Chapter 12
Transplantation of Embryonic Stem Cells in Traumatic Brain Injury

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Contents

Abstract Traumatic Brain Injury (TBI) is one of the leading causes of death and disability worldwide. Experimental research during the past decade has increased our understanding of the pathophysiology of TBI and allowed us to develop neuroprotective pharmacological therapies. However, to date, no therapeutic approach has been proven effective in reversing the pathologic cellular sequelae underlying the progression of cell loss and in improving neurobehavioral outcome. Embryonic stem cells (ESC) are pluripotent cells with the ability to differentiate into any brain-tissue-specific cell type. Therefore ESC may possess great therapeutic potential in brain injury. Therefore ESC transplantation has begun to be evaluated in several models of experimental TBI, with promising results. The following is a compendium of these new and exciting studies, including a critical discussion of the rationale and hazards associated with ESC transplantation techniques in experimental TBI research.

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Abbreviations

TBI	Traumatic Brain Injury
ESC	Embryonic stem cells
BBB	Blood-brain barrier
CCI	Controlled Cortical Impact
CNS	Central nervous system
FP	Lateral fluid percussion brain injury
IFN	Gamma interferon-gamma
TNFalpha	Tumor necrosis factor-alpha

12.1 Introduction

Traumatic Brain Injury (TBI) is one of the leading causes of death and disability worldwide (Bruns et al. 2003). Thus, it is a highly relevant medical and socio-economic problem of modern society. During the last two decades, acute pre- and in hospital care, time management, diagnostic procedures, and rehabilitation strategies have substantially improved the level of care and outcome following TBI (Maegele et al. 2007). But still, to date, no therapeutic approach has been proven effective in reversing the pathologic cellular sequelae underlying the progression of cell loss and in improving neurobehavioral outcome. As the brain has limited capacity for self-repair, restorative approaches with focus on replacement and repair of dysfunctional and dead cells by transplantation of embryonic stem cells (ESC) following TBI has been studied. The therapeutic potential of these cells has been examined in experimental brain injury using a variety of approaches. An overview of current pre-clinical knowledge with respect to ESC replacement into the experimentally injured brain is presented.

12.2 Traumatic Brain Injury—A Disease Characterized by Cell Loss

The pathologic sequelae after TBI are separated into the following: The primary injury with immediate and nonreversible mechanical damage to the central nervous system (CNS) that occurs at the moment of impact, and secondary or delayed injury: An unclarified pathologic cascade that is initiated at the moment of the traumatic insult and progresses up to months or even years. This secondary injury to the CNS is a complex network of interacting structural, functional, cellular, and molecular changes, including breakdown of the blood-brain barrier (BBB), edema formation with brain swelling, impairment of energy metabolism, changes in cerebral perfusion, ionic dyshomeostasis, activation of destructive neurochemicals and enzymes, generation of free radicals, and genomic changes. Alone or in combination these events may lead to delayed cell death (Laurer et al. 1999).

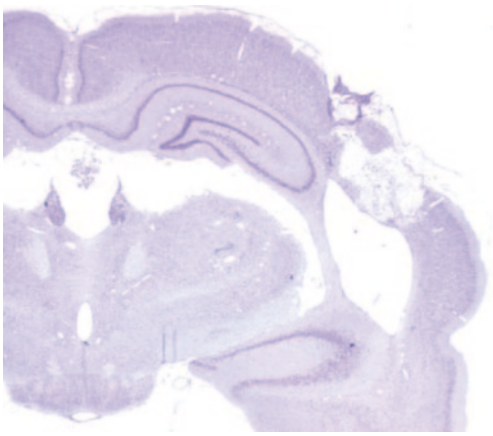

Fig. 12.1 Cresyl-Violet-Staining: Histological evaluation at 6 weeks after lateral Fluid-Percussion brain injury. Ventriculomegaly, shrinkage of the hippocampal cell layer, and necrosis is shown

Today consensus is reached that the stimulation of regenerative potentials within the injured adult central nervous system requires one or more of the following processes (Schouten et al. 2004):

- Cellular replacement,
- delivery of neurotrophic factors,
- removal of growth inhibition,
- promotion of axonal guidance,
- adequate intracellular signaling,
- bridging and artificial substrates, and
- modulation of the host immunoresponse.

The development of clinically relevant experimental models of TBI has greatly increased our understanding of the pathophysiology of TBI. Behavioral evaluation following both clinical and experimental TBI in rodent models demonstrates that long term impairments of cognitive and neuromotor function occur. It is proven that enduring cognitive, neurobehavioral, and histopathological changes persist for up to 1 year following severe experimental brain injury in rats (Pierce et al. 1998). The extent of cognitive dysfunction following experimental TBI appears to be related to both injury severity and neuronal loss in the CA3 and dentate hilar regions of the hippocampus (Hicks et al. 1993). While mild experimental TBI in rat only induces cognitive deficits associated with regional neuronal loss in the hippocampus, severe lateral fluid percussion (FP) injury in the rat (McIntosh et al. 1989) resulted in neurological motor deficits with an initial but incomplete recovery, persisting up to 1 year. Yet cognitive deficits endured up to 1 year post-injury. Histological evaluation demonstrated ongoing axonal degeneration in the striatum, corpus callosum, and injured cortex continuing up to 1 year and for up to 6 months post-injury in the thalamus (Pierce et al. 1998). In addition, ventriculomegaly, thalamic degeneration, shrinkage of the hippocampal pyramidal cell layer, progressive bilateral neuronal death in the dentate gyrus, reactive astrocytosis, and progressive atrophy of the cortex, thalamus, hippocampus, and septum have been reported up to 1 year following lateral Fluid Percussion brain injury in rats (Fig. 12.1) (Bramlett et al. 2002).

