Comparative analysis of the characteristics of radial glia like neural stem cells isolated from different brain areas

PhD theses

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Introduction

All neuroectodermal cell types of the nervous system can be generated by neural stem/ progenitor cells. The stem cells of the central nervous system are radial glial cells. In the early embryonic brain long radial projections of radial glia cells overcomes the developing cortex, and express glial (BLBP, GLAST, GFAP- in primates expression) and stem cell markers (nestin, assymetric cell division). In addition to the general properties found in all radial glial cells, these cells show different gene expression patterns depending on the brain region they are located. These so-called region-specific genes also determine the type of differentiated neural cells and the phenotype of the neuron's neurotransmitter. At the end of embryonic development, some of the radial glial cells are transformed into ependyma cells, and another group converts to astroglia cell. However, a significant group of radial glial cells retains its dividing capability and the characteristics of stem/ progenitor cells and through the ependyma cell layer forms the secondary germinal layer, the subventricular zone. From the subventricular zone GABAergic interneurons of the anterior cerebral cortex, nerve cells of a striatum and most of the forebrain glial cells are derived. Today, we also know that self-renewing cells that are capable of multi-directional development - tissue stem cells - are present in highly differentiated, matured central nervous system. In two physiologically important areas of the rodent brain tissue the stem cell characteristic of radial glial cells are preserved, and new neurons are formed in the adult subventricular zone (SVZ) of the forebrain and in the subgranular zone (SGZ) of the hippocampus. The progenitor cells that migrate from the site of origin as so called "dormant cells" can also be found in the functioning brain parenchyma at various stages of nerve tissue development and maybe induced by stimulation to be neurogenic. Our knowledge raises the possibility of clinical neuronal replacement, but immediately raised the question: if there is tissue-forming cell capacity, why the possibility of regeneration in the central nervous tissue is low compared to other tissues. Are the autonomic, internal developmental possibilities of neuronal stem cells limited? Or do neural tissue stem cells have a broad developmental potential, but does the microenvironment of the central nervous tissue inhibit the potential for cell development?

During my work, I have isolated cell populations of radial glia of the central nervous tissue of the mouse to investigate their cellular biological properties and cell-forming capacity in a well-defined environment in vitro. By characterizing and influencing in vitro neurons / progenitor cells, I sought to find out how the fate of our central nervous tissue neural stem cells can be influenced by changing the environmental effects. I examined the effects of ionic

stimulation affecting all stages of cell development in the regulation of motility of the progenitor cells.

Objectives

During my PhD work I set the following goals:

• Isolation of radial glia-like neural stem cells from different areas of embryonic and adult brain; production of neural stem cell clones

• Comparison of cellular biological characteristics and gene expression patterns of cloned radial glial-type stem cells of different origin

• Comparison of the ability to differentiate of cloned radial glial-like neural stem cells

• Analysis of the effect of ionic stimulation on cell migration on radial glial-type neural stem cells expressing channelrhodopsin photosensitive ion channel

Materials and methods

Creating AK-cycl [RGDfC] -based breeding surfaces

A 10 µg/ml solution of AK-cyclo [RGDfC] was prepared immediately before use. Polystyrene or glass culturing surfaces were coated with the peptide by adding 0.25 µg of peptide to each cm^{2}. At room temperature, the culture surfaces were incubated for 30 minutes and the solution was aspirated. Under sterile conditions, the coated surfaces were dried.

Preparation of embryonic and adult primary cell suspension

Time pregnant hGFAP-GFP, wild-type CD1, CD1/EGFP, and B6;129S-Gt(ROSA)26Sor$^{tm32(CAG-COP4*H134R/EY FP)Hze}$/J mice were anesthetised by ketamin (100 µ g/g)/xylazin (10 µ g/g) injection at the 14-17.5 embryonic day, or at the 62 day of post-embryonic development. The forebrain of embryos (10-25) and the desired brain areas of adult mice were isolated from the sub-ventricular zone of the anterior lateral ventricle, the hippocampus, the parietal cortex, and the dorso-lateral part of the colliculus superior. Meninges were removed under a microscope. Tissues were triturated by using Pasteur pipette in DMEM to acquire one- cell suspension. In the case of tissue sections from adult brain besides mechanical dissociation, enzymatic dissociation was also performed using the Neural Tissue Dissociation Kit (Miltenyi Biotec) according to the manufacturer's instructions. The resulting cell suspensions were filtered

through a 45µm pore size filter. The individual cells in the filtrate were counted with a hemocytometer.

