Embryonic stem cells develop into functional dopaminergic neurons after transplantation in a Parkinson rat model

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Parkinson`s disease

- Degeneration of the Substantia nigra, loss of midbrain dopamine neurons
  
  DA ↓ → ACH↑ → 5HT ↓ NA ↓

  ⇒ Detraction of motor function as well as mental, sensory and vegetative regions
Aim of the research

• Recent treatment with L-DOPA alleviates the symptoms and decelerates illness but the patient only gains another 10 years at most.

• Does development of new DA neurons via engrafting embryonic stem cells represent a sustained advancement in healing Parkinson`s disease?
Propagation and Preparation of ES cells

- Breeding mouse ES cell line D3 in presence of human leukemia inhibitory factor
- Trypsinization to separate cells
- Seeding without leukemia inhibitory factor
- Again trypsinization and trituration to receive low cell densities
- Determination of viability and concentration by a homocytometer
6-OHDA Lesion and Amphetamine Rotations

- unilateral stereotaxic injections of the neurotoxin 6-OHDA into the median forebrain bundle, coordinates were set according to the atlas of Paxinos and Watson
- Amphetamine (4mg/kg i.p.) releases DA from the undamaged nigrostriatal nerve terminals and causes the rotation towards the lesioned side
- >500 turns ⇒ >97% DA lesion ⇒ selected for grafting
• **Transplantation procedures:**
  Stereotaxic injection of 1µl (0.25µl/min) ES cells (1000-2000) into 2 sides of the right striatum.

  25 rats received cell injection,
  13 rats received medium injection (sham surgery).

  6 rats showed no graft survival, 5 rats died because of teratoma-like tumors.

• **Immunosuppression:**
  by injections of cyclosporine A.

• **Cell Counting:**
  3-dimensional Abercrombie method with a fluorescence detecting confocal laser microscope.
Detection of DA neurons

- TH-positive cells (green) coexpressed the neuronal marker NeuN (red):

- Triple labeling (white) TH/DAT/AADC:
  - TyrosineHydroxylase-positive cells:
  - TH cells coexpressing DATransporter:
  - TH cells coexpressing AromaticAcidDecarboxylase:
Detection of DA neurons in the ventral striatal region (A10)

- calretinin and calbindin are typical DA neuron markers

- *purple* cells show coexpression of all 3 DA neuron specific markers
Expression of neuronal markers in the substantia nigra

- TH positive neurons (green) coexpressing the A9 (substantia nigra) marker aldehyde dehydrogenase 2 (AHD 2, yellow coexpression)

- 5-HT neurons (green) also coexpressed aromatic acid decarboxylase (yellow)

- 5-HT neurons (green) and TH neurons (red)
Identification of mouse ES cell-derived DA neurons

- Mouse specific M6 antibodies (*yellow* coexpression with TH)

- Mouse specific inclusions after Hoechst staining (*blue*), coexpression with TH (*red*)

- Astrocytes (glial fibrillary acidic protein-positive, *green*) and neurons (NeuN, *red*), that show mouse specific intranuclear fluorescent inclusions (*blue*)
Statistical Analysis of the rotational behavior

- Significant decrease in amphetamine-induced turns of animals with ES cell neuronal grafts
- Posttransplantational number of rotations decreases in comparison to pretransplantation of ES cell engrafted animals
Functional assay

How can you measure metabolic changes in the brain caused by DA release in response to amphetamine?

→ PET (positron emission tomography); produces slices of living organisms and shows biochemical and physiological actions via detecting activated substances

→ MRI (magnetic resonance imaging); shows metabolic activities of cerebral cross sections by detecting changes of a magnetic field
• $[^{11}\text{C}]\text{CFT}$, a specific DAT ligand showed specific binding in the right stiatum (*left*).

• The increase of $[^{11}\text{C}]\text{CFT}$ binding was correlated with the presence of postmortem TH-immunoreactive neurons in the graft (*right*).
• Percentage of change in rCBV in response to amphetamine for control (upper) and an ES cell-derived graft (lower).

• The response on the grafted (red line) and the normal (contralateral) (blue line) striata was similar, no changes in sham grafted animals (green line).
Main results/Summary

- ES cells differentiate into mesencephalic dopaminergic-like phenotypes after transplantation to the adult 6-OHDA-lesioned brain and become integrated into the host circuitry.

- 6-OHDA-lesion results in a complete absence of CBV regulation and induces motor asymmetry of the animals in response to amphetamine.

⇒ Parkinson rats show behavioral recovery, reduced asymmetry and improved physiological & functional response to DA-releasing agents after ES cell grafts.
Discussion and Perspectives

• There are a lot of factors that could be responsible for the neuralizing effect such as noggin, follistatin, cerberus and chordin, but the reasons for grafted ES cells specifically becoming DA neurons in vivo are not known. Low cell density benefits the development into neurons.

• This form of Parkinson`s disease in rat is man-made. The reasons why dopaminergic neurons degenerate at the „real“ form of PD are not known. Won`t these destroying factors kill the new developed DA cells as well?