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Research Report

Ex vivo cell-mediated gene therapy for metachromatic leukodystrophy using neurospheres

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ABSTRACT

Metachromatic leukodystrophy (MLD) is an autosomal recessive disease caused by mutations in the gene encoding the lysosomal enzyme arylsulfatase A (ASA). In MLD, accumulation of the substrate, sulfated glycoprotein, in the central and peripheral nervous systems results in progressive motor and mental deterioration. Neural progenitor cells are thought to be useful for cell replacement therapy and for cell-mediated gene therapy in neurodegenerative diseases. In the present study, we examined the feasibility of ex vivo gene therapy for MLD using neural progenitor cells. Neural progenitor cells (neurospheres) were prepared from the striatum of E14 embryo MLD knockout mice or GFP transgenic mice and were transduced with the VSV pseudotyped HIV vector carrying the ASA gene (HIV-ASA). For in vivo study, neurospheres from GFP mice were transduced with HIV-ASA and inoculated into the brain parenchyma of adult MLD mice. HIV vector-transduced progenitor cells retained the potential for differentiation into neurons, astrocytes and oligodendrocytes in vitro. Expression of ASA in neurospheres transduced with HIV-ASA was confirmed by spectrophotometric enzyme assay and Western blotting. In vivo, GFP-positive cells were detectable 1 month after injection. These cells included GFAP- and MAP2-positive cells. Immunohistochemistry using anti-ASA antibody demonstrated localization of ASA in both GFP-positive and -negative cells. Partial clearance of accumulated sulfatide was confirmed in vivo in MLD knockout mice. The present findings suggest that ASA enzyme is released from migrated neurospheres and is able to digest sulfatide in surrounding cells. Our results suggest the potential of genetically engineered neural progenitor cells (neurospheres) for ex vivo therapy in MLD.

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1. Introduction

Metachromatic leukodystrophy (MLD) is an autosomal recessive inherited lysosomal disorder caused by a deficiency of the lysosomal enzyme arylsulfatase A (ASA; EC3.1.6.8), which catalyzes the degradation of galactosyl-3-sulfate ceramide (sulfatide), a major sphingolipid of myelin. This disease is characterized pathologically by degeneration of myelin in both the central and peripheral nervous systems, and clinically by progressive motor and mental deterioration, and finally lethal neurological symptoms.

Three types of MLD are clinically recognized based on age at disease onset and symptom severity. The most common type, the late infantile type, appears from 18 to 24 months of age and is diagnosed based on gait disturbances from infancy, and ataxia, spastic quadriplegia and optical atrophy are subsequently observed. In the juvenile type, the disease appears between the ages of 4 and 16 years. In the adult type, some patients show psychiatric symptoms (Von Figura et al., 2001; Gieselmann et al., 1998; Gieselmann, 2003). No effective therapy, other than allogeneic bone marrow transplantation (BMT), has yet been reported. Moreover, it is crucial to perform BMT at presymptomatic stages of MLD in order to achieve the best neurological outcome (Krivit et al., 1990).

Enzyme replacement therapy is very effective in a limited number of disorders, including type-1 Gaucher disease and Fabry disease (Barton et al., 1991; Schiffmann et al., 2001; Eng et al., 2001; Brady and Schiffmann, 2000). However, this therapy is not available for lysozomal diseases in which the central nervous system is widely affected, such as MLD and Krabbe disease. This has encouraged investigators to seek other strategies to treat these disorders.

Gene therapy experiments for MLD using various viral vectors have been undertaken (Matzner et al., 2000, 2002; Wei et al., 1994; Consiglio et al., 2001). Improvement of neurological symptoms was reported with retroviral transduction of bone marrow progenitor cells with the ASA gene in MLD knockout mice (Matzner et al., 2000; Matzner et al., 2002). Direct injection of a lentiviral vector containing the ASA gene into brains of MLD knockout mice resulted in protection of cells in the CNS and improvement in movement disturbance (Consiglio et al., 2001).

An alternative strategy for neurological disorders, neural cell replacement therapy using brain tissue from aborted embryos, has been studied mainly for Parkinson's disease, and the effectiveness of this therapy has been discussed (Piccini et al., 1999; Bjorklund and Lindvall, 2000). Multipotential neuronal cells have been also studied (so-called neurospheres). These are isolated from mammalian CNS cells and can differentiate into neurons and glial cells in vitro (Reynolds and Weiss, 1992).

Currently, transplantation of cells transduced with a therapeutic gene is promising as a novel therapeutic strategy for genetic diseases characterized by enzyme deficiency. The present study was designed to determine the feasibility of cell-mediated gene therapy for MLD using multipotent neurospheres as carrier cells.

2. Results

2.1. *Lentiviral-vector-transduced NPCs retained the potential for differentiation into MAP2+, GFAP+ and GalC+ cells in vitro*

Cells harvested from the corpus striatum of mouse embryo at 13–15 days of gestation were cultured in specific medium, as described above, and formed neurospheres after 6–8 days. Neurospheres derived from MLD knockout mice were transduced with lentivirus vector containing the GFP gene as a marker. Neurospheres showed very high sensitivity to lentiviral vectors, as reported previously (Falk et al., 2002; Ostenfeld et al., 2002; Hughes et al., 2002) (Fig. 1a). Transduced cells were maintained on polyethylenimine-coated dishes with addition of 10% fetal bovine serum (FBS) as neuroproliferative medium. Three days later, cells were stained with MAP2 antibody (neuron marker), GFAP antibody (astrocyte marker) and GalC antibody (oligodendrocyte marker). Cells positive for each antibody were observed (Fig. 1b). Phenotypically, the various types of cells expanded continuously for as long as 7 weeks in vitro, thus demonstrating that neurospheres contained neural stem cells and neural progenitor cells and that they possessed pluripotency to differentiate into neurons and glial cells. Moreover, these cells showed that transduction with the lentivirus vector did not change morphology and that differentiation potential was preserved.

2.2. *Expression of ASA on neurospheres from MLD knockout mice after lentiviral transduction*

Neurospheres derived from MLD knockout mice were transduced with lentivirus vector containing the ASA gene. ASA enzyme activity was measured by colorimetric assay after transduction. ASA activity of transduced neurospheres was elevated to 5-fold the level of that in non-transduced MLD mice (transduced, 254.42 ± 49.0 nmol/h/mg protein; non-transduced, 50.45 ± 11.6 nmol/h/mg protein; C57-BL6 (normal control), 322.65 ± 12.25 nmol/h/mg protein), which was as high as 80% of that in normal controls (Fig. 2a). On Western blotting analysis, the 50-kDa band corresponding to ASA protein was confirmed after lentivirus transduction with the ASA gene (Fig. 2b).

2.3. *Neurospheres from GFP mice were transduced with HIV-ASA and inoculated into brains of MLD mice*

Neurospheres derived from GFP mice were transduced with lentiviral vector having the ASA gene. ASA activity of transduced cells increased by about 5-fold when compared with that in wild-type cells (3722 ± 1202 and 570 ± 288 nmol/h/mg protein, respectively) (Fig. 2c). These ASA-expressing neurospheres (5×10^4) were stereotaxically inoculated into the hippocampus of MLD knockout mice. Four weeks after transplantation, numerous ASA-transduced GFP-positive cells were detected at the site of injection and also continuously along with the corpus callosum (Fig. 3b). A few GFP-positive cells were found at

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