Animal testing was carried out with the permission of the local authorities (license number 22.1 / 3894/003/2009), paying particular attention to the ethical rules of animal testing as recommended by the Council of the European Community (86/609 / EEC and 2010/63 / EU).

Creating Radial Glial Cell Cultures

The cell suspensions isolated from the embryonic and adult brain were centrifuged (120g, 10 min) and resuspended in the basic RG1 medium containing 50/50% DMEM / F12 (Sigma) and 1% B27 (Gibco, Invitrogen) supplement. The cells were then seeded at 2 x 10^5 cells/cm^2 on the AK-cyclo [RGDfC] -covered surfaces. After transplanting the cells, the base RG1 medium was supplemented with 20ng / ml EGF (Preprotech).

The culture medium was replaced every 2 days for embryonic cell cultures. Before adding the new nutrient solution to the cells, the weakly adherent cells were washed with PBS. PBS was not washed on adult cell cultures and only half of the medium was replaced every 2 days during the first week.

After one week, when the embryonic cell cultures were confluent and colonies developed in adult cell cultures, the cells were digested with trypsin (0.05% trypsin, 1mM EDTA in PBS, 1 minute at room temperature) and washed from the substrate with basic RG1 medium. The resulting cell suspensions were distributed and transferred to a fresh AK-cyclo [RGDfC] - based culture dish at a density of 10^5cells/cm^2. After the first passage, the cell cultures were distributed every 2. or 3. days and transplanted into a new culture dish. After 3-4 passages, cell cultures were apparently homogeneous, showing morphology characteristic of radial glia.

Production of one cell-derived clones

After 4 passages, the cells were digested with trypsin (0.05% trypsin, 1mM EDTA in PBS, 1 min at room temperature) and excised so that the individual cells were spaced apart from each other on the AK-Cyclo [RGDfC] coated surfaces of 90mm or 60mm Petri dishes. 4-6 hours later, the adherent individual cells were isolated with a cloning ring. After the colonies developed in each cloning ring, they were considered as one cell-derived clones.

Transfection with pTurbo- Cre plasmid

Radial glial-like cells isolated from B6; 129S-Gt(ROSA)26Sor$^{tm32(CAG-COP4*H134R/EYFP)Hze}$/J transgene mice were transfected by pTurbo-Cre plasmid (chicken Cre-actin promoter controlled Cre expression) using Lipofectamin Plus reagent (Invitrogen-Thermo Fisher Scientific) according to the manufacturer's instructions. From then the transfected cells in the culture pots were protected from light by aluminum foil and a blue filter was used during microscopic examinations.

Sorting ChR2-eYFP positive and negative radial glial cells with FACS

One week after transfection, cells were sorted by FACS based on their fluorescence. A suspension of 1-1.5 $\times$ 10^6 cells was prepared by dissolving in 2ml sorting buffer (1mM EDTA and 0.4% bovine serum albumin in PBS).

We checked the integrity of the cell preparation with the FACS (BD FACSAria II, BD Biosciences) and determined the optimal parameters for separating eYFP positive and negative cells.

Differentiation of radial glial cells

To generate large amounts of nerve cells, EGF was withdrawn from confluent radial glia-like cell cultures. 5-7 days after EGF withdrawal, the resulting neurons were analyzed in live cell cultures and after immunocytochemical staining.

To differentiate into astroglia, we supplemented the medium with 5% fetal calf serum (Sigma). The presence of GFAP positive astroglial cells was detected 3 days after the addition of fetal calf serum.

Differentiation of oligodendroglia cells was induced by a 5 + 4 day protocol. Briefly, the base RG1 medium was supplemented with FGF2 (10 µg / ml; Peprotech), PDGF (10 µg / ml; Sigma) and forskolin (10 µM; Sigma) and cultured under these conditions for 5 days. The medium was then replaced with DMEM / F12 1/1, supplemented with 3,3,5-triiodothyronine (T3; 30 ng / ml; Sigma) and ascorbic acid (200 µM; Sigma). On day 9, the presence of oligodendroglia cells was confirmed by immunocytochemical staining.

