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The potential of stem cells for the treatment of brain tumors and globoid cell leukodystrophy

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Abstract

Stem cells of different origin are under careful scrutiny as potential new tools for the treatment of several neurological diseases. The major focus of these reaserches have been neurodegenerative disorders, such as Huntington Chorea or Parkinson Disease (Shihabuddin et al., 1999). More recently attention has been devoted to their use for brain repair after stroke (Savitz et al., 2002). In this review we will focus on the potential of stem cell treatments for glioblastoma multiforme (Holland, 2000), the most aggressive primary brain tumor, and globoid cell leukodystrophy (Krabbe disease), a metabolic disorder of the white matter (Berger et al., 2001). These two diseases may offer a paradigm of what the stem cell approach may offer in term of treatment, alone or in combination with other therapeutic approaches. Two kinds of stem cells will be considered here: neural stem cells and hematopoietic stem cells, both obtained after birth. The review will focus on experimental models, with an eye on clinical perspectives.

Glioblastoma multiforme

Glioblastoma multiforme (GBM, grade IV astrocytoma) can be divided into two subtypes based on clinical characteristics: primary and secondary GBM (Maher et al., 2001). Primary GBM arises as a *de novo* process, in the absence of a pre-existing low-grade lesion, whereas secondary GBM develops progressively from low-grade astrocytoma, generally over a period of 5–10 yr. Genetic studies of GBMs indicate that there are distinct genetic pathways involved in the initiation and progression of these neoplasms.

The p53 tumor suppressor is a transcription factor that regulates cell-cycle progression and apoptosis in response to many external insults, such as DNA damage and oncogenic mutations (Vogelstein et al., 2000). p53 mutations are present in both low- and high-grade astrocytomas. These findings led to the hypothesis that these common mutations are involved in early phases of tumor formation.

Genetic analysis of astrocytomas indicates that RAS-mediated signalling is also involved in the initiation of astrocytoma development. The small GTP-binding protein, RAS, is an important downstream effector of the growth-factor–RTK signalling pathway, and can activate at least three downstream cascades: RAF–MEK–MAPK (mitogen-activated protein kinase), phosphatidylinositol 3-kinase (PI3K)–AKT and CDC42–RAC–RHO. The growth-factor–RTK–RAS signalling cascade is one of the most frequently targeted genetic pathways in human cancers, possibly because activating mutations render cancer cells independent of exogenous growth factors. Elevated expression of growth factors and their cognate RTK receptors, including platelet-derived growth factor (PDGF) and platelet-derived growth-factor receptor (PDGFR), are found in every grade of astrocytoma. Furthermore, PDGF and PDGFR are often co-expressed in the same tumor cells, indicating that astrocytoma cells establish an autocrine stimulatory loop (Hermanson et al., 1992). These observations indicate that the PDGF/PDGFR-mediated signalling cascade could be involved in the initiation of astrocytoma development.

Genetic pathways that are specifically disrupted in high-grade but not low-grade astrocytoma are considered to be involved in tumor progression. To main-

tain tissue homeostasis, normal cells have several mechanisms to regulate cell-cycle progression and to prevent uncontrolled proliferation. One of these regulatory stages takes place at the G1/S-phase checkpoint. The tumor suppressor retinoblastoma (RB) is a key regulator of such checkpoint. A hallmark of high-grade astrocytomas is high mitotic activity. It is, therefore, not surprising that the RB–CDK–CKI (cyclin-dependent kinase inhibitor) regulatory circuit is frequently disrupted in these tumors (Ueki et al., 1996). In total, mutations in INK4A/CDK4/RB are detected in more than 80% of GBMs and in 50% of anaplastic astrocytomas. By contrast, such mutations are rare in low-grade astrocytomas.

Although primary and secondary GBMs have similar histopathological characteristics and clinical outcomes, the kinetics of tumor development in these two subtypes are dramatically different. Primary GBMs arise rapidly (<3 months) without clinical or histological evidence of pre-existing low-grade lesions, which makes it difficult to distinguish between genetic alterations that contribute to the initiation of primary GBMs and those that are associated with the progression of primary GBMs. Mutations in the gene that encodes INK4A – most of which are homozygous deletions – are common in primary GBMs (40%) and rare in secondary GBMs (4%). *TP53* mutations, by contrast, are frequently detected in secondary GBMs (>60%), but are less common in primary GBMs (10%). Furthermore, in these tumors, mutations in *TP53* and the gene that encodes INK4A are mutually exclusive, although INK4A is involved in the RB-mediated cell-cycle regulatory pathway (Fulci et al., 2000). This apparent contradiction is probably reconciled by the identification of a second transcript, ARF, from the *CDKN2A* locus. ARF stabilizes p53 proteins by antagonizing MDM2 (amplification and overexpression of *MDM2* are detected in primary GBMs that lack *TP53* mutations), which targets p53 for ubiquitin-mediated degradation. So, in secondary GBMs, *TP53* is directly mutated, whereas in primary GBMs, the p53 pathway is altered, resulting either from loss of ARF or from upregulation of *MDM2*. Homozygous deletion of the *CDKN2A* locus ablates both INK4A and ARF function, simultaneously dismantling both RB and p53 pathways. This might explain why primary GBMs manifest so rapidly. In mice, simultaneous disruption of both *Nf1* and *Trp53* genes results in the development of high-grade astrocytomas in certain genetic backgrounds, whereas stepwise loss of Nf1 and p53 function does not (Reilly et al., 2000). These mouse studies support the concept that simultaneous loss of two key growth-regulatory pathways in a cell might present more favourable conditions for the development of cancer.

