CEREBROSPINAL FLUID DERIVED FROM PROGRESSIVE MULTIPLE SCLEROSIS PATIENTS PROMOTES NEURONAL AND OLIGODENDROGLIAL DIFFERENTIATION OF HUMAN NEURAL PRECURSOR CELLS IN VITRO

Barbara Cymring, Massimiliano Cristofanilli,* Amy Lu, Hannah Rosenthal, Saud A. Sadiq
Multiple Sclerosis Research Center of New York

INTRODUCTION

Adult multipotent neural precursor cells (NPCs) have the capacity for self-renewal and differentiation into functional brain cells (e.g. neurons, astrocytes or oligodendrocytes) within discrete tissue-specific germinal niches.

Due to their intrinsic plasticity, NPCs can be considered an essential part of the cellular mechanism(s) by which the central nervous system (CNS) tries to repair itself after an injury.

Although developing evidence indicates that endogenous neurogenesis and gliogenesis occur as part of an intrinsic self-repair process during the course of inflammatory CNS disorders, such as multiple sclerosis (MS), there are no convincing explanations about the overall incapacity of the endogenous stem cells to promote full and long-lasting CNS repair in progressive forms of MS.

Many reports suggest that endogenous NPCs, while contributing to CNS repair in MS, may also become the target of the disease itself.

In this study, we investigated the effect of applications of embryonic-derived neural stem cells (ES-NSCs) in vitro.

METHODS

CSF from primary progressive (PPMS) and secondary progressive (SPMS) MS patients or control CSF was diluted in the culture media (5%) and administered on cultured ES-NSCs.

In this experiment, we collected at 14 days post treatment and analyzed by FACS, q-RT-PCR, immunocytochemistry (ICC), and western blot (WB).

RESULTS

A highly pure population of sox2+/Nestin+ NSCs with the ability to differentiate in the mature brain cells (neurons, astrocytes, and oligodendrocytes) was used in this work (Figure 1).}

METHODS

CSF from primary progressive (PPMS) and secondary progressive (SPMS) MS patients or control CSF was diluted in the culture media (5%) and administered on cultured ES-NSCs.

CSF-media was replaced every other day and samples were collected at 14 days post-treatment and analyzed by FACS, q-RT-PCR, immunocytochemistry (ICC), and western blot (WB).

CONCLUSIONS

CSF-MS reduced the proliferation of ES-NSC and increased their differentiation toward neuronal and oligodendroglial cells compared to control CSF.

Table 1. Patient demographics

<table>
<thead>
<tr>
<th>Controls (n = 19)</th>
<th>SPMS (n = 18)</th>
<th>PPMS (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (range)</td>
<td>65 (25–66)</td>
<td>55 (20–77)</td>
</tr>
<tr>
<td>Gender (M:F)</td>
<td>7:12</td>
<td>7:11</td>
</tr>
<tr>
<td>Median EDSS (range)</td>
<td>7.25 (3.5–9.0)</td>
<td>6.5 (2–9.0)</td>
</tr>
</tbody>
</table>

Figure 1. Characterization of the embryonic-derived neural stem cells ENStem-A and their neuroepithelial differentiation potential. Cells seeded in adherent condition, with proliferation media, form rosette like structures visible in phase contrast. A and B are Sox2+ and Nestin+ in immunofluorescent staining. Two weeks after growth factor removal, MAP2+ neurons, GFAp+ astrocytes, NG2+ OPCs, and MBP+ myelinating oligodendrocytes are present.

Figure 2. FACS quantification of ENStem-A survival and proliferation after CSF treatment. CSF from progressive MS patients reduced cell proliferation but did not affect cell survival. Error bars represent SEM. *p<0.023, one way ANOVA [post-hoc analysis: Tukey HSD Test].

Figure 3. Western blot characterization of ENStem-A after CSF treatment and growth factor removal. Compared to control CSF (C), CSF from progressive MS patients increased the amount of neuron (TuJ1 and MAP2) and oligodendrocytic (MBP) proteins. For each cell type, values were expressed as percentage of total cell count and normalized to media treated samples. Error bars represent SEM. **p<0.01, one-way ANOVA [post-hoc analysis: Tukey HSD Test].

Figure 4. Characterization of the differentiated neuronal and oligodendroglial cells compared to control CSF.

Figure 5. Quantification of immunolabeled ENStem-A after CSF treatment and growth factor removal. CSF from progressive MS patients increased the numbers of ENStem-A-derived neurons and oligodendrocytes. For each cell type, values were expressed as percentage of total cell count and normalized to media treated samples. Error bars represent SEM. **p<0.01, one-way ANOVA [post-hoc analysis: Tukey HSD Test].

CNS repair in MS, may also become the target of the disease itself.

A highly pure population of sox2+/Nestin+ NSCs with the ability to differentiate in the mature brain cells (neurons, astrocytes, and oligodendrocytes) was used in this work (Figure 1).