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Progenitor Cells in the Brainstem and Filum Terminale

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ABSTRACT

Neurogenesis prevails in the adult mammalian CNS. New neurons are constitutively formed *in-vivo* in the subventricular zone (SVZ) of the lateral ventricle wall and in the subgranular zone of the dentate gyrus. However, other regions along the entire neuroaxis have been found to harbor cells with the ability to form neurospheres, self-renew and differentiate into neurons, oligodendrocytes and astrocytes.

Adult rodents were subjected to axotomies of the hypoglossal nerve (n.XII) followed by assessment of the endogenous neural progenitor cell (NPC) response and the fate of grafted adult eGFP SVZ NPCs in the brainstem. Sox2 expressing endogenous NPCs were abundant in the ependymal region of the central canal and 4th ventricle and activated after n.XII avulsion injury, which induced massive loss of motor neurons, but not after transection where the cell loss was minimal. Activation included NPC proliferation and subsequent migration to the hypoglossal nucleus (nucl.XII) where they expressed astrocytic markers. However, neurogenesis failed and no neuroprotective effect on motor neurons was observed. Therefore, a transplantation model was developed; SVZ NPCs were harvested from inbred eGFP transgenic animals and cells grafted to the nucl.XII of their wt siblings after n.XII injuries. The SVZ NPCs from transgenic animals where characterized *in-vitro* prior to grafting, and found to have same NPC characteristics as cells from wt animals. Upon transplantation after avulsion injury NPCs survived, differentiated into neurons, oligodendrocytes and astrocytes and integrated with the host circuitry. Grafted NPCs expressed VEGF and GPx1 that after avulsion exerted a neuroprotective effect on motor neurons. Moreover, we suggest that GPx1 expression contributed to the survival of transplanted cells in the nucl.XII post avulsion. This was in contrast to the results on grafting after transection since NPCs did not survive to the same extent and only differentiated into cells with astrocytic phenotype. Also, some of the cells continued to express Sox2. The cells did not integrate and no neuroprotective effects were found. Differences in activation after the two types of axotomies were correlated to levels of motor neuron death in the nucl.XII, and hence differences in the microenvironment with regards also to levels of free radicals and inflammatory compounds.

The beneficial effects after grafting post avulsion initiated the development of an autologous transplantation model where the distribution of NPCs in the filum terminale was investigated. Sox2 and Mushasi-1 expressing cells in the human filum terminale were found, i.e. cells expressing NPC markers. The cells were abundant and when isolated and propagated *in-vitro* they formed neurospheres, proliferated and differentiated into neurons and glia. Clonal expansion indicated a relatively strong self-renewal capacity. The cells responded to the addition of PDGF-BB with an increase in neuronal cell numbers.

In summary, the findings suggest a repair strategy based on transplantation of adult NPCs in neural repair after severe motor nerve injuries. The filum terminale is a potential source of NPCs in future autologous cell therapies, even in human.

Key words: *brainstem, filum terminale, ependymal layer, subventricular zone, neural progenitor cells, nerve injury, motor neurons, transplantation, differentiation, integration*

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