Amplification of the gene that encodes epidermal growth-factor receptor (EGFR) is found in 40% of primary GBMs, but is rare in secondary GBMs Schlegel et al., 1994). The specific role of the EGFR signalling pathway in primary GBMs is consistent with the observation that *EGFR* amplification is associated with mutation in the gene that encodes INK4A and is mutually exclusive with the *TP53* mutation (Hayashi et al., 1997). Moreover, most tumors with *EGFR* amplifications (77%) have additional genetic alterations, most of which are intragenic rearrangements that lead to a truncated and constitutively active EGFR (Frederick et al., 2000).

Overexpression of this truncated EGFR confers a growth advantage and tumorigenic properties in glioma cell lines (Nishikawa et al., 1994). Overexpression of this mutant form of *EGFR* in the Ink4a–Arf mutant background leads to the development of gliomas, supporting a model in which the cooperation of EGFR activation and INK4A–ARF deficiency is necessary to tumor formation (Holland et al., 1998).

Loss of the long arm of chromosome 10 is the most common genetic alteration that is associated with GBMs (Albarosa et al., 1996). Several genetic loci that are associated with these tumors have been identified in this region. Among them, loss of *PTEN* – phosphatase and tensin homologue on chromosome 10, is found in more than 30% of primary GBMs (Chiariello et al., 1998), but is rare in secondary GBMs (4%) (Tohma et al., 1998). The PTEN protein can function as both a protein and lipid phosphatase, and its activity seems to be essential for tumor suppression as many mutations are found in its phosphatase domain. Some mutant forms of the PTEN protein still retain protein phosphatase activity, suggesting that the ability to dephosphorylate lipid might be more important for tumor suppression (Myers et al., 1998).

The identification of phosphatidylinositol (3,4,5)-triphosphate (PIP3, a PI3K product) as a PTEN substrate indicates that PTEN could function as a negative regulator of a well-known growth-control signalling pathway – the PI3K–AKT pathway. This is confirmed by the observation that enhanced AKT activity has been detected in PTEN-deficient tumors and cell lines from both humans and mice (Wu et al., 1998). Furthermore, overexpression of constitutive *Akt*, as well as oncogenic *Ras*, in mouse neural stem cells leads to

the development of GBMs, supporting the idea that the PI3K–AKT pathway is pivotal in the aetiology of GBMs (Holland et al., 2000; Uhrbom et al., 2002).

Globoid cell leukodystrophy (GLD or Krabbe disease)

Globoid cell leukodystrophy (GLD or Krabbe disease) is an autosomal recessive disease involving the white matter of the central and peripheral nervous systems that usually starts during the infancy (Online Mendelian Inheritance in Man, #245200; http://www.ncbi.nlm.nih.gov/omim). GLD is characterized by generalized rigidity and, subsequently, optic atrophy, deafness and cachexia. In a fraction of cases symptoms may start during childhood or even adulthood and be more benign. GLD is caused by mutations of the lysosomal enzyme galactocerebrosidase (GALC) encoded by a gene of 17 exons with an open reading frame of 669 codons (Suzuki and Suzuki, 1970, 1971; Young et al., 1972).

More than 40 mutations had been identified in patients with diverse clinical types of globoid cell leukodystyrophy (Wenger et al., 2000; Selleri et al., 2000). Relationships between the phenotype and the genotype are not direct. Particularly difficult to explain is the phenotype of late-onset patients who carried on both alleles mutations that completely abolish enzyme activity. These observations point to the possibility that other genetic factors besides mutations in the galactocerebrosidase gene may contribute to the phenotype in late-onset GLD.

An autosomal recessive leukodystrophy of the mouse, 'twitcher', is very similar histopathologically to Krabbe disease (Duchen et al., 1980). The 'twitcher' mouse is an enzymatically authentic model of human GLD (Kobayashi et al., 1980), as are disorders in sheep and dog. Progressive accumulation of a cytotoxic metabolite, galactosylsphingosine (psychosine), was found in the brain of the twitcher. Similar abnormal accumulation was also found in the brain of the genetic galactosylceramidase deficiency disease in the dog and in human patients. Galactosylsphingosine was absent in the brains of normal and heterozygous mice.

Transplantation of enzymatically normal congenic bone marrow in the twitcher mouse increased galactosylceramidase levels in the CNS. There was a gradual disappearance of globoid cells, the histologic hallmark of Krabbe disease, and the appearance of foamy macrophages capable of metabolizing the storage product. By immunohistochemical labeling, it was shown that these macrophages in the CNS were of donor origin. Extensive remyelination was observed in the CNS (Ichioka et al., 1987). Further studies found that bone marrow transplantation in the twitcher mouse resulted in an increase in the galactosylceramidase activity in the CNS to 15% of normal donor levels with a prevention of paralysis of the hind legs and a prolonged survival from 30–40 days to more than 100 days in some instances (Hoogerbrugge et al., 1988).

