and by other conditions. Transplanted cells must undergo differentiation and replace defective recipient cells, or promote differentiation of endogenous cells, integrate into structural assemblies without impairing cytoarchitectonics of the recipient brain, functionally integrate in the host nervous network, stable secrete therapeutic factors under regulatory control (spontaneously or after genetic modification *ex vivo*). The migration of maturing neurons from the ventricular zone into appropriate compartments of the brain is guided by the radial glia [31], which rapidly degrades during the postnatal ontogeny. Hence, migration of donor cells from the potential inoculation zone to lesions in adult recipients should proceed in another way.

There are two migration pathways independent on the radial glia. During the formation of the cortex some neuroblasts migrate perpendicularly to the radial glial network (tangential migration) [42]. Neural precursors from the subventricular growth zone migrate to the olfactory bulb "by chain", *i.e.* as a chain of cells compactly adhering to each other and surrounded by glial cells. These cells are believed to use partner cells as the migration substrate, while polysialylated nerve cell adhesion molecule (PSA-NCAM) is the main regulator of these cell-cell interactions. Therefore, neuronal migration does not always require the participation of the radial glia or preexisting axon bonds. "Chain" migration along the rostral migration tract is maintained throughout the life. The existence of nonradial forms of migration allows to develop a strategy of target delivery of transplanted neural precursors in mature nervous system.

Various approaches were tried for solution of such wide-scale problems in recent years. Primary neural tissue, xeno- and allogenic neural cells, immortalized strains of neural precursors, and neural cells multiplied under the effect of growth factors were used in experimental models simulating pathological processes in the CNS. These studies determined future strategy for selection of adequate cell material for transplantation. Encouraging results were obtained, to this or that extent meeting the above listed requirements. It became obvious that the previous efforts were insufficient and that transplantation of neural SC and their committed descendants seem to be the most promising approach. Obtaining of undifferentiated neural precursors from fetuses seems to be logical. These cells are sufficiently heterogeneous by their strain specificity and proliferative potential, even in the same parts of the brain. One of the main difficulties at the initial stage was the absence of reliable specific strain markers needed for identification and isolation of these cells. Detection of mitogenic effect of some growth factors on neural precursors in the culture opened new vistas for *in vitro* studies of these cells. After expansion, neural precursors retained their intrinsic capacity to differentiate into neurons, astrocytes, and oligodendrocytes, *i. e.* showed the true properties of SC. Hence, neural SC completely meet the definition of SC.

Neural SC are undifferentiated, *i. e.* morphologically differ from mature cells and do not carry their markers. They proliferate and are capable of autoregeneration, multipotent, *i. e.* can give rise to functionally mature descendants of the neural line *in vitro* and *in vivo*.

Transplantation of neural SC from 9-week human embryos into the cerebral lateral ventricle of adult rats demonstrated a high plastic potential of these cells, developing morphogenetic processes in a new microenvironment, such as migration of nerve and glial cells, differentiation of the neurons and glia. Donor cells survive in a xenogenic recipient without immunosuppressive therapy for at least 20 days.

Epigenetic stimulation and subsequent immortalization helped to obtain proliferating cells committed to the neural phenotype. This limitation can be prevented by deriving neural precursors from totipotent embryonic SC. In this case proliferation to any desired number of cells occurs before neural differentiation, and multiplied cells are rapidly converted in the neural phenotype. Hence, *in vitro* differentiation of embryonic SC into neural precursors provides an unlimited source of neural SC. Embryonic SC can be isolated from blastocyst; in the presence of LIF they are capable of unlimited division, retaining their totipotency. Neural differentiation of embryonic SC *in vitro* is induced by retinoic acid. After transplantation into the