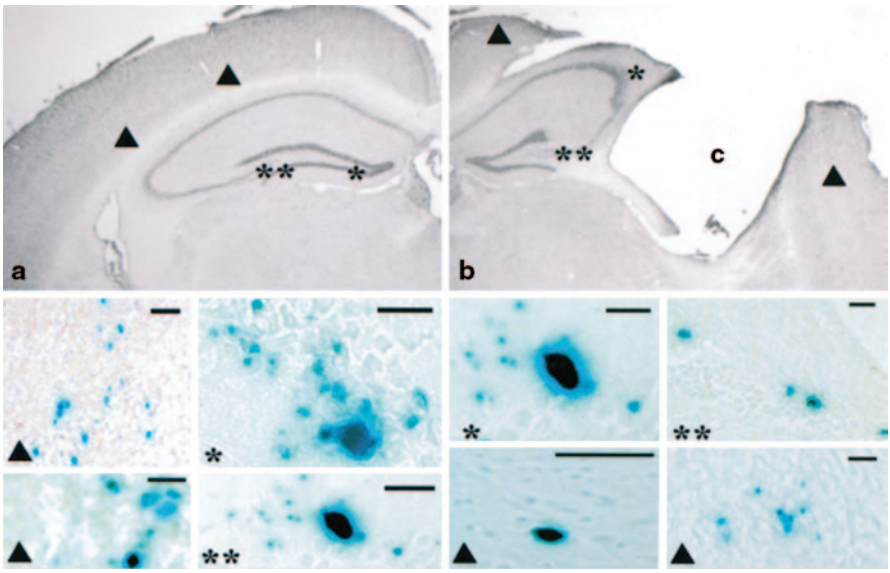

Fig. 12.2 Detection of neural stem cells (NSCs) (C17-2) applying X-gal staining after 13 weeks following transplantation. NSCs were transplanted either contralateral—into the healthy hemisphere- (**a**) or ipsilateral—into the injured hemisphere- (**b**). After transplantation into the contralateral hemisphere (**a**), NSCs were localized in the cortex (▲), dentate gyrus (*) or CA3 (**) region of the hippocampus. After ipsilateral hemispheric transplantation (**b**), X-gal- cells were located in the cortex (▲) surrounding the injury cavity (C), the granule cell layer of the dentate gyrus (**), and the CA3 region (*) of the hippocampus. (Adapted from Riess et al. 2002)

Using another TBI inducing model, the Controlled Cortical Impact (CCI) in mice and rats, histological findings could be confirmed by the detection of substantial tissue loss in the region of impact and selective hippocampal neuronal cell loss have also been observed (Fig. 12.2), and have been related to behavioral deficits seen in this model (Dixon et al. 1999; Riess et al. 2002).

As the brain has limited capacity for self-repair, mature neurons have no ability to regenerate, and endogenous progenitor cells, although present in multiple locations in the adult mammalian brain, appear to have limited ability to generate new functional neurons in response to injury. For this reason, there is great interest in the possibility of repairing the nervous system by transplanting cells that can replace those lost due to the damage (Bjorklund et al. 2002), or to manipulate endogenous progenitor cells to increase their neurogenic potential or their ability to facilitate regeneration. On the base of current neuropathological knowledge of TBI, a variety of potentially beneficial cellular replacement strategies should be considered. For further information see Schouten et al. (Schouten et al. 2004). One of these options is the use of embryonic stem cells. They show a great potential in the experimental phase on cellular levels as well as in animal trials, but ethical obstacles could be not dismissed in human.

12.3 Embryonic Stem Cells for Transplantation

Embryonic stem cells (ESCs) are nontransformed, pluripotent cells that are derived directly from the inner cell mass of the blastocyst (Evans et al. 1981). These cells are able to participate fully in embryonic development when they were reintroduced into the blastocyst. In vitro, ESCs give rise to cell types of all three primary germ layers in a way that recapitulates events of embryogenesis (Doetschman et al. 1985).