RT-PCR analysis

Cell RNA was isolated using the Rneasy Mini Kit (Quiagen) according to the manufacturer's instructions. Reverse transcription was performed from 1µg RNA using the "First beach cDNA synthesis kit" (Fermentas). Hotstart Taq (Quiagene) polymerase was used for the PCR

reaction. The cDNA content of the samples was diluted to the same level on the basis of the ratio of the "home" gene hypoxanthine guanine phosphoribosyl transferase (hprt) PCR products. The polymerase chain reaction (PCR) parameters were optimized for each primer pair. The TC-512 machine was used for reverse transcription and PCR reaction. The PCR reaction products were run in 0.5% Etidium Bromide (Promega) on a 1% agarose (Promega) gel and visualized by UV scanning and then photographed with a CCD camera.

Immunocytochemical staining

Cultures were fixed in PBS with 4% PFA solution at room temperature for 20 minutes. For permeabilization of the cells, 0.1% Triton-X 100 (Promega) solution was used for 10 minutes. The non-specific antibody binding sites were blocked with 2% BSA (Sigma) for 1 hour at room temperature. The cultures were incubated overnight at 4 ° C with the first layer of antibody in blocking solution. For the second and / or biotin enhancement, the third layer antibodies were used for 1-1 hours at room temperature.

When elicited with DAB (3,3'-diaminobenzidine), the cultures were incubated for 45 min with ABC reagents (Vector) dissolved in PBS after biotinylated second layer antibody. The peroxidase reaction was initiated by incubation in 0.55 mg / ml DAB (Sigma) and 0.3% H2O2 (Sigma) in PBS at room temperature for 5 to 25 minutes. The reaction was stopped by PBS washing with 0.1% Na azide (Sigma). In the double DAB staining, the first immune response was developed in a solution containing 0.1 M Ni2 +, resulting in a black precipitate. The second immunoreaction was performed in the same manner as previously described.

The stained cultures were coated with Mowiol (Sigma) containing bisbenzimide (Hoechst 33342 [Sigma]) core staining for fluorescent staining. Paintings containing the Ni-DAB precipitate, after dewatering the section with an ascending alcohol line, were covered with Depex (Serva). Microscopic images were taken with a Zeiss Axiovert 200M microscope.

Chromosome counting

Cell division and blocking of metaphase chromosomes were performed with 0.2 μg / ml colchicine (Sigma) for 2 hours. The cells were then digested with trypsin and washed from the substrate with a basic RGl medium. The suspended cells were hypotonized with 0.56% KCl and distilled water for 10-10 minutes. Hypotonized cells were fixed on ice with methanol / acetic acid 3: 1 for 20 minutes. Fixed cells were dropped from a 50cm height defatted slide,

and metaphase chromosomes that were visible from the disintegrated cells were examined by phase contrast microscopy.

Microscopic determination of expression of ChR2-eYFP fusion protein

Using AxioVision 4.8 (Zeiss) the cells were plotted and the area of the plotted spots and fluorescence intensity were determined. The fluorescence intensity values were related to 1 μm^2 cell area for both non-induced and induced cells.

Videomicroscopy

Microscopy video recordings of E17.5 ChR2 + and ChR2- non-induced radial glial cells and induced cells were stored at 37 ° C in a mobile mini-incubator containing 5% CO2 and 95% air fixed it to the stage of the microscope. Video recordings were made using a Zeiss Axiovert 200M inverted fluorescent microscope.

An epifluorescent filter was used for light stimulation and fluorescence imaging (excitation: λ: 470 ± 40 nm, emission: λ: 525 ± 50 nm). Cells were stimulated at 300ms for every 5 minutes with fluorescent light intensity at 0.13 mW/mm^2 for 12 hours. Phase contrast and epifluorescence images were taken with a 10X lens at the end of each exposure period.

Analysis of cell movement

The video footage was opened with the WTrack program and 20 cells were selected for each start image of the movement which was tracked.

The movement of the selected cells was followed by clicking on the center of the cell, the coordinates which were recorded from the image of the WTrack program. The real displacement was determined by magnification and resolution: 1 pixel corresponds to 0.645 micrometers. Using the corrected cell coordinates we determined the distance between the two centers of the cell based on the two-dimensional euclidean distance d (pi, pi + 1) = q (xi + 1 - xi) 2 + (yi + 1 - yi) 2 ∗ 0.645. The data showed the displacement in µm / 5 min. Summing the shift from picture to image dtotal = N X −1 i = 1 q (xi + 1 - xi) 2 + (yi + 1 - yi) 2 ∗ 0.645 determined the total distance of cell movement within 12 hours for each monitored cell.