Five children, 1 with the infantile type and 4 with late-onset disease were treated by allogeneic hematopoietic stem cell transplantation (Krivit et al., 1998). Four of the patients had clinical CNS abnormalities before transplantation. In all 4 cases, CNS deterioration was reversed. In the patient with the infantile form of the disease, the expected decline in CNS function had not occurred by the age of 16 months, 14 months posttransplantation. The authors concluded that CNS manifestations of Krabbe disease can be reversed or prevented by allogeneic hematopoietic stem cell transplantation.

Neural stem cells (NSC) for the treatment of gliomas and GLD

A stem cell is an unspecialised cell which has the ability to renew itself indefinitely, and, under appropriate conditions, give rise to a wide range of mature cell types in the human body. As any disorder involving loss of, or injury to, normal cells could be a candidate for stem cell replacement therapy, the potential of stem cells is wide. The issue of stem cell research is politically and ethically debated, as so much emphasis has been placed on the use of stem cells derived from early human embryos. As a result, stem cell technology is in between destructive human embryo research on the one hand, and the magnitude of the potential benefits to patients, on the other. However, stem cells may be derived from a variety of sources, including not only early embryos, but also fetal tissue and some adult tissues (eg, bone marrow and blood). Recently, a renewable resource of neural stem cells was discovered in the adult human brain (Eriksson et al., 1998). These cells may be a candidate for cell-replacement therapy for nervous system disorders. The ability to isolate these cells from the adult human brain raises the possibility of autologous (self-to-self) transplantation, circumventing the issues

surrounding transplantation of embryonic stem cells into the human central nervous system (CNS).

NSCs have been isolated from various regions of the embryonic, fetal and adult human brain, including the hippocampus, the ventricular/ependymal zone, and, more recently, the cortex and the amygdala (Arsenijevic et al., 2001; Carpenter et al., 1999; Fricker et al., 1999; Svendsen et al., 1999; Uchida et al., 2000).

NSCs have been isolated from brain tissue obtained from patients undergoing surgical procedures involving removal of brain tissue for the treatment of epilepsy, tumors, or trauma. These studies demonstrate that the adult human brain contains a renewable source of neural stem cells, which can be successfully isolated through various surgical techniques.

A key step in the perspective of stem cell therapy is to show that the harvested cells are sustainable and expandable in long-term culture systems, and that they can be instructed to form specific neural cell types. Embryonic and fetal neural stem cells have shown remarkable functional stability and renewal capacity for extended culture periods of up to two years (Vescovi et al., 1999; Villa et al., 2000). They spontaneously differentiated into the three fundamental neuronal lineages (neurones, astrocytes and oligodendrocytes) and were able to achieve full neuronal maturation. It has been reported that cultures of neural progenitor cells obtained from the embryonic human forebrain can be expanded up to 10^7-fold in culture in the presence of epidermal growth factor, basic fibroblast growth factor, and leukemia inhibitory growth factor (Fricker et al., 1999). Such culture systems could provide an almost unlimited source of neural stem cells for cell-replacement strategies.

If adult neural stem cells are to be used in clinical trials they must also be amenable to expansion into clinically significant quantities. These cells, however, seem to have a limited life-span in the culture dish and it remains to be determined whether they are stable at later passages (Kukekov et al., 1999; Roy et al., 2000). Adult human neural cells do, however, exhibit many of the promising characteristics of embryonic and fetal stem cells.

NSCs could be transplanted as undifferentiated cells whose subsequent differentiation would be controlled by cues derived from the patient's brain (Uchida et al., 2000). Alternatively, they could be pre-differentiated in the culture dish into a desired neuronal type, which could then be transplanted back into the host brain.

It is of major interest to investigate the capacity of NSCs to engraft into the brain in a functionally meaningful manner in well-characterised animal models of CNS dysfunction.

Studies have shown that stem cells derived from the embryonic or fetal human brain can successfully graft into the developing rodent CNS. Once transplanted, these cells survived, migrated and integrated seamlessly into the host tissue, giving rise to cells from all three fundamental neuronal lineages. Transplantation studies in the adult CNS are more challenging as the environment is fully established, developmental cues are restricted, and space is more limited. Nevertheless, investigators have shown that stem cells isolated from the embryonic human brain survived and differentiated into neurons and glia when grafted into various regions of the adult rat brain (Fricker et al., 1999). Significantly, transplants of these cells were able to improve cognitive function in aged rats (Qu et al., 2001).

A frequent concern is that the long-term propagation of stem cells *in vitro* could induce tumor formation. For instance, extensive culturing of rodent neural stem cells has been shown to lead to genetic changes that altered cell growth and differentiation. However, tumor formation has not been observed in any culture systems of human CNS stem cells, or after transplantation into any animal models to date.

Experimental animal studies are casting considerable light on many facets of adult neurogenesis. For example, it has been suggested that an interplay between astrocytes and the microenvironment may bring about adult neurogenesis (Alvarez-Buylla and Garcia-Verdugo, 2002).

Another study has concluded that hippocampal astrocytes provide a unique niche for adult neurogenesis (Song et al., 2002). These researchers further postulate that the capability for adult neurogenesis may lie in regionally specified astrocytes in the adult CNS providing appropriate signals. Such possibilities could also prove significant in humans.