striatum damaged by quinoline and 6-hydroxydopamine these cells can differentiate into dopaminergic and serotoninergic neurons. An effective method for obtaining neural precursors from embryonic SC has been described by O. Brustle [7]. At first, embryonic SC are cultured with LIF; differentiation starts after transfer into medium without LIF. Cells are aggregated, forming embryonic corpuscles, which are then placed into medium preserving neuronal precursors, and then the proliferation of neural SC is induced in the presence of FGF-2. Removal of the mitogenic factor stimulates differentiation of neural SC into neurons and glia (Fig. 3). After intraventricular implantation into rat embryonic brain, neural precursors (descendants from embryonic SC) migrate from the ventricle into various compartments of recipient brain (cortex, striatum, septum, thalamus, hypothalamus, and cerebellum). Cells remaining in ventricular cavities form epithelial structures resembling the neurotube and individual islets of non-neural tissue. Donor cells penetrating into recipient brain parenchyma form all 3 main types of nervous system cells. Some embryonic SC, derivatives of cortical neurons, have long apical dendrites, pyramidal cell bodies, and basal axons projecting into the corpus callosum. Donor astrocytes extend their processes to the nearest capillaries, and donor oligodendrocytes closely contact with myelin muffs, being evidently involved in the formation of myelin. Hence, neural precursors obtained from embryonic SC *in vitro* can adequately react to signals from the microenvironment, directing regional migration and differentiation, and can provide the neurons and glia to many areas of developing brain.

We should like to emphasize that efficiency of obtaining neural SC and other precursors from embryonic SC depends exclusively on the possibility of obtaining highly purified neural SC. Undifferentiated embryonic SC transplanted to an adult immunocompetent recipient can form teratomas and teratocarcinomas [10]. Even a negligible admixture of poorly differentiated cells in donor cell suspension can result in the transplant tumorigenicity or induction of nonneural tissue. Therefore, homogenous populations of neural precursor cells should be obtained before using embryonic SC as an alternative source of donor tissue. This becomes possible with the use of external signals determining the specific neural phenotype in the course of normal embryogenesis. Another approach consists in elimination of undesirable cell population by strain-specific selection [18]. When neomycin resistance gene is inserted into locus Sox with subsequent induction of embryonic SC differentiation and selection in medium containing G418, Sox-negative cells are eliminated and only neural cell population is retained. It remains unclear whether the cells selected by the present

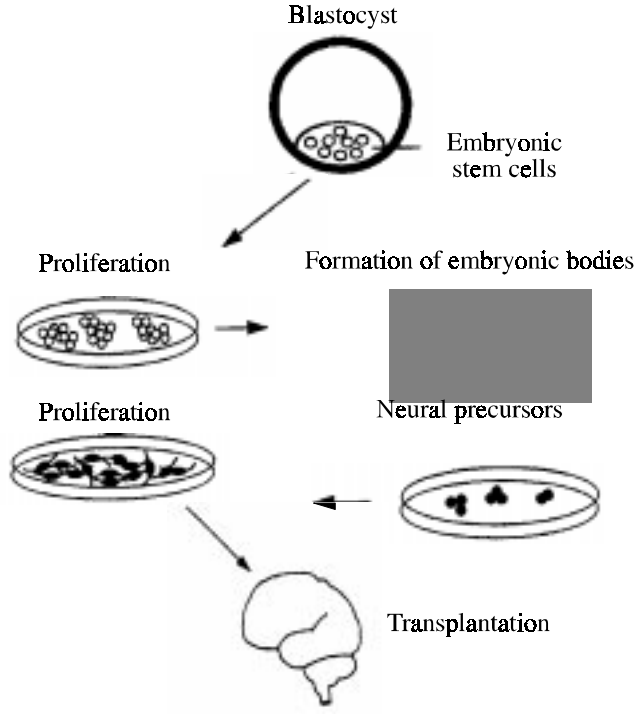

Fig. 3. Preparation of neural precursors from embryonic stem cell.

moment express genes specific for neural subpopulations and are sufficiently mature for migration and integration into recipient brain structures.

Selection of microenvironment in the recipient is another important preliminary condition of culturing precursor cells from embryonic SC to be used as donor material. Insufficient exposure with brain parenchyma can result in failure of neural differentiation program with the formation of structures characteristic of the neurotubule [7].