ESCs cultured under specific conditions will differentiate into neuons, astrocytes, or oligodendrocytes, depending on specific factors (Schouten et al. 2004). Due to their ability to differentiate into any tissue-specific cell type, ESCs may therefore possess great therapeutic potential in brain injury. Because TBI is associated with a massive loss of multiple cell types due to primary mechanical tissue disruption, bleeding, and secondary insults such as edema and rise of intracranial pressure leading to cell necrosis. In such case, an entire tissue segment including neurons, glia and vascular structures has to be treated by cells, able to differentiate into all lost cell types (Schouten et al 2004; Riess et al 2007; Molcanyi et al. 2013). Furthermore, ESCs provide a unique cellular system for experimental dissection of lineage specification and determination. Moreover, understanding and controlling ESC differentiation is an important step toward harnessing their potential to differentiate in any cell type of need for biomedical purposes. ESCs have been isolated from mice, monkeys, and humans (Schouten et al. 2004).

12.4 Host Environment Impairs ESC Survival But Induces ESC Differentiation and Trophic Factor Release

It has been suggested that stem cells hold great potential for the repair of the damaged nervous system. Migration and differentiation of stem cell derived precursors or progenitors seem to be accompanied by an improvement of neurological motor function as well as sensorimotor functional recovery (Riess et al. 2002, 2007). Despite these promising reports, it has to be noted that the number of surviving and differentiating cells after implantation is mostly reduced and therefore cannot completely explain the functional recovery reported. The cell survival and differentiation seems to be not just defined but also restricted by the host environment. This assumption is underlined by reports describing the post-traumatic brain as a hostile environment due to the onset of an acute inflammatory response associated with an activation of immune competent cells, the release of immune mediators, the breakdown of the blood-brain barrier as well as the infiltration of peripheral blood cells and neurotoxic components (Kelly et al. 2004; Lenzlinger et al. 2001). Furthermore, the cellular immune responses, particularly the recruitment of macrophages has been shown to be responsible for loss of cells due to extensive phagocytosis of the transplanted ESCs following TBI (Molcanyi et al. 2007). This potential detrimental effect of the post traumatic brain environment was also shown *in vitro*. Inflammatory cytokines released following brain trauma such as interferon-gamma

(IFN gamma) or tumor necrosis factor-alpha (TNFalpha) seem to inhibit the generation of neurospheres and to exhibit cytotoxic effects on neural stem cells (Wong et al. 2004).

Hence, pathophysiological changes associated with TBI may affect the survival, migration and differentiation of transplanted ESCs (Bentz et al. 2010) examined the effect of trauma associated environmental alterations on stem cell survival and differentiation. They added tissue extract after FP-brain injury (TBE) or healthy rat brains (HBE) to undifferentiated murine embryonic stem cells (CGR8) cultured in feeder-free conditions. Time-dependent survival, proliferation and differentiation of murine ESCs were examined over a period of 7 days. Hereby omission of serum from the culture medium induced neural differentiation of ESCs, as indicated by a significant time dependent down-regulation of oct −4 with a concomitant up-regulation of nestin after 7 days. Pronounced cell loss largely due to apoptotic cell death was observed additionally. In TBE treated cells, on the other hand, a significant amplification of apoptotic cell death, enhancement of nestin and MAP2 expression and marked morphological changes such as axonal-like outgrowth was observed within 3 days of conditioning. Treatment of ESCs with HBE resulted in less pronounced neuronal differentiation processes. Axonal-like outgrowth was not noticed. The pronounced expression of nestin was enhanced in the CGR8 cells following 3 days of incubation with TBE (Fig. 12.3). Furthermore TBE treated cells also expressed Map2. Cells treated with HBE or untreated cells also expressed nestin and Map2. However, analysis of fluorescence intensity revealed significant more intensive nestin and Map2 signals in TBE treated cells than in untreated cells or cells incubated with HBE (Fig. 12.3). The expression of nestin and Map2 confirmed the observed tendency of cells, in particular of TBE treated cells, towards neuronal development.

In this experiment, the authors suggest that during the early acute phase of traumatic brain injury the cerebral environment is disposed to detrimental as well as potent protective signals that seem to rapidly induce neurogenic processes.

In another experiment (Bentz et al. 2007) compared the capacity of two different ES cell lines to secrete neurotrophins in response to cerebral tissue extract derived from healthy or FP brain injury in rats. The intrinsic capacity of the embryonic cell lines BAC7 (feeder cell-dependent cultivation) to release brain-derived neurotrophic factor (BDNF) or neurotrophin-3 (NT-3) exceeded the release of these factors by CGR8 cells (feeder cell-free growth) by factors of 10 and 4, respectively. Nerve growth factor (NGF) was secreted only by BAC7 cells. Conditioning of cell lines with cerebral tissue extract derived from healthy or brain injured rat brains resulted in a significant time-dependent increase in BDNF release in both cell lines. The increase in BDNF release by BAC7 cells was more pronounced when cells were incubated with brain extract derived from injured brain. Neutrophin-3 and NGF release was inhibited when cell lines were exposed to cerebral tissue extract. The magnitude of the response to cerebral tissue extract was dependent on the intrinsic capacity of the cell lines to release neurotrophins. In this experiment the authors were able to show, that significant variations in the intrinsic capability of different stem cell lines to produce neurotrophic factors exist. In this experiment the authors