NSC biology is poised to make an impact on clinical neural transplantation programs. However, there is a danger that, similarly to what happened in the field of gene therapy, the rush to apply stem cell therapies in actual patients may lead to scientifically ill-founded clinical trials that lack adequate support from rigorous preclinical research.

While the results with NSCs (both embryonic and adult) have been very promising thus far, there are still hurdles to be overcome. Trials in human patients

should not be initiated until the *in vitro* manipulation of NSCs becomes more sophisticated. More clinically relevant animal studies should be carried out and clinical trials should not be initiated on the basis of results from a limited number of rodent studies. Neurological testing should show *significant* and *long-lasting* functional recovery after transplantation experiments in well characterised animal models of human CNS disorders (Bjorklund and Lindvall, 2000).

As outlined before, glioblastomas have a very poor prognosis. Gene therapy of glioblastomas is limited by the short survival of viral vectors and by their difficulty to reach glioblastoma cells infiltrating the brain parenchyma. Neural stem/progenitor cells can be engineered to produce therapeutic molecules and have the potential to overcome these limitations because they may travel along the white matter like neoplastic cells and engraft stably into the brain. Retrovirus-mediated transfer of the gene for interleukin-4 is an effective treatment for experimental glioblastomas. We then transferred the gene for interleukin-4 into C57BL6J mouse primary neural progenitor cells and injected those cells into established syngeneic brain glioblastomas (Benedetti et al., 2000). This led to the survival of most tumor-bearing mice. We obtained similar results by implanting immortalized neural progenitor cells derived from Sprague-Dawley rats into C6 glioblastomas. We also documented by magnetic resonance imaging the progressive disappearance of large tumors, and detected 5-bromodeoxyuridine-labeled progenitor cells several weeks after the injection.

Our results showed that neural progenitor cells engineered to release high levels of IL-4 can have a strong anti-tumor effect and be more effective than retrovirus-mediated, *in vivo* transfer of IL-4. Indeed, GL261 cells co-injected with retroviral packaging cells for *in vivo* transfer of IL-4 (E86.L4SN.IL-4) produced mice that survived significantly longer than control mice ($P < 0.004$; not shown) but significantly shorter than mice injected with neural progenitor cells with one of six (treated with retrovirus) and six of seven (treated with neural progenitor cells) surviving at 3 months; $P < 0.004$). Experiments with rats have shown a similar trend: retroviral transfer of the gene for IL-4 into established C6 tumors is followed by long-term survival of 40% of the rats, whereas using ST14A cells as vehicles for IL-4 production, we found survival of 65% of the rats.

Several factors may contribute to the anti-tumor action of neural progenitors. Neural progenitor cells can engraft stably and are less or not susceptible to immune rejection. Moreover, progenitor cells may migrate away from the injection site, mimicking the behavior of infiltrating glioblastoma cells. For BrdU-labeled ST14A cells injected into the rat striatum, there was diffusion from the injection site and a tendency to migrate along the white fibers and the corpus callosum. The presence of ST14A.IL-4.3 in the context of the tumor confirmed this observation. Furthermore, progenitor cells, while differentiating, may release factors with an anti-proliferative effect, as indicated by *in vivo* and *in vitro* that we have previously provided and on what we are now investigating using DNA microarray technology. Finally, in the perspective of a clinical use of neural stem/progenitor cells, it is particularly useful that their anti-tumor effect is obtained in the absence of viral-mediated gene transfer *in vivo*, thus avoiding the potential dangers of this procedure.

Our findings have supported a new approach for gene therapy of brain tumors, based on the grafting of neural stem cells producing therapeutic molecules. Aboody et al. (2000) demonstrated that NSCs, when implanted into experimental intracranial gliomas *in vivo* in adult rodents, distribute themselves quickly and extensively throughout the tumor bed and migrate uniquely in juxtaposition to widely expanding and aggressively advancing tumor cells, while continuing to stably express a foreign gene. The NSCs 'surround' the invading tumor border while 'chasing down' infiltrating tumor cells. When implanted intracranially at distant sites from the tumor (e.g., into normal tissue, into the contralateral hemisphere, or into the cerebral ventricles), the donor cells migrate through normal tissue targeting the tumor cells (including human glioblastomas). In their experiments NSCs delivered a therapeutically relevant molecule-cytosine deaminase- obtaining a quantifiable reduction in tumor burden. Ehtesham et al. (2002) inoculated intracranial glioma-bearing mice with interleukin 12 (IL-12) producing NSCs. Intratumoral therapy with IL-12-secreting NSCs prolonged survival compared to treatment with nonsecretory NSCs or saline. NSCs demonstrated strong tropism for disseminating glioma, and IL-12-secreting NSC therapy was associated with enhanced T-cell infiltration in tumor microsatellites and long-term antitumor immunity. These results also confirmed that the use of tumor tracking NSCs represents a potent new therapeutic modality for glioma.