Another source of neural precursors was found in the bone marrow. Recently the existence of mesenchymal SC giving rise to all types of connective tissue cells was proven [29]. Mesenchymal SC present in the bone marrow stroma undergo neural differentiation under certain conditions. Human and mouse bone marrow stromal cells, cultured *in vitro* with EGF or BDNF, express nestin, marker of neural precursors. Cells with markers of the glia (GFAP) and neurons (nuclear protein NeuN) appeared under certain conditions of culturing. After transplantation into the lateral brain ventricle of syngeneic newborn mice, highly purified promarked mesenchymal bone marrow SC migrate, populating the forebrain and cerebellum without impairing the intact cytoarchitectonics of recipient brain. Some mesenchymal SC are differentiated into mature astrocytes in the corpus striatum and molecular layer of the hippocampus. Mesenchymal SC populate Culley islets, olfactory bulb, external and internal granular layers of the cerebellum, and are differentiated into

neurons in the reticular formation [13]. This gave grounds to use mesenchymal SC as cell vectors for treating CNS diseases. Mesenchymal SC from human bone marrow can differentiate into macroglia *in vitro* and after transplantation integrate in the rat brain structures. Similarly, mesenchymal SC isolated from human bone marrow and transplanted into the hippocampus of adult rats migrate into brain parenchyma and are differentiated into neurons and glia [53].

Genetic modification of neural SC. Growth stimulatory signals external for the cells (epigenetic) and various methods of modifying the genome for attaining cells expansion apparently converge on common pathways through which the expression of various regulatory components of cell cycle is modified.

Temporary reverse blocking of neural cells differentiation in culture is needed for optimizing the methods for identification, cloning, expansion, preservation, and modification of some characteristics of these cells. Epigenetic effect can be regulated by adding mitogens into culture or their removal from *in vitro* system. In addition, genetic regulatory elements can be inserted, whose products "automatically" react with cell cycle proteins in an autoregulatory mode. Transduction of such genes in precursor cells isolated from developing CNS is a reliable, effective, and convenient method for isolation and production of neural SC. Viral oncogene, adenoviral (SV40) large T antigen gene (T-ag) are widely used for producing cells from various compartments of the CNS. The product of T-ag mutant allele, tsA58, is a thermosensitive protein stable at permissive temperature (33°C) and inducing cell cycle, but degrading at 37°C in culture or after transplantation into the brain of warm-blooded animals [40].

Genetically modified cell clones behave like stable cell strains, without signs of transformation *in vivo* or *in vitro.* In the culture they are subjected to contact inhibition and cannot grow in semiliquid agar. Transplantation of these transfectants is not associated with tumor development, and donor cells gradually insert in the recipient tissue without impairing the organ cytoarchitectonics. Donor neural SC do not deform the integration zone and with equal rights compete for space with host precursor cells. Follow-up of bromdeoxyuridine-labeled donor cells showed that the wave of their mitoses is sharply arrested 48-72 h after adoptive transfer - a mirror-image of their behavior in a culture caused by contact inhibition [39]. No neurological abnormalities appeared in recipient mice; CNS areas with integrated transplant developed normally. Clones of neural CS rapidly migrated from the site of inoculation, often leaved the respective germinal zones, migrated along the rostral tract, and were adequately incorporated in cerebral tissue. Genetically modified clones and cell strains of neural SC are incorporated into multiple areas of fetal, newborn, adult, and even aging recipient CNS, showing capacity to adequate integration and differentiation. After transplantation, *e. g.* into cerebral ventricles the cells migrate without impairing the blood-brain barrier and become integral components of brain tissue. Donor neurons get respective synapses and express ionic channels. Astroglia originating from neural CS stretches its processes to cerebral vessels, with the blood-brain barrier remaining intact. Donor oligodendrocytes express the myelin major protein and myelinize neuronal processes.

Neural SC clone C17.2 modified by transfection of *c-fos* cell oncogene, implanted in the hypothalamic suprachiasmatic nucleus, cyclically positively regulates the expression of *c-fos* in circadian light exposure of animals, which indicates functional insertion of this clone into recipient nervous network [49]. Subpopulation of clone C17.2 persistently takes root in the forebrain in the middle of gestation period and can be recultured after removal from a recipient reaching adult age in repeated implantation to an absolutely different region of the CNS of other mice at different stages of ontogeny, showing wide-range differentiation potential typical of these cells [38,39].

Hence, some neural SC retain multipotency after intracerebral transplantation. They give rise to different types of neurons in the CNS areas of neurogenesis, while in the areas of completed neurogenesis they differentiate only into glial cells. Purkinje cells can originate from neural SC in the cerebellum of 12-14-day mouse embryos, *i. e.* in the same terms as analogous cells in the recipient, but at later stages of ontogeny only minor insertion neurons and the glia can originate from the same clone [49]. In adult brain neurogenesis proceeds only in the hippocampus, supraventricular zone, and olfactory bulb. Endogenous precursors migrating from the supraventricular zone to the olfactory bulb become granular neurons. After transplantation of neural SC clone into adult mouse ventricles these cells mix with recipient supraventricular zone neural SC, migrate into the olfactory bulb via the rostral migration tract, and are differentiated into granulation neurons [36,37]. If the cells of this neural SC clone penetrate from adult animal brain ventricles into the neocortex or other compartments of the brain, where gliogenesis predominates, they produce solely glial cells [37,49].

