ABSTRACT

The aim of this work was to elucidate the differentiation mechanisms of neural stem/progenitor cells *in vitro* and their potential to survive and differentiate *in vivo*, after transplantation into the injured rat cortex. Immunohistochemistry was used for cell identifications, and the properties of K^+ and Na^+ voltage-gated ion channels were studied using the patch-clamp technique.

We have demonstrated that immortalised green fluorescent protein (GFP)/NE-4C neural stem cells derived from the neuroepithelium of p53$^-$deficient mouse embryos at embryonic day (E)9 are able to differentiate into neurons *in vitro*. After transplantation into the site of a photochemical lesion of adult rats, GFP/NE-4C cells survive and give rise to neurons, astrocytes and oligodendrocytes.

Primary embryonic neural stem cells were isolated from D6/GFP mice, in which GFP is expressed under the control of D6, a promoter of the *mDach1* gene, which is involved in the development of the cortex. At E12, D6 is specifically expressed in the neural stem cells of the dorsal telencephalon, from which cortical neurons arise. We have shown that D6/GFP neural stem cells isolated from E12 embryos are able to give rise to neurons and glial fibrillary acidic protein (GFAP)-positive cells *in vitro* and that after transplantation into the non-injured brain or into the site of a photochemical lesion these cells survive and differentiate exclusively into GABA-responding maturing neurons.

Sonic Hedgehog (Shh) and Wnt-7a are morphogenes involved in the early phases of embryonic development, and they play various roles in the development of the telencephalon. Their role in postnatal neurogenesis is extensively studied. Therefore, we focused on exploring the role of these two morphogenes in neural stem cells proliferation and differentiation *in vitro*. We used neonatal neural stem cells transduced with GFP and Shh (Shh-expressing cells) or GFP and Wnt-7a (Wnt-7a-expressing cells). Cells expressing GFP only were used as a control. We have shown that both Shh and Wnt-7a increase the expression of neuronal markers during *in vitro* differentiation and that Wnt-7a increases the incidence of cells displaying a neuron-like current pattern in differentiated cells.