Statistical analysis

Statistical analyzes were performed by statistical programming R. The graphs are represented by the ggplot2 package. In all cases, $p <0.05$ (*), $p <0.01$ (**) and $p <0.001$ (***) were considered statistically significant. The total distance traveled by the cells within 12 hours

was classified into 3 categories. Data were plotted and statistical significance was determined by Pearson Chi-squared test in each event table, followed by paired comparisons with a post hoc Fisher test, where the p-values were adjusted using the Bonferroni method. Illumination-related electrophysiological responses and total distance data were plotted as boxplots, and significance was determined by the Wilcoxon-Mann-Whitney test. The significance of the distribution of the 5-day induced ChR2 + and ChR2 cells with or without 12-hour illumination was determined on the basis of the Kruskal-Wallis ranking test and the Dunn test.

Results

Radial glia-like stem cells from different ages and brain regions

Isolation of radial glia-like neural stem cells from embryonic forebrain

Cell cultures were prepared from the forebrain of 14-day mouse embryos (E14) carrying the hGFAP-GFP construct. In primary cell cultures, cells were organized into aggregates 2-3 days after isolation from the brain tissue. From day 4 an intensively dividing cell population began to penetrate between the aggregates on the AK-cyclo (RGDfC) -based substrate. In serum-free medium (DMEM-F12-B27), in the presence of one added growth factor (EGF), the cells were stably attached to the surface and continuously divided. By maintaining the cells stable in the absence of serum a AK-cyclo (RGDfC) -based substrates were allowed to develop an adhesion-based cell selection method and define (serum free) culturing assay of cells.

The majority of cells adhering to the surfice expressed the GFP protein, indicating that the cells attached to the AK-cyclo (RGDfC) represented an undifferentiated cell type.

Cells were also isolated from the ventral and dorsal forebrain areas of transgenic mouse strain (E14.5) expressing the wild-type CD1 mouse embryos (E14) from the forebrain and GFP cells in order to further characterize the undifferentiated cell population and to maintain the planned implantation experiments. method.

Apparently homogeneous cell populations were tested by immunohistochemistry. Most of the cells showed a common nesistin and RC2 positivity of radial glia stem cells. The composition of the cultures became morphologically completely homogeneous, with all the cells showing prolonged morphology of radial glial cells in vitro. There was no spontaneous differentiation between adherent cells.

One cell-derived clones also exhibited characteristics of radial glial cells based on their immunohistochemical and mRNA profiles.

After multiple in vitro passages, region specific gene expression only partially reflected the origin of radial glial cells. Only Ngn2 was related tot he in vivo expression, Ngn2 was only expressed in dorsal forebrain clones. The other region-specific genes (Em2, Dlx2 gene) were expressed in all clones, regardless of their origin.

Isolation of radial glia-like neural stem cells from adult brain

Primary cell cultures were also prepared from adult mouse brain (P62): subventricular zone of the lateral ventricle (SVZ), hippocampus (HC) and non-neurogenic zones forebrain cortex (CTX), midbrain (MES). Only a fraction of the grafted cells (<1%) were initially adhered to the cultured surfaces coated with AK-cyclo (RGDfC). However, these few adherent cells were able to divide within 1-2 days and created small colonies. The cells were continuously divided and formed a monolayer in 10 days in the serum-free medium (DMEM-F12-B27) in the presence of EGF on a AK-cyclo (RGDfC) -based substrate. Stably dividing, radial glia-type cultures were used to produce one cell-derived clones.

One cell-derived clone also expressed characteristics of radial glial cells based on their immunohistochemical and mRNA profiles. Of the region-specific genes (dlx2, emx2, pax6, gbx2) tested, proneural genes ngn2 and mash1 were expressed in all clones although, their expression in the adult brain was not completely overlapping. The hox2b gene indicating the rhombencephalic region was not expressed in the clones. Surprisingly, we could not detect the expression of nkx2.1 characteristic of SVZ. At the same time, the math2 gene, except of midbrain cells, was not transcribed in cells of any clone,so the majority of radial glial cells do not carry the characteristics of direct neuronal development.

Differentiation of Radial Glial Cells into Different Nervous Cell Types

Various differentiation techniques have been used to generate all three major neural tissue types in cultures of radial glial cells.

Development of astroglia cells

To the semi-confluent culture of radial glial cells, GFAP positive, flattened astroglia cells were generated over 4 days by the addition of 10% serum. Neuronal cells and oligodendroglia cells could not be identified in the cultures.