Gene therapy for GLD could complement bone marrow transplantation being pointed at the amelior-

ation of the symptoms due to CNS involvement. Our strategy for the treatment of *twitcher* has been partly based on the implantation into the peri-ventricular region of the brain of neural stem/progenitor cells engineered to produce high levels of GALC. The cDNA encoding human GALC was first transduced into ST14A cells causing a 4-fold increase of GALC activity (Torchiana et al., 1998). *Twi/twi* fibroblasts were also transduced by the same retrovirus producing cells obtaining an activity of 45.0 nmol hr^{-1} mg^{-1} protein (background activity was zero). In the first experiment we injected 2×10^5 ST14A neural progenitor cells, trasduced to overproduce galactocerebrosidase, in the periventricular region of normal and t*wi/twi* mice. The second series of experiments was performed injecting syngeneic fibroblasts overexpressing GALC into the left emisphere of *twi/twi* mice.

Control animals (n = 5) survived 31 ± 2.8 days, the group injected with ST14A.GALC cells (n = 6) survived 32.3 ± 3.0 days and the group injected with GALC.fibroblasts (n = 3) 37.5 ± 2.1. Differences in survival were not statistically significant.

Under these experimental conditions, the injection of neural progenitor cells overexpressing GALC did not affect the phenotype of *twitcher*. The survival of treated mice was similar to controls and their weight as well as the onset and the severity of twitching were not modified after cell injection. The difficulty in tracking down ST14A cells after their injection into the brain of *twitcher* suggests that these cells were attacked and eliminated by the immune system quite rapidly. Indeed they could only be found in the brain of control mice while in *twitcher*, possibly because of the up-regulation of the immune system, these xenogeneic cells were removed. In agreement with this, syngeneic fibroblasts could be detected after two weeks and their injection was associated with longer survival.

These data stressed the relevance that the immune system may have in deciding the fate (and consequently the therapeutic potential) of engineered neural progenitor cells, challenging the idea of brain immune privilege (Carson and Sutcliffe, 1999). In *twitcher*, the immune system is up-regulated by the occurrence of macrophages trying to cope with the accumulation of undegraded substrate(s). Transferring the *twitcher* genotype on a MHC II-negative backgroud decreased the severity of the phenotype (Matsushima et al., 1994).

Presently, we are using primary NSC derived from C57BL6J mice for *ex vivo* transduction with a lentiviral vector transducing the GALC gene and subsequent injection in the periventricular region of twitcher mice. The effects of repeated injection of these cells are also under scrutiny.

Hematopoietic stem cells (HSC) for the treatment of gliomas and GLD

Microglia are the resident immunological effector cells of the central nervous system (CNS). They are activated in response to minor pathological changes in the CNS, and they have a key role in the defense of the neural parenchyma against infection, inflammation, ischemia, trauma, brain tumors and neurodegeneration. Recently, cells derived from bone marrow (BM) were found to enter the brain in adult life to differentiate into microglia, astrocytes and neurons (Brazelton et al., 2000; Eglitis and Mezey, 1997; Mezey et al., 2000).

A recent study by Priller et al. demonstrates that upon adoptive BM transplantation, genetically-modified hematopoietic cells differentiate into CNS microglia in a site-selective manner (Priller et al., 2001). The immediate precursors of these microglia most likely are mature BM mononuclear cells, which have been shown to enter the brain and engraft after transplantation into lethally irradiated mice. Microglial engraftment was specifically enhanced by neuropathology, suggesting that myeloid cells may be used as vehicles for gene delivery to the CNS. It is conceivable that BM stem cells sense injury to the nervous system, mobilize into the blood and undergo microglial differentiation at the sites of CNS damage. The authors could not exclude the possibility that whole body irradiation promoted the engraftment of BM-derived cells in the CNS. Recent experimental evidence in BM chimeras, however, suggests that the number of cells that engraft in the CNS is the same, irrespective of whether the animals were irradiated or not.

A remarkable feature of CNS microglial engraftment is the propensity of gene-modified hematogenous cells to be attracted to sites of neuronal injury. Thus, BM-derived cells were found to differentiate into microglia in the ischemic hemisphere following stroke. The site-specific influx of BM-derived cells despite an intact blood–brain barrier suggests that neurons may signal damage to circulating cells via specific molecular mediators such as monocyte chemoattractant protein-1 (MCP-1) (Flugel et al., 2001). Interestingly, MCP-1 is secreted by glioma

cell lines, suggesting that glioma, similarly to other pathological situations into the brain, may attract bone-marrow derived monocytes (Desbaillets et al., 1994; Kuratsu et al., 1993). It is therefore conceivable that molecular engineering of HSC might be considered to deliver potentially therapeutic genes to gliomas. Presently we have set-up the conditions to investigate whether GFP-expressing HSC may target an experimental glioma established before BMT.

Using GFP transgenic mice as donors, the distribution of hematogenous cells after transplantation of GFP+ bone marrow cells was investigated in the twitcher mice in the laboratory of Dr Suzuki (Wu et al., 2000). Bone marrow transplantation was carried out at 8 postnatal days. In twitcher chimeric mice examined before 30 postnatal days, numerous GFP+ cells were detected in spleen and peripheral nerve but only a few were detected in the liver, lung, and spinal white matter. In contrast, at 35 to 40 postnatal days when demyelination was evident, many GFP+ cells with ameboid form were detected in the white matter of the spinal cord, brainstem, and cerebrum. Approximately half of these GFP+ cells were co-labeled with the cell type-specific antigen for macrophage-microglia lineage cells Mac-1(CD11b). In twitcher chimeric mice examined after 100 postnatal days, the majority of GFP/Mac-1 double-positive cells displayed the morphological features of ramified microglia with fine delicate processes and was distributed diffusely in both gray and white matter. These authors suggest that a significant number of donor hematogenous cells are able to infiltrate into the brain parenchyma, repositioning themselves into areas previously occupied by microglia, and to ameliorate lethality.

