6 Summary

Adenoviral infections of neurons, glial cells and ventricle ependyma cells in situ as a prospect for an in-vivo genetic therapeutical approach in neurodegenerative diseases

In this study the characteristics of transduction as well as the efficacy of transduction of different stem cell populations are being investigated by using a 3rd generation adenoviral vector, a so called gutless adenoviral vector.
These cells include glial restricted precursor cells of rat and human origin as well as murine embryonic stem cells.
As representatives for an adult stem cell population the possibility of a transduction of the autologous available mesenchymal stem cells deriving from the human bone marrow stroma are examined.
For cell transduction, vectors with different reporter sequences are applied, resulting in the possibility of measuring the transduction success after infection by the transgene expression.
The reporter genes include the green fluorescent protein (GFP), the LacZ (ß-galactosidase) as well as the human secreted placenta alkaline phosphatase (SEAP). Applying increasing numbers of infectious particles (10, 50 and 100 MOI) a concentration-dependent transduction rate can be ascertained in the different investigated cell populations. The highest transduction rates are achieved in association with the glial restricted neural precursor cells.
Following infection of the neural differentiated embryonic stem cells similarly a tendency towards a preferred transduction of glial differentiated cells is observed. The use of adult stem cells also results in transduction rates up to 85%.
In further investigations glial restricted neural precursor cells, after having been infected with the adenoviral vector are injected into the striatum of adult rats. This procedure was performed in order to test the possibility of an ex-vivo gene therapeutical use in association with glial cells as possible carrier cells. The results show that the transduced cells are integrated into the receiving tissue and during the investigation period of six weeks only migrate into the surrounding tissue to a restricted extent.
At a closer look the injected cells show normal characteristics of differentiation along the glial cell linage as it has been described for astrocytes in-vivo.

The second part of this study focuses on the direct vector injection into the central nervous system. Therefore the 3rd generation adenoviral vector is injected into different brain areas. Using immunohistochemical investigations using antibodies against NeuN and GFAP it can be shown that after injection into the striatum analogous to the situation in-vitro a preferential transduction of astrocytes is achieved. Within this brain compartment a diffusion of the vector up to a distance of 1000 µm from the injection site can be determined.

Following injection of vector particles into the corpus callosum, a vector distribution within the entire fibre tract of the white substance including the contralateral hemisphere is observed.

With these results it can be shown that the allocation of the vector is dependent on the structure and features of the receiving tissue.

Injecting the adenoviral vector into the cerebrospinal fluid results in transgene expression (GFP, LacZ and SEAP) in the ependymal cells surrounding the entire ventricle.

These results suggest that the vector distributes within the cerebrospinal fluid and therefore would result in a generalized transduction of ependymal cells.

After installing a permanent catheter in the lateral ventricle cerebrospinal fluid may be removed in a continuous manner in order to analyse/evaluate the marker enzyme SEAP.

Using a luminometric screening procedure the enzyme SEAP can be demonstrated up to a period of 42 days following vector injection into the cerebrospinal fluid.

Summarizing the results, the use of an adenoviral vector at least under experimental circumstances might be a pioneer therapeutical option for diseases of the central nervous system.