Oligodendroglia cell formation

With a 2-step protocol, O4 positive early oligodendroglia cells were differentiated from all clones. However, the amount of these oligodendroglia cells was different in each clone. In dorsal and ventral embryonic cultures, 6-12% of the cells were converted to oligodendroglia. The ventral clone generated twice as many oligodendroglia cells as the dorsal clone. Oligodendroglia formation of the clone from the cerebral cortex was the highest (23%). O4 positive cells were also high in cultures derived from adult hippocampus (16%). The amount of oligodendroglia cells produced was very low (1-2%) in adult SVZ and midbrain derived cultures.

Neuronal formation

Clones isolated from both adult and embryonic brain were able to generate neuronal cells. Five to seven days after EGF withdrawal, bipolar cells appeared at the top of a flattened layer of stem cells. Cells with nerve cell morphology showed neuron-specific βIII tubulin positivity. Their shape, extension length and branching showed significant differences depending on the origin of the radial glial cells. The cells with the most advanced nerve cells, with long branching systems, differentiated from the adult midbrain clone. Radial glia-like cells from the anterior cerebral cortex and the sub-ventricular zone developed evidently underdeveloped neuronal forms of bipolar shape during the 8 days of the study.

Relatively large amounts of nerve cells (~ 20%) were differentiated from clones isolated from all embryonic clones and from adult clones from the subventricular zone (~ 15%), hippocampus (~ 15%), and from the midbrain (~ 20%). In the cultures of the clone isolated from the adult cerebral cortex, significantly less neurons (~ 5%) were produced by the same induction method.

Neurotransmitter phenotype of nerve cells formed in vitro from radial glial cells of different origin

RT-PCR studies have shown that the gene coding the vesicular GABA transporter (vGAT), which is characteristic of GABA release, is expressed in all radial glial cells, regardless of their origin. The expression of other neurotransmission genes was significantly dependent on the origin of the radial glial cell and the age of the source brain tissue. In the clone of radial glia from embryonic brain express the VGAT gene, the vglut1and vglut2 coding the glutamate vesicular transporter and the gene coding the tyrosine hydroxylase (TH) of

catecholamine metabolism. Expression of the corresponding proteins in embryonic RG1 neurons was also detected in neurons from radial glial cells of adult origin. Type 1 glutamate transporter (vGLUT1) was expressed only in the hippocampal clone. Tyrosine hydroxylase (TH) is expressed in clones isolated from the adult subventricular zone. The presence of TH protein was confirmed by immunocytochemistry. The expression of noradrenergic, serotonergic and cholinergic nerve genes was not observed in clones.

Influence of ion-induced stimuli on migration of radial glial cells and their progenies

Isolation of radial glial-type cells expressing Channelrhodopsin from embryonic mice

Primary cell cultures were prepared from the forebrain of mouse embryos (E17.5) containing the LoxpStoploxpChR2 (H134) -EYFP construct. The ChR2 (H134) -EYFP fusion protein is driven by the CAG promoter, so it is stably expressed in all cells of the body. Similarly to cell cultures from the previously described embryonic brain, the cells initially clustered into aggregates on the AC-cyclo (RGDfC) -based substrate and then overgrown the substrate. Homogeneous and confluent cultures after a few passages the expression of the chanelrhodopsin channel was induced by pTurbo- Cre plasmid. After 1 week, approximately 5% of the cells showed fluorescence of eYFP. Cells expressing eYFP, thus containing channelrhodopsin, as well as cells that did not express eYFP, were separated by FACS. Neither morphological nor immunocytochemical differences were found between cultures containing ChR2 + and ChR2 cells. Radial glia markers were also detected by the immunocytochemical method in ChR2 + and ChR2 cells.

Nuclear cell formation of Channelrhodopsin2 + radial glial cells

If EGF was withdrawn from the cultures in confluent state, on the 5th day, the βIII tubulin-positive neurons appeared in the eYFP positive (ChR2 +) and eYFP negative (ChR2-) cells. Due to the interpretation of further studies, we also had to examine whether the degree of ChR expression changes in neurons differentiating cells. At the same magnification, we made flursescent images of ChR2 + induced and non- differentiated radial glial-type cultures. Axiovision program measures the area of each cell and the fluorescence intensity of the cell area. The mean and standard deviation of the specific fluorescence intensity of 20-20 non-induced radial glial cells or radial glial origin were determined. According to the data, the specific fluorescence intensity does not change significantly during neuronal development.