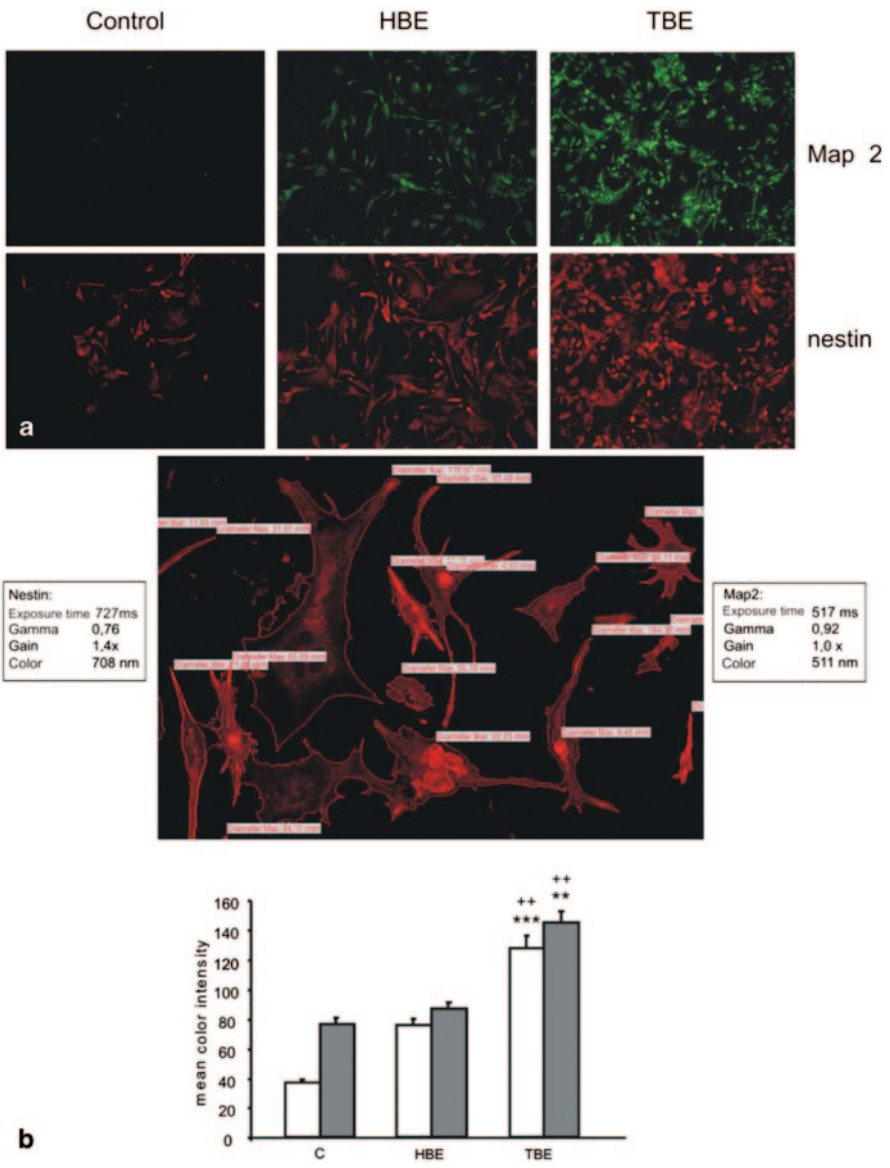

Fig. 12.3 Brain-extract modulation of stem cell differentiation at day 3 following conditioning. CGR8 stem cells were conditioned with brain extract derived from traumatized rats (TBE) or brain extract from healthy animals (HBE). Alternatively, cells were grown in serum-free medium (differentiation control) or under normal/standard conditions (+FCS, +lif). **a** Expression of *nestin* (*red*), and Map2 (*green*) was assessed by immunocytochemistry. **b** Quantification of optical density of nestin (*white bars*) or MAP2 (*grey bars*) signals were evaluated. Data are presented as mean ± s.e.m of raw data. Significant difference from control ($^{**}=p<0.01$; $^{***}=p<0.001$) or HBE conditioned cells ($++=p<0.01$). (Adapted from Bentz K et al. 2010)

were able to show significant variations in the intrinsic capability of differ-190 ent stem cell lines to produce neurotrophic factors. Furthermore, a significant modulation of neurotrophic factor release was observed following conditioning of cell lines with tissue extract derived from rat brains.

12.5 ESC Transplantation Following TBI

The cell loss due to TBI based on the primary (mechanical) direct trauma and on the developing oedema leading to apoptosis and necrosis of multiple cell types. Therefore the choice of cell population utilized in replacement therapies after TBI might be critical. Pluripotent ESCs derived from the inner mass of the blastocyst are able to differentiate into any tissue-specific cell type and may therefore possess great therapeutic potential in brain injury, since a variety of cell types are damaged or destroyed following cerebral trauma. In accordance with this assumption pluripotent murine embryonic stem cells (D3 ES cell line) have been shown to survive and differentiate following transplantation into rat brains in an experimental stroke model (Erdö et al. 2003).