We have confirmed that BMT may significantly ameliorate survival in twitcher mice (Pellegatta et al, unpublished). Studies with GFP transgenic mice are ongoing to verify whether in our experimental set-up the timing and the relevance of microglial infiltration into the CNS is similar to what described by Wu et al. (2000). Our goal is to establish the conditions for *ex vivo* transduction by lentiviral (or retroviral) vectors of the GALC gene into immune-selcted HSC and subsequent transplantation into twitcher mice. If increased survival will be comparable or superior to that obtained by BMT, this form of autologous transplantation could be considered as an option for the treatment of Krabbe disease.

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References

Aboody KS, Brown A, Rainov NG, Bower KA, Liu S, Yang W, Small JE, Herrlinger U, Ourednik V & Black PM et al. (2000) From the cover: neural stem cells display extensive tropism for pathology in adult brain: Evidence from intracranial gliomas. Proc Natl Acad Sci USA 97: 12846–12851.

Albarosa R, Colombo BM, Roz L, Magnani I, Pollo B, Cirenei N, Giani C, Conti AM, DiDonato S & Finocchiaro G (1996) Deletion mapping of gliomas suggest the presence of two small regions for candidate tumor-suppressor genes in a 17-cM interval on chromosome 10q. Am J Hum Genet 58: 1260–1267.

Alvarez-Buylla A & Garcia-Verdugo JM (2002) Neurogenesis in adult subventricular zone. J Neurosci 22: 629–634.

Arsenijevic Y, Villemure JG, Brunet JF, Bloch JJ, Deglon N, Kostic C, Zurn A & Aebischer P (2001) Isolation of multipotent neural precursors residing in the cortex of the adult human brain. Exp Neurol 170: 48–62.

Benedetti S, Pirola B, Pollo B, Magrassi L, Bruzzone MG, Rigamonti D, Galli R, Selleri S, Di Meco F, De Fraja C et al. (2000) Gene therapy of experimental brain tumors using neural progenitor cells. Nat Med 6: 447–450.

Berger J, Moser HW & Forss-Petter S (2001) Leukodystrophies: Recent developments in genetics, molecular biology, pathogenesis and treatment. Curr Opin Neurol 14: 305–312.

Bjorklund A & Lindvall O (2000) Cell replacement therapies for central nervous system disorders. Nat Neurosci 3: 537–544.

Brazelton TR, Rossi FM, Keshet GI & Blau HM (2000) From marrow to brain: expression of neuronal phenotypes in adult mice. Science 290, 1775–1779.

Carpenter MK, Cui X, Hu ZY, Jackson J, Sherman S, Seiger A & and Wahlberg LU (1999) *In vitro* expansion of a multipotent population of human neural progenitor cells. Exp Neurol 158: 265–278.

Carson MJ & Sutcliffe JG (1999) Balancing function vs. self defense: The CNS as an active regulator of immune responses. J Neurosci Res 55: 1–8.

Chiariello E, Roz L, Albarosa R, Magnani I & Finocchiaro G (1998) PTEN/MMAC1 mutations in primary glioblastomas and short-term cultures of malignant gliomas. Oncogene 16: 541–545.

Desbaillets I, Tada M, De Tribolet N, Diserens AC, Hamou MF & Van Meir EG (1994) Human astrocytomas and glioblastomas express monocyte chemoattractant protein-1 (MCP-1) *in vivo* and *in vitro*. Int J Cancer 58: 240–247.

Duchen LW, Eicher EM, Jacobs JM, Scaravilli F & Teixeira F (1980) Hereditary leucodystrophy in the mouse: The new mutant twitcher. Brain 103: 695–710.

Eglitis MA & Mezey E (1997) Hematopoietic cells differentiate into both microglia and macroglia in the brains of adult mice. Proc Natl Acad Sci USA 94: 4080–4085.

Ehtesham M, Kabos P, Kabosova A, Neuman T, Black KL & Yu JS (2002) The use of interleukin 12-secreting neural stem cells for the treatment of intracranial glioma. Cancer Res 62: 5657–5663.

Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn AM, Nordborg C, Peterson DA & Gage FH (1998) Neurogenesis in the adult human hippocampus. Nat Med 4: 1313–1317.

Flugel A, Hager G, Horvat A, Spitzer C, Singer GM, Graeber MB, Kreutzberg GW & Schwaiger FW (2001) Neuronal MCP-1 expression in response to remote nerve injury. J Cereb Blood Flow Metab 21: 69–76.

Frederick L, Wang XY, Eley G & James CD (2000) Diversity and frequency of epidermal growth factor receptor mutations in human glioblastomas. Cancer Res 60: 1383–1387.

Fricker RA, Carpenter MK, Winkler C, Greco C, Gates MA & Bjorklund A (1999) Site-specific migration and neuronal differentiation of human neural progenitor cells after transplantation in the adult rat brain. J Neurosci 19: 5990–6005.