Response of cells expressing Channelrhodopsin to light stimulation

Patch clamp measurements confirmed that blue light (λ: 488 nm) induces cation currents in chanelrhodopsin-expressing cells. No cell-activating cation influx appeared in cells expressing chanelrhodopsin. According to the data, the amplitude of the cation influx increased with the increase of the luminous intensity, no biologically relevant increase was detected above 0.5 mW/mm^2.

The effect of induced cation flow on the movement of radial glial cells

Repeated illumination of different intensities of 300 ms for different periods of 5 minutes was found to cause ChR2 + cells to be damaged after 400 minutes and die within 12 hours if the intensity of the illumination light is greater than 0.25mW/mm^2. Based on light sensitivity data, light pulses of 0.13 mW/mm^2 each for a duration of 300 ms were repeated every 5 minutes for 12 hours for motility studies. After each pulse, phase contrast was recorded from the cells. Along with the same illumination sequences, we investigated the displacement of ChR2 + and ChR- cells in the induced and non-induced radial glial state and on days 1 and 5 of induction of neuronal differentiation.

Analysis of cell movement data

In the visible light series, the shift of the center of each cell was monitored using the WTrack software. We determined the x, y coordinates of the cell centers in each image, corrected with similar coordinates of an apparently immobile reference point. Following the temporal changes in the location coordinates of each cell, we plotted the pathway and trajectory of the cell movement. From the trajectory curves it could be read from the eye that the cell-crossed path significantly decreases during the period of nerve cell conversion. This reduction of motion on day 5 of nerve cell induction was more pronounced in ChR2 + cells than in ChR2 cells.

The observed cell movements were further analyzed in distribution studies. Knowing the displacement of the center of each cell within 12 hours, we determined the "high migration rate" of "non-migrating", with a distance of less than 200 μm and with a distance greater than 400 μm. cellular activity in different cell populations at different stages of in vitro cell development. Migration analysis has shown that there are practically no "stationary" cells in non-induced radial glial cell cultures, non-induced radial glial-like cells are migratory cells, regardless of the presence of induced cationic currents. On Day 1 of neuronal induction the migration activity of ChR2 + cells increased compared to the activity of non-induced cells, while the migration activity of ChR2- cells decreased. On day 5 of induction, the motility of

Chr2 + and Chr2 cells was also significantly reduced compared to the non-induced cell migration activity. ChR2 + cells showed significantly less migration activity on day 5 of neuronal differentiation than ChR2 cells. The number of cells that migrated at a distance greater than 400μm in 12 hours was significantly reduced on day 5 of differentiation into nerve cells. The length of the total cell displacement was then determined and it was also seen that the average pathway length decreased with the progression to nerve cell differentiation in both the channelrhodopsin-expressing and the channelrhodopsin-non expressing cultures. On the 5th day following induction, however, the migration pathway of chanelrhodopin-expressing cells was also significantly reduced compared to chanelrhodopin-expressing (control) cells. To exclude the effect of the ChR2-eYFP construct inserted into the genome on motility, a series of experiments were performed in which the movements of ChR2-expressing (ChR2 +) cells were analyzed without targeted illumination. ChR2 +, 5-day induced cell migration was reduced without direct exposure to ChR2 cells. However, exposure to blue light increased significantly the decrease in activity.

Findings

In our laboratory, using a synthetic peptide conjugate (AK-cyclo (RGDfC)), we have developed a reliable isolation method and defined serum-free culture conditions for cloning of progenitor cells. We have created an in vitro system in which radial glial-like cells stably express the channelrhodopsin-sensitive ion channel so that the effect of ion-shifting by opening the channel can be studied in cell culture under defined conditions.

Based on the results of my PhD thesis, the following statements can be made:

• There is a method of isolating neural stem cells from embryonic and adult brain with high efficiency.

• In vitro cultured cells isolated from embryonic and adult brain can be maintained by appropriate culture methods; their cloned populations can be grown in a long-term, stable manner under defined conditions.

• Various region-specific genes that are not expressed in vivo are expressed in radial glia-like stem cells from brain regions of different ages, regardless of their origin.

• The in vivo regional characteristics of doral embryonic origin of the pronural ngn2 gene remain in vitro

• The ability of radial glial cells to differentiate depends on the brain area of the cells and the age of the brain tissue.

• The migration activity of radial glial-type cells stably expressing Channelrhodopsin2 (H134) photosensitive ion channel is not influenced by the opening of the cation channel in the stem cell state;

• Ion stimulation influences cellular properties in a state of development; in neuronal precursors, it is highly probable that the phenotype of the more mature nerve cell appears.

Publication List:

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