Furthermore, enhanced green fluorescent protein (eGFP)-transfected D3 ESCs have been shown to migrate along the corpus callosum to the ventricular walls and to populate the border zone of the damaged brain tissue. They were also found on the hemisphere opposite to the implantation site, indicating the highly migratory behavior of implanted ESCs (Hoehn et al. 2002). In another murine model of TBI (induced by injecting phosphate-buffered saline into the left frontal and right caudal cortex) (Srivastava et al. 2006) analyzed the feasibility of ESC transplantation with the focus on the migration in response to lesions induced in brain tissues, and the mechanism of their in vivo differentiation into neighboring neural cells. They demonstrate that undifferentiated ESCs migrate within 1 week after injection to the damaged regions of brain tissue, engraft, and proliferate. Behavioral assessment was not performed. They conclude that damaged brain tissue provides a niche that attracts ESCs to migrate and proliferate. It has to be taken into consideration that the highly proliferative characteristics (self-renewal) of ESCs combined with the ability to differentiate into all embryonic germinal layers (pluripotency) present a potential threat of tumor development (teratoma, teratocarcinoma) when they are transplanted into the adult CNS. Tumorigenesis has been observed after implantation of undifferentiated human ESCs into healthy rat brains, giving rise to teratomas and malignant teratocarcinomas (Thomson et al. 1998). Accordingly, Erdö et al. (2003) compared the tumorigenic outcome after implantation of D3 ESCs in a homologous (mouse to mouse) vs. xenogeneic (mouse to rat) stroke model. In injured and healthy mouse brains, both transplanted undifferentiated and pre-differentiated murine ESCs produced highly malignant teratomas, while mouse ESCs xenotransplanted into injured rat brain migrated towards the lesion and differentiated into neurons at the border zone of the ischemic infarct. This suggests that tumorigenesis may be related to the host animal rather than to the differentiation status of the implanted cells.

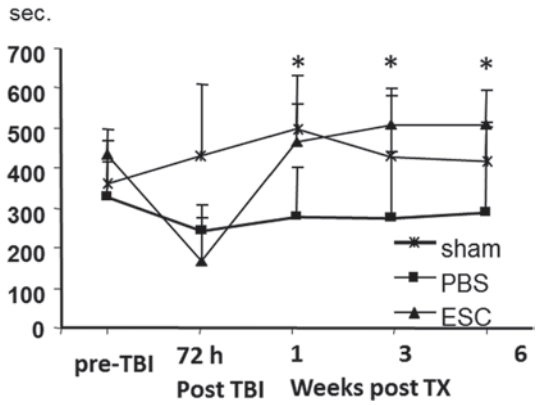

Fig. 12.4 Evaluation of time-dependent modification in locomotive motor function using the rotarod test. *Sham-operated animals. ▲ animals treated with embryonic stem (*ES*) cells following fluid percussion injury; ■ control animals treated with phosphate-buffered saline (*PBS*). Times of two performances on the Rotarod were calculated and are expressed as sum ± SE. $^{*}p \leq 0.5$ when injured ES-cell transplanted animals are compared to their respective PBS-injected animals. (Adapted from Riess et al. 2007)

Based upon these findings of Erdö undifferentiated murine ESCs of the D3 line stably transfected with the pCX -(ß-act-) were implanted into the ipsilateral cortex of rats (Fig. 12.5a) 72 h after lateral fluid-percussion brain injury of moderate severity to examine their effects on neurofunctional recovery. The chosen transplantation paradigm based upon reports demonstrating that the initial inflammatory response decreases 3 days after trauma to the brain and the development of astroglial scar begins to build thereafter (Okano 2002). The relatively early time point was chosen in order to avoid the peak of any inflammatory reaction and to allow for the migration and differentiation of stem cells that might be obstructed by the later formation of astroglial scar.

Neuromotor function was assessed at 1, 3, and 6 weeks after transplantation using a Rotatrod and a Composite Neuroscore test. During this 6 weeks of observation transplanted animals showed a significant improvement in the Rotarod Test (Fig. 12.4) and in the Composite Neuroscore Test as compared to controls.

At Five days after transplantation (TX), ESCs were detected as cell clusters in 100% of transplanted animals (Fig. 12.5b). At 7 weeks following transplantation, only a few transplanted cells were detected in one animal (Fig. 12.5c).

There was also tumor formation detected in this animal (Fig. 12.6), although a tumor-suppressive effect of xenotransplantation has been suggested (Erdö et al. 2003). Neither differentiation nor migration of cells was observed at any time point. Due to the unexpected tumor formation, Molcanyi et al. (2009) critically investigated tumorigenesis and possible mechanisms of tumor-suppressive effects following xenotransplantation of D-3 murine ESCs into uninjured adult rat brains lacking any preliminary inflammatory potential. In this experiment in 5 out of 8 healthy animals tumor formation was observed within 2 weeks of ESC implantation (Figs. 12.7

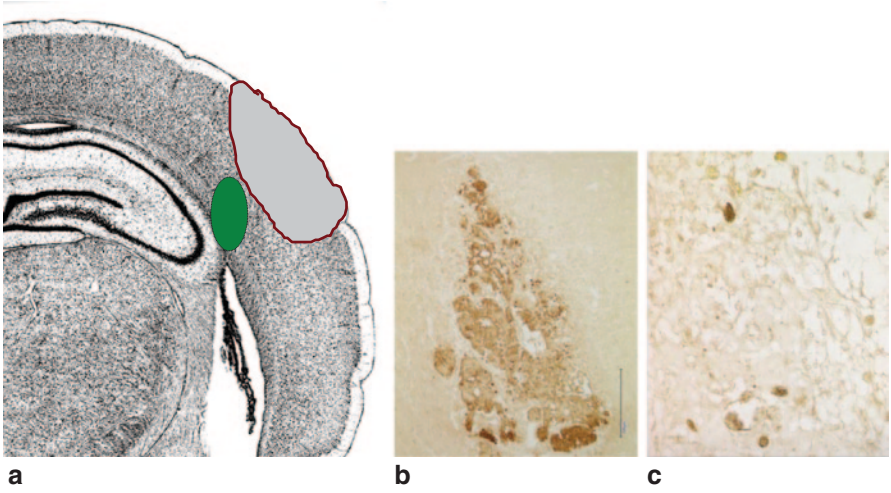

Fig. 12.5 **a**, **b**, **c** Schematic representation of injury and transplantation site. Immunohistochemical analysis of embryonic stem (*ES*) cell location at 1 week in cluster formation, and 7 weeks post-transplantation. (Adapted from Riess et al. 2007)

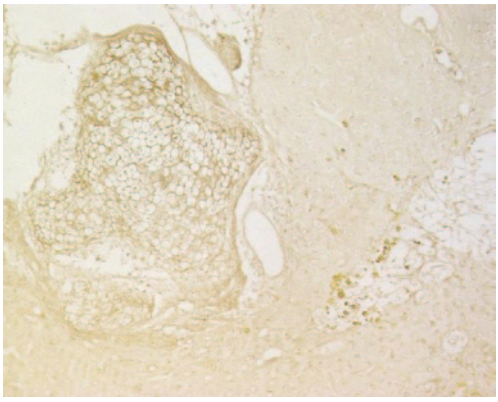

Fig. 12.6 Seven weeks after ESC-Transplantation histological evaluation reveals tumor formation (chondroma). (Adapted from Riess et al. 2007)

and 12.8), indicating the tumorsuppressive effect mainly occur after transplantation of ESC s into injured rat brains. Tumor-suppressive effects, reflected by Erdö could possibly be ascribed to immunomodulatory activity of macrophages scavenging the tumorigenic fraction of the implanted cells (Fig. 12.9) (Molcanyi et al. 2009). Macrophage activation could also be additionally triggered by potential impurity stem cell graft caused by feeder cells, the stem cells have been initially raised on (Molcanyi et al. 2014). In the same time, macrophage populations may become a major source of methodological pitfalls interfering with correct identification of implanted stem cells (Molcanyi et al. 2013).

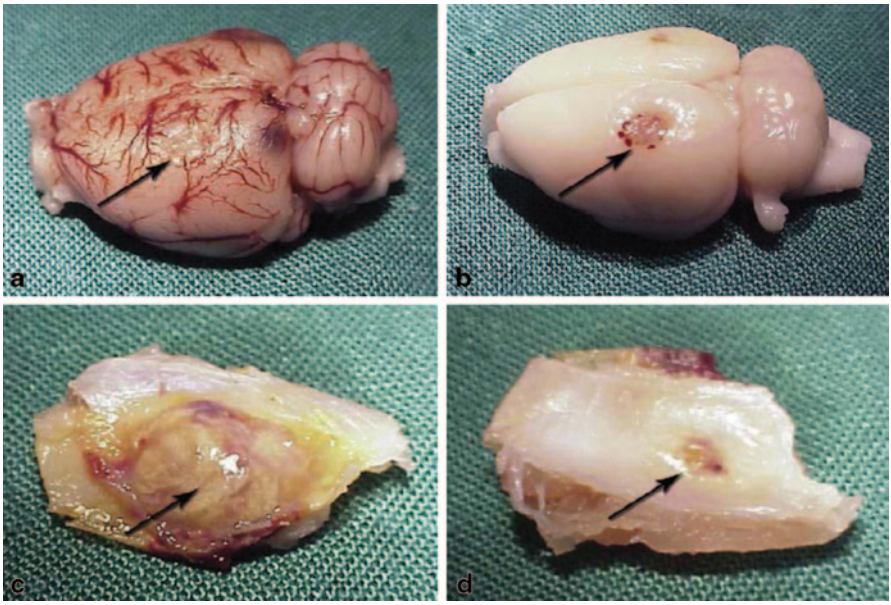

Fig. 12.7 Autopsy view—pathological changes in brains that received ESC grafts. **a** Brain without transcardial perfusion, because the animal died 8 days after ESC transplantation. Rough area with firm surface texture, lacking superficial vasculature was later histologically identified as tumor. **b** Brain perfused with PFA solution (2 weeks after ESC transplanation) shows typical pathological signs of tumor, including the vascular reorganization at the border between tumor and healthy tissue. **c** Tumor formation found on top of the skull, communicating with the brain tumor lying underneath via craniotomy opening. **d** Craniotomy opening (view from inside of scull). (Adapted from Molcanyi et al. 2009)

This is in accordance with studies suggesting that low incidence of tumorigenesis following ESC xenotransplantation may be due, in part, to the removal of implanted cells by activated inflammatory cells in the injured brain (Molcanyi et al. 2007).

Molcanyi examined the time dependent fate of the ESCs following ipsi- and contralateral transplantation into rat brains following FP brain injury. Double-staining for GFP and macrophage antigens revealed stem cell clusters embedded and phagocytosed by infiltrated and activated macrophages, indicating the loss of implanted stem cells was due to an early posttraumatic inflammatory response (Fig. 12.9). When ESCs were implanted into completely intact healthy brains macrophage infiltration was less pronounced. The authors therefore suggested that the massive macrophage infiltration at graft sites might be ascribed to the combined stimulus exhibited by the FP brain injury and the substantial conglomeration of ESCs. Macrophage activation could also be additionally triggered by potential impurity stem cell graft caused by feeder cells, the stem cells have been initially raised on (Molcanyi et al. 2014). In the same time, macrophage populations may become a major source of methodological pitfalls interfering with correct identification of implanted stem cells (Molcanyi et al. 2013).

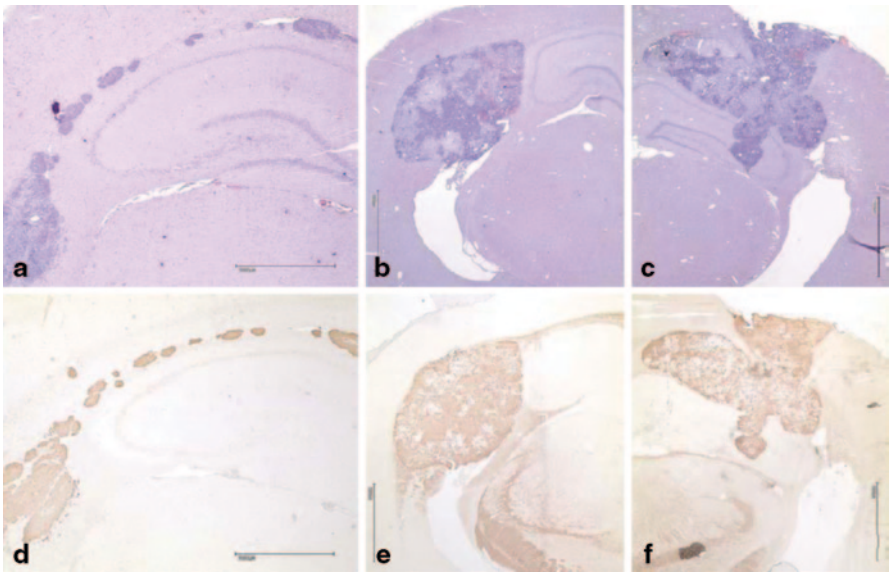

Fig. 12.8 Gross morphology of tumor formations. **a** Coronal section stained with HE reveals the main cell cluster implanted in the lateral parahippocampal cortex and several smaller clusters spreading along corpus callosum (8 day survival). **b** Tumor formation, stained by HE, exerting pressure on the hippocampus and lateral ventricle (2 week survival). **c** Tumor formation, stained by HE, showing an infiltrative growth into hippocampus and cortex (2 week survival). **d**, **e**, **f** Corresponding adjacent sections labelled with anti-GFP antibody confirmed the tumor structures to be arising from implanted GFP-transfected ESCs. (Adapted from Molcanyi et al. 2009)

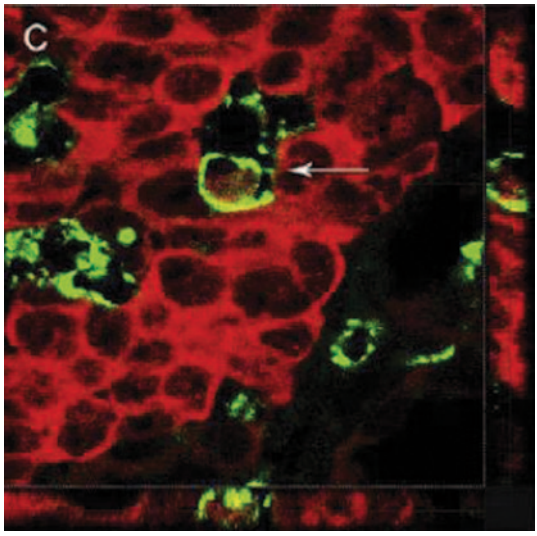

Fig. 12.9 Phagocytosis of the implanted ES cells (appear *red*—anti-GFP-Cy3) by macrophages (*green*—anti-ED -1-FITC) -confocal imaging. High magnification of the *red* stem cell localized inside of a *green* phagocyting macrophage (*thin arrow*). (Adapted from Molcanyi et al. 2007)

Previous studies have also shown that the grafting procedure itself, i.e. needle insertion followed by pressure exerted by cell graft infusion, may provoke a notable cellular response in terms of macrophage invasion and microglia activation. The extensive loss of ESCs and the apparent lack of differentiation and migration of these cells following implantation into injured rat brains indicate that the significant improvement of motor function that was observed within 5 days after implantation is not attributed to the functional integration of ESCs into the neuronal cerebral network. *In vitro* studies demonstrated that the mechanism underlying stem-cell-mediated functional improvement might be partially due to the release of trophic factors by implanted cells. Incubation of D3 ESCs with extract derived from injured rat hemispheres resulted in a rapid time-dependent and significant release of BDNF into the medium (see previous chapter: Host environment induces cell differentiation and neurotrophic factor release, but also impairs ESCs survival), indicating that similar to the observations made by Chen et al. (2002), the improvement of neuronal function following transplantation of embryonic stem cells might be triggered by the release of protective neurotrophic factors.

Conclusions

The development of therapeutic strategies for neuro-protection and regeneration following TBI has been an active field of research over the past decades. By focusing on the posttraumatic cellular pathophysiological sequelae, our understanding of mechanisms leading to brain damage after TBI has greatly increased, and more neuroprotective agents have been developed and tested in both experimental and clinical settings. The use of ESCs in cell replacement therapy has received a great deal of scientific and public interest in the recent years. This is due to the remarkable pace at which paradigm-changing discoveries have been made regarding the neurogenic potential of embryonic, fetal, and adult stem cells.

Unfortunately, in studies on day 5 after implantation, surviving undifferentiated embryonic stem cells were detected in large clusters but 7 weeks later only a few GFP-positive ESCs were detectable at the implantation site. (Molcanyi et al. 2007). This lack of survival and/or integration of ESCs may be related to the severity of injury as described before (Shindo et al. 2006).

Additionally the cell survival and differentiation seems to be not just defined but also restricted by the host environment. This assumption is underlined by reports describing that the post-traumatic brain is a hostile environment due to the onset of an acute inflammatory response associated with an activation of immunocompetent cells, the release of immune mediators, the breakdown of the blood-brain barrier as well as the infiltration of peripheral blood cells and neurotoxic components (Kelly et al. 2004; Lenzlinger et al. 2001).

Furthermore, the cellular immune responses, particularly the recruitment of macrophages has been shown to be responsible for loss of cells by extensive phagocytosis of the implanted ESCs following TBI (Molcanyi et al. 2007). The observed

functional improvements must therefore be regulated by mechanisms that are closely associated with the transplantation of ESCs but are independent of stem cell integration and differentiation. Plausible mechanisms may include the production and/or secretion of trophic factors by ESCs. Tissue culture experiments revealed, that post-traumatic host environment induces rapid ESC differentiation and neurotrophic factor release, but also impairs the survival of ESCs (Bentz et al. 2010).

Tumorigenesis is also an important issue in cell transplantation. It has to be taken into consideration that the highly proliferative characteristics of ESCs combined with the pluripotency present a potential threat of tumor development when they are transplanted into the CNS. Xenogeneic transplantation of undifferentiated murine ESCs into an intact healthy rat brain leads to the onset of tumorigenesis. The absence of tumor formation, observed in other studies, could partially be explained in a successful removal of tumorigenic cell pool by phagocytosis, the prevention of tumorigenesis via xenotransplantation as described before seems unreliable.

The repair of the traumatically injured brain is challenging due to the complex network of interacting structural, functional, cellular, and molecular changes following TBI leading to cell death. The restoration of brain function after TBI requires more than cellular replacement. An understanding of graft integration and the mechanisms underlying transplant-induced behavioral recovery is essential in the development of more effective therapeutic approaches to CNS injury. In addition, combined therapies including cellular replacement, growth factor infusion, gene therapy, environmental enrichment, and manipulation of plasticity are likely to improve the already encouraging data concerning the development of clinically effective transplantation based treatments for the traumatically injured CNS.

The effects of neurotrophic factors expressed by transplanted ESCs following transplantation to the injured brain seem to be crucial. Also important for the therapeutic effects of ESCs are the secondary effects as e.g. inflammatory responses caused by ESC transplantation. Furthermore phagocytosis of the ESCs is important to prevent the host brain of tumor formation. These findings provide evidence for the therapeutic potential of ESC transplantation after TBI in rats, but also raise serious safety concerns for human trails. However, the therapeutic use of undifferentiated ESCs is seriously limited due to the risk of tumor development, and in addition the clinical transplantation of ESC-derivatives is complicated by ethical and immunological concerns.

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