Fulci G, Labuhn M, Maier D, Lachat Y, Hausmann O, Hegi ME, Janzer RC, Merlo A & Van Meir EG (2000) p53 gene mutation and ink4a-arf deletion appear to be two mutually exclusive events in human glioblastoma. Oncogene 19: 3816–3822.

Hayashi Y, Ueki K, Waha A, Wiestler OD, Louis DN & Von Deimling A (1997) Association of EGFR gene amplification and CDKN2 (p16/MTS1) gene deletion in glioblastoma multiforme. Brain Pathol 7: 871–875.

Hermanson M, Funa K, Hartman M, Claesson-Welsh L, Heldin CH, Westermark B & Nister M (1992) Platelet-derived growth factor and its receptors in human glioma tissue: Expression of messenger RNA and protein suggests the presence of autocrine and paracrine loops. Cancer Res 52: 3213–3219.

Holland EC (2000) Glioblastoma multiforme: The terminator. Proc Natl Acad Sci USA 97: 6242–6244.

Holland EC, Celestino J, Dai C, Schaefer L, Sawaya RE & Fuller GN (2000) Combined activation of Ras and Akt in neural progenitors induces glioblastoma formation in mice. Nat Genet 25: 55–57.

Holland EC, Hively WP, DePinho RA & Varmus HE (1998) A constitutively active epidermal growth factor receptor cooperates with disruption of G1 cell-cycle arrest pathways to induce glioma-like lesions in mice. Genes Dev 12: 3675–3685.

Hoogerbrugge PM, Suzuki K, Poorthuis BJ, Kobayashi T, Wagemaker G & Van Bekkum DW (1988) Donor-derived cells in the central nervous system of twitcher mice after bone marrow transplantation. Science 239: 1035–1038.

Ichioka T, Kishimoto Y, Brennan S, Santos GW & Yeager AM (1987) Hematopoietic cell transplantation in murine globoid cell leukodystrophy (the twitcher mouse): effects on levels of galactosylceramidase, psychosine, and galactocerebrosides. Proc Natl Acad Sci USA 84: 4259–4263.

Kobayashi T, Yamanaka T, Jacobs JM, Teixeira F & Suzuki K (1980) The Twitcher mouse: an enzymatically authentic model of human globoid cell leukodystrophy (Krabbe disease). Brain Res 202: 479–483.

Krivit W, Shapiro EG, Peters C, Wagner JE, Cornu G, Kurtzberg J, Wenger DA, Kolodny EH, Vanier MT, Loes DJ et al. (1998) Hematopoietic stem-cell transplantation in globoid-cell leukodystrophy. N Engl J Med 338: 1119–1126.

Kukekov VG, Laywell ED, Suslov O, Davies K, Scheffler B, Thomas LB, O'Brien TF, Kusakabe M & Steindler DA (1999) Multipotent stem/progenitor cells with similar properties arise from two neurogenic regions of adult human brain. Exp Neurol 156: 333–344.

Kuratsu J, Yoshizato K, Yoshimura T, Leonard EJ, Takeshima H & Ushio Y (1993) Quantitative study of monocyte chemoattractant protein-1 (MCP-1) in cerebrospinal fluid and cyst fluid from patients with malignant glioma. J Natl Cancer Inst 85: 1836–1839.

Maher EA, Furnari FB, Bachoo RM, Rowitch DH, Louis DN, Cavenee WK & DePinho RA (2001) Malignant glioma: Genetics and biology of a grave matter. Genes Dev 15: 1311–1333.

Matsushima GK, Taniike M, Glimcher LH, Grusby MJ, Frelinger JA, Suzuki K & Ting JP (1994) Absence of MHC class II molecules reduces CNS demyelination, microglial/macrophage infiltration, and twitching in murine globoid cell leukodystrophy. Cell 78: 645–656.

Mezey E, Chandross KJ, Harta G, Maki RA & McKercher SR (2000) Turning blood into brain: Cells bearing neuronal antigens generated *in vivo* from bone marrow. Science 290: 1779–1782.

Myers MP, Pass I, Batty IH, Van der Kaay J, Stolarov JP, Hemmings BA, Wigler MH, Downes CP & Tonks NK (1998) The lipid phosphatase activity of PTEN is critical for its tumor supressor function. Proc Natl Acad Sci USA 95: 13513–13518.

Nishikawa R, Ji XD, Harmon RC, Lazar CS, Gill GN, Cavenee WK & Huang HJ (1994) A mutant epidermal growth factor receptor common in human glioma confers enhanced tumorigenicity. Proc Natl Acad Sci USA 91: 7727–7731.

Priller J, Flugel A, Wehner T, Boentert M, Haas CA, Prinz M, Fernandez-Klett F, Prass K, Bechmann I, De Boer BA et al. (2001) Targeting gene-modified hematopoietic cells to the central nervous system: use of green fluorescent protein uncovers microglial engraftment. Nat Med 7: 1356–1361.

Qu T, Brannen CL, Kim HM & Sugaya K (2001) Human neural stem cells improve cognitive function of aged brain. Neuroreport 12: 1127–1132.

Reilly KM, Loisel DA, Bronson RT, McLaughlin ME & Jacks T (2000) Nf1;Trp53 mutant mice develop glioblastoma with evidence of strain-specific effects. Nat Genet 26: 109–113.

Roy NS, Benraiss A, Wang S, Fraser RA, Goodman R, Couldwell WT, Nedergaard M, Kawaguchi A, Okano H & Goldman SA (2000) Promoter-targeted selection and isolation of neural progenitor cells from the adult human ventricular zone. J Neurosci Res 59: 321–331.

Savitz SI, Rosenbaum DM, Dinsmore JH, Wechsler LR & Caplan LR (2002) Cell transplantation for stroke. Ann Neurol 52: 266–275.

Schlegel J, Merdes A, Stumm G, Albert FK, Forsting M, Hynes N & Kiessling M (1994) Amplification of the epidermal-growth-factor-receptor gene correlates with different growth behaviour in human glioblastoma. Int J Cancer 56: 72–77.

Selleri S, Torchiana E, Pareyson D, Lulli L, Bertagnolio B, Savoiardo M, Farina L, Carrara F, Filocamo M & Gatti R et al. (2000) Deletion of exons 11–17 and novel mutations of the galactocerebrosidase gene in adult- and early-onset patients with Krabbe disease. J Neurol 247, 875–877.

Shihabuddin LS, Palmer TD & Gage FH (1999) The search for neural progenitor cells: Prospects for the therapy of neurodegenerative disease. Mol Med Today 5: 474–480.

Song H, Stevens CF & Gage FH (2002) Astroglia induce neurogenesis from adult neural stem cells. Nature 417: 39–44.

Suzuki K & Suzuki Y (1970) Globoid cell leucodystrophy (Krabbe's disease): Deficiency of galactocerebroside beta-galactosidase. Proc Natl Acad Sci USA 66: 302–309.

Suzuki Y & Suzuki K (1971) Krabbe's globoid cell leukodystrophy: Deficiency of glactocerebrosidase in serum, leukocytes, and fibroblasts. Science 171: 73–75.

Svendsen CN, Caldwell MA & Ostenfeld T (1999) Human neural stem cells: isolation, expansion and transplantation. Brain Pathol 9, 499–513.

Tohma Y, Gratas C, Biernat W, Peraud A, Fukuda M, Yonekawa Y, Kleihues P& Ohgaki H (1998) PTEN (MMAC1) mutations are

frequent in primary glioblastomas (*de novo*) but not in secondary glioblastomas. J Neuropathol Exp Neurol 57: 684–689.

Torchiana E, Lulli L, Cattaneo E, Invernizzi F, Orefice R, Bertagnolio B, Di Donato S & Finocchiaro G (1998) Retroviral-mediated transfer of the galactocerebrosidase gene in neural progenitor cells. Neuroreport 9, 3823–3827.

Uchida N, Buck DW, He D, Reitsma MJ, Masek M, Phan TV, Tsukamoto AS, Gage FH & Weissman IL (2000) Direct isolation of human central nervous system stem cells. Proc Natl Acad Sci USA 97: 14720–14725.

Ueki K, Ono Y, Henson JW, Efird JT, Von Deimling A & Louis DN (1996) CDKN2/p16 or RB alterations occur in the majority of glioblastomas and are inversely correlated. Cancer Res 56: 150–153.

Uhrbom L, Dai C, Celestino JC, Rosenblum MK, Fuller GN & Holland EC (2002) Ink4a-Arf loss cooperates with KRas activation in astrocytes and neural progenitors to generate glioblastomas of various morphologies depending on activated Akt. Cancer Res 62: 5551–5558.

Vescovi AL, Parati EA, Gritti A, Poulin P, Ferrario M, Wanke E, Frolichsthal-Schoeller P, Cova L, Arcellana-Panlilio M, Colombo A & Galli R (1999) Isolation and cloning of multipotential stem cells from the embryonic human CNS and establishment of transplantable human neural stem cell lines by epigenetic stimulation. Exp Neurol 156, 71–83.

Villa A, Snyder EY, Vescovi A & Martinez-Serrano A (2000) Establishment and properties of a growth factor-dependent, perpetual neural stem cell line from the human CNS. Exp Neurol 161: 67–84.

Vogelstein B, Lane D & Levine AJ (2000) Surfing the p53 network. Nature 408: 307–310.

Wenger DA, Rafi MA, Luzi P, Datto J & Costantino-Ceccarini E (2000) Krabbe disease: Genetic aspects and progress toward therapy. Mol Genet Metab 70: 1–9.

Wu X, Senechal K, Neshat MS, Whang YE & Sawyers CL (1998) The PTEN/MMAC1 tumor suppressor phosphatase functions as a negative regulator of the phosphoinositide 3-kinase/Akt pathway. Proc Natl Acad Sci USA 95: 15587–15591.

Wu YP, McMahon E, Kraine MR, Tisch R, Meyers A, Frelinger J, Matsushima GK & Suzuki K (2000) Distribution and characterization of GFP(+) donor hematogenous cells in Twitcher mice after bone marrow transplantation. Am J Pathol 156: 1849–1854.

Young E, Wilson J, Patrick AD & Crome L (1972) Galactocerebrosidase deficiency in globoid cell leucodystrophy of late onset. Arch Dis Child 47: 449–450.