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Abstracts

Talks

Regulation and function of neurogenesis in the adult Hippocampus

Fred H. Gage
Salk Institute for Biological Studies, La Jolla, USA

Most neurons in the adult central nervous system (CNS) are terminally differentiated and are not replaced when they die. Evidence now exists that small populations of neurons are formed in the adult olfactory bulb and hippocampus. In the adult hippocampus, newly born neurons originate from putative stem cells that exist in the subgranular zone of the dentate gyrus. Progeny of these putative stem cells differentiate into neurons in the granular layer within a month of the cells' birth, and this late neurogenesis continues throughout the adult life of all mammals. Stems cells can be harvested from a variety of brain and spinal cord regions genetically modified and transplanted back to the brain and spinal cord where they can differentiate into mature glia and neurons depending on the local environment. In addition, environmental stimulation can differentially effect the proliferation, migration and differentiation of these cells in vivo. These environmentally induced changes in the structural organization of the hippocampus, result in changes in electrophysiological responses in the hippocampus as well as in hippocampal relate behaviors. We are studying the cellular; molecular as well as environmental influences that regulate neurogenesis in the adult brain and spinal cord. These adult stem cells from the brain can be genetically modified and transplanted to the adult intact and damaged brain, thus can be a delivery system therapeutic genes. In addition these cells can be targeted with retroviruses that to express factors that may be important in the repair process following injury. The potential of these cells, in vitro and in vivo will be discussed

Generation of dopamine neurons from embryonic stem cells for transplantation in Parkinson´s disease

Anders Björklund
Wallenberg Neuroscience Center, Lund University

Clinical trials have shown that transplanted mesencephalic dopamine (DA) neurons can survive and restore lost function in patients with advanced Parkinson´s disease (PD). In open-label trials long-lasting symptomatic improvement has been reported in a majority of the grafted patients, and some have shown substantial, therapeutic benefit that have lasted for more than 10 years. The ethical and practical problems associated with the use of fetal tissue is today the most serious obstacle to further developments of this approach. Further progress towards a cell replacement therapy in PD, therefore, is critically dependent on the development of new procedures that will allow generation of DA neurons in large numbers from stem cells. Andersson et al (2006) have recently identified two transcription factors, Lmx1a and Msx1, that seem to function as a key intrinsic determinant in the development of midbrain DA neurons. Expression of Lmx1a or Msx1 in mouse embryonic stem (ES) cells is sufficient to drive the ES cell-derived neurons with very high efficiency into a fully differentiated midbrain dopaminergic phenotype. *In vivo*, the Lmx1a- and Msx1-transduced ES cells develop into fully mature DA neurons, of both the A9 and A10 subtypes, and grow to form an extensive axonal terminal network throughout the host striatum. The results obtained so far indicate that genuine and fully functional midbrain DA neurons can be generated in unlimited numbers from mouse embryonic stem cells using this approach. With the differentiation protocol used so far, however, many of the transplants show some degree of tumor formation, suggesting that the differentiated ES cell cultures may contain cells with tumorgenic properties. This problem should be possible to avoid by using either cell sorting to eliminate the proliferative cell component, or by generating stable non-tumorgenic neural stem cell cultures from the Lmx1a and/or Msx1 transduced ES cells, i.e., culture protocols that will allow the residual ES cells to be eliminated from the neural progenitor cell preparation.

Neuronal Birth and Death in the Substantia Nigra: Can you tell the difference?

Günter U. Höglinger
Experimental Neurology, Philipps University, Marburg, Germany.