Adaptation and integration of neural SC after transplantation seem to be stable and for the life. It seems that donor cells do not induce rejection mediated by the host immune system despite the fact that they are genetically modified. Donor cells were detected in recipients for at least 2 years after transplantation without essential decrease in their amount. Undif-

ferentiated neural SC do not express the histocompatibility complex (classes I and II) molecules at a level sufficient for induction of immune rejection [36].

Neural SC and prospects of replacement cell therapy in CNS disease. Since the time when isolation and cloning of mammalian neural SC became a reality, these cells and their committed descendants were expected to be used in replacement cell therapy for various CNS diseases. The capacity of neural SC to give rise to integral components of the host brain cytoarchitectonics allows their use for therapeutic replacement of absent or defective neural cells. Neural SC can be used as cell vectors for stable *in vivo* expression of foreign genes participating in the development of the nervous system or possessing therapeutic significance. The products of these genes can compensate for various biochemical defects of the CNS, if necessary. Due to high migration capacity and insertion in various regions of the brain after implantation into germinal zones, neural SC can globally restore cell and enzyme deficit. Neural SC are apparently tropic to pathologically altered regions of the CNS and manifest a trophic function in these regions, modifying the routes of recipient cell differentiation and thus compensating for deficient specific pools of neural cells. Neurogenic signals to recapitulation of neurogenesis are realized in some neurodegenerative processes, and neural SC can react to these signals.

The therapeutic potential of neural SC is illustrated by numerous data obtained in experimental diseases of the CNS in laboratory animals [28]. In adult rats with ischemic stroke induced by occlusion of the middle cerebral artery intracysternal injection of a clone of mouse neural SC leads to a decrease of degeneration area in the brain. The infarction zone decreases still more if neural SC are combined with FGF-2. Immunocytochemical analysis showed migration of neural SC to infarction zone and integration of donor cells to recipient brain parenchyma [22]. Implantation of immortalized neuroepithelial mouse cell strain MNR36 in the rat brain parenchyma in experimental stroke led to improvement of the sensorimotor function, and intraventricular injection of donor cells to improvement of the cognitive function [25]. As was mentioned above, bone marrow can be the source of neural precursors. Human bone marrow cells transplanted to rats restore the cerebrocortical function impaired by ischemia. Donor cells implanted into the area adjacent to the focus of lesion take in and migrate to the zone of degenerative changes [53]. In traumas of the cerebral cortex in rats, intracranial injection of homologous bone marrow cells leads to partial restoration of motor function. Donor cells proliferate, undergo neural differentiation into neurons and astrocytes, and migrate towards the focus of lesion [21]. It was shown that human neural SC clones implanted into the striatum of adult rats with experimental stroke can replace lost or damaged CNS cells and partially restore the impaired brain functions [52]. Replacement cell therapy with neural SC was for the first time used clinically in the treatment of patients with stroke involving the basal ganglia of the brain. Implantation of donor cells resulted in clinical improvement in the majority of patients [12].

The possibility of using neural SC for the treatment of ataxia-telangiectasia syndrome was demonstrated in mutant nr and pcd mice. Purkinje cells disappear from the brain of these animals during the first weeks of postnatal development. After inoculation of mouse neuronal SC clones to nr and pcd mutants, donor cells are differentiated into Purkinje cells and granular neurons. Coordination abnormalities are partially corrected and tremor is decreased in pcd mutants. Similar effect was reproduced in transplantation of cloned human neural SC to primates, in which degeneration of Purkinje cells was induced by onconase. Donor neural SC were widely distributed in the cerebellar parenchyma, being detected in the granular, molecular layers, and in the Purkinje cell layer [26,33].

The same authors transplanted clones of mouse and human neural SC to mouse and primates, respectively, with degeneration of dopaminergic neurons induced in the mesostriatal system by injection of methyl-phenyl-tetra-pyridine (Parkinson's disease model) [27]. Neural SC, transplanted 8 months after destruction of dopaminergic neurons, integrated in recipient CNS. One month after implantation of human neural SC to primates, donor cells were situated bilaterally along the midbrain. Some donor neurons expressed tyrosine hydrolase. No immunosuppressive therapy was administered, and no signs of transplant rejection were seen [33]. In rats injected with 6-hydroxydopamine (model of Parkinson's disease) adaptation to the host brain microenvironment was determined by the conditions of neural SC culturing before transplantation. Neural SC proliferating *in vitro* under the effect of EGF compensated for deficit of dopaminergic neurons in the damaged striatum more effectively than cells from 28-day cultures. This is believed to be due to the loss of capacity to perceive appropriate differentiation signals in the course of *in vitro* division of neural precursors. The therapeutic effect of genetically modified neural SC is illustrated by the data obtained on an experimental model of type VII mucopolysaccharidosis in mice. These disease is caused by deletion mutation of β-glucuronidase gene and manifests by neurodegeneration in mice and progressive delay of intellectual development in humans [36]. After implantation of neural SC-transfectants secreting β-glucuronidase into brain ventricles of defective recipient

mice at birth, donor cells appeared in the germinal zone and then spread in the brain parenchyma, persistently correcting lysosome preservation in the mutant brain. Retrovirus-transduced neural SC implanted to fetuses and newborn mice effectively provided expression of β-hexosaminidase β-subunit in recipients with mutation leading to pathological accumulation of G_{M2}-ganglioside (Tay-Sachs disease model) [15]. Neural SC were used for neurotrophin-3 release in rats with spinal hemisection and brain asphyxia [11, 36] and for expression of nerve growth factors NGF and BDNF in the septum and basal ganglia [23, 24], tyrosine hydroxylase in the striatum [3], riline in the cerebellum [4], and basic myelin protein in the brain [49].

CONCLUSION

Replacement of neural cells in CNS diseases is the key problem of neurotransplantology in the new millennium. Discovery of neural SC allowed radical revision of the strategy of search for optimal ways of its solution. Obvious progress was attained in discovery of new sources of biomaterial and in development of model systems allowing evaluation of the therapeutic potential of neural precursors. The efficiency of epigenetic effect on neural SC and other neural precursors is proven, which will soon make large-scale reproduction of these cells and their differentiated descendants a routine procedure.

An unprecedented possibility of purposeful controlled modification of cells to have any cell components thus appears. Genetic modification of neural precursors can provide stable committed modification of phenotype resistant to external factors, which is essential in pathological processes associated with production (in recipient) of factors preventing survival and differentiation of donor cells. Due to capacity of neural SC to adapt in germinal zones, migrate and integrate in various compartments of the CNS, neural SC can be used in the treatment of not only local, but extensive (stroke or asphyxia), multifocal (disseminated sclerosis), and even global (majority of hereditary metabolic disorders of neurodegenerative detentias) pathological processes as well. Human neural SC can produce factors cross-correcting genetic defects of brain cells and serve as the optimal cell vectors for disseminated release of products of various genes expressed in the CNS. Due to their high plasticity, transplanted neural SC can functionally integrate into cell ensembles of host brain, and therefore their activity as transfectants (cell vectors passively releasing foreign gene products) will allow restructuring of the neuronal network elements by the feedback mechanism with its reconstruction.

Donor neural SC can provide regeneration of damaged recipient CNS sites through the production of various factors. If the deficit of these factors cannot be compensated because of their insufficient production by donor cells, neural SC can be genetically modified before transplantation, *i. e.* they can be turned into a "factory" for local production of substances mobilizing recipient's pool of silent neural precursors promoting differentiation of immature cells, induction of countercurrent growth of recipient nerve fibers, or preventing degeneration caused by inefficiency of trophic factors or enzymes in the microenvironment.

The use of clones of neural SC retaining plasticity of parental strain holds good promise. These clones are convenient for gene transfection, persistently express these genes, and after genetic modification cross-correct the genetic defects in the host CNS. High capacity to migration and replacement of host neural cells along with the above listed advantages make them the most adequate material for replacement cell transplantation.

Hence, we can expect that in the nearest future transplantation of neural SC will be widely used in the therapy of heretofore incurable diseases of the CNS.

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