FGF2, but not EGF, induces multiciliated ependymal cells to dedifferentiate and adopt radial glial features in vitro

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Abstract

Multiciliated ependymal cells form an epithelium lining most of the ventricular cavities of vertebrates brain. Although considered postmitotic and completely differentiated, ependymal cells maintain some phenotypic characteristics of neural stem cells. Therefore, under specific conditions they behave as neural stem cells, developing radial glia characteristics, and undergoing asymmetric division. Our group is searching for factors that promote dedifferentiation of ependymal cells in vitro. We developed a simple method to obtain pure cultures of non-adherent multiciliated ependymal cells from adult rat. These cultures were used to investigate the effect of FGF2 on the differentiation state and the aggregation of ependymal cells. Thus, FGF2 treated ependymal cells lose cilia and become motile, and after 7 days they aggregate to form irregular spheres (40-50 µm). These aggregates were stained for FGF2 receptor expression, and anti-FGF2 neutralizing antibody was used. In both conditions the aggregation effect of FGF2 was abolished. No cell proliferation was observed during spheres formation, at least in such experimental conditions. Spheres were analyzed by immunocytochemistry using radial glia markers. They were positive for GAP43, GABA and BLBP. In vivo data suggest that FGF2 promotes the identity loss in multiciliated ependymal cells, in vitro, which are transformed into radial glia features.

Introduction

Ependymal cells cover most of the ventricular cavities and the central canal of the spinal cord of the vertebrate nervous system. They form a simple cuboidal epithelium that separates the central canal fluid (CSF) and the nervous parenchyma. Ependymal cells form part of the adult neurogenic niche in the subventricular zone (SVZ) (Sotelo et al. 1997) where they are in intimate contact with the neural stem cells (NSC) and their progeny. Recent findings demonstrate that ependymal cells are quiescent in normal conditions, and that they do not fulfill the defining criteria of stem cells (Carlen et al. 2009). However, several evidences suggest that they may behave as NSC under specific circumstances, such as nervous tissue injury (Nazar and Tater 1993), stroke (Carlen et al. 2009; Zhang et al. 2007), carcinogenic transformation (Naylor et al. 2005), astrocyte signalling blockade (Carlen et al. 2009), and exposure to growth factors (Gregg and Weiss 2003). In addition, a hallmark of all stem cells is the asymmetric cell division, a feature that has also been observed in ependymal cells after brain injury (Gleason et al. 2008). And finally, it has been demonstrated that ependymal cells can proliferate under certain conditions such as injury of the nervous system (Gleason et al. 2008, Mehler et al. 2008, Ceballos et al. 2008, Ceballos et al. 2009, Mehler-Matute et al. 2009) and stroke (Carlen et al. 2009; Li et al. 2002; Zhang et al. 2005, 2007), rendering a progeny consisting of neurons (Carlen et al. 2009) or astrocytes and oligodendrocytes (Mehler et al. 2008). These features make ependymal cells a possible candidate for cell replacement or nervous tissue repair. Our group developed a method to obtain pure primary cultures of ependymal cells from animals to study the factors that could trigger these changes. We used both FGF2 and EGF as they induce the development of neurospheres from NSC.

Results

FGF2 induces aggregation of isolated ependymal cells in vitro

<table>
<thead>
<tr>
<th>Experimental conditions</th>
<th>FGF2</th>
<th>Heparin</th>
<th>DMDSO</th>
<th>FGF2 inhibitor</th>
<th>Anti-FGF2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>&lt;50 ng/ml</td>
<td>&lt;10 µg/ml</td>
<td>&lt;10 µg/ml</td>
<td>&lt;50 ng/ml</td>
<td>&lt;50 ng/ml</td>
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<tr>
<td>Experimental</td>
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<td>&gt;10 µg/ml</td>
<td>&gt;10 µg/ml</td>
<td>&gt;50 ng/ml</td>
<td>&gt;50 ng/ml</td>
</tr>
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Cells/aggregates from three stages were fixed in formalin and used for:
- Immunostaining with glial radial markers.
- In the case of the cell aggregation stage, projected area quantification was performed both in cells and aggregates.

Conclusions

- The effect of both FGF2 and EGF on the differentiation status of ependymal cells was tested on pure cultures.
- FGF2, but not EGF, induces ependymal cells to aggregate into spheres.
- Cells in the spheres express radial glia markers (BLBP, GLAST and GFAP).
- Under the action of FGF2, cells from aggregates display cellular processes and adopt an astrocytic morphology.