The recent years have provided great advances in our knowledge on neurogenesis occurring in the adult mammalian brain. Neural stem or precursor cells, respectively, with the capacity to give rise to new neurons, astrocytes and oligodendrocytes have been shown to be present in the subependyma of the lateral ventricles and the dentate gyrus of the hippocampus the brain throughout adulthood. Interestingly, presence of precursor cells with similar properties has also been demonstrated in the parenchyma of many other brain regions, including retina, cortex, corpus callosum, striatum, midbrain and spinal cord. In culture, it has been shown that these cells exhibit alt least limited self-renewal capacities and generate cells of the three neural lineages. In vivo, however, constitutive generation of new neurons under physiological conditions has up to now only been convincingly demonstrated to occur in the olfactory bulb with cells arising from the subventricular zone (SVZ) of the lateral ventricles and in the dentate gyrus of the hippocampus. Despite the presence of cells with neurogenic potential, all other regions of the adult mammalian brain appear to exhibit strong inhibitory cues to prevent neurogenesis from occurring under physiological conditions. The hope to develop novel cell replacement strategies for neurodegenerative disorders has driven exhaustive investigations to study, if the diseased brain might release the potential of endogenous stem or precursor cells to replace the lost neurons by newborn neurons in an anatomically and functionally appropriate way. While some of these studies provided encouraging results suggesting lesion-induced neurogenesis to occur under certain conditions, recent studies raised the awareness that degenerating neurons might activate the mitotic cell cycle prior to their demise and might therefore be mistaken as newborn neurons. In the light of these novel findings, I will critically review the presently available evidence for the existence of lesion-induced neurogenesis, with a particular focus on the dopaminergic neurons of the substantia nigra.

Functional characterization of genes underlying Morbus Parkinson in mouse models

Wolfgang Wurst
Institute of Developmental Genetics, Helmholtz Zentrum München-National Research Centre for Environment and Health, Munich, Max Planck Institute of Psychiatry, Munich, Technical University of Munich, Munich, Germany

Parkinson´disease (PD) is a neurodegenerative movement disorder characterized by the loss of dopaminergic neurons in the substantia nigra. In recent years several genes have been associated with the etiology of this disease amongst which are Pink-1, LRRK-2 and DJ-1. The precise and detailed functions of these genes in PD is, however, still unkown. Therefore analysis of the phenotype of animal models carrying mutations in these genes is of uttermost importance for understanding their role in the etiology and course of the disease. In order to do so we started out to generate different genetic animal models for PD. Here we present our first data on the analysis of DJ-1 knock-out mice.
Loss-of-function mutations in the DJ-1 (PARK7) gene are linked to familial early-onset parkinsonism. The DJ-1 mRNA is ubiquitously expressed during mouse embryonic development. In the adult brain it can be found at high levels in the cortex, hippocampus, amygdala, substantia nigra, locus coeruleus, and the nuclei of the cranial nerves. Furthermore in other tissues such as kidney, pituitary, gonads, and muscle DJ-1 is also strongly expressed. Within the framework of a large insertional mutagenesis screen we obtained an ES cell clone (BayGenomics) harbouring a gene trap vector pGT1xlf integration into the DJ-1 gene. The insertion of the genetrap vector led to a fusion transcript and a truncated protein lacking the carboxy-terminal domain required for dimerization and stability of the protein. The DJ-1 deficient mice are viable, fertile, and show no gross anatomical or neuronal abnormalities. DAT and TH immunoreactivity revealed that the numbers of dopaminergic neurons in the ventral midbrain of mutant mice are reduced about 10%-20%, whereas noradrenergic neurons in the locus coeruleus were normal. Other markers of dopaminergic neurons such as Nurr1, Lmx1b, and other neurotransmitter systems such as GABAergic and serotonergic ones were unaffected in the DJ-1 mice. Studies of the mitochondrial electron transport chain in different tissues of the DJ-1 deficient mice showed that the activities of all mitochondrial complexes were slightly but significantly increased. This indicates that the missing protective function of DJ-1 against oxidative stress in these mice may be compensated by a tighter coupling of electron transport. Behaviourally the DJ-1 deficient mice showed only a mild reduction of spontaneous locomotor activity, but had a clear impairment in social memory and cognitive functions, which may represent early indicators of PD.

Reduced neurogenesis in models of Parkinson disease

Jürgen Winkler
Department of Neurology, University of Regensburg, Regensburg, Germany

Neurogenesis persists in the adult mammalian brain in the subventricular zone (SVZ)/ olfactory bulb system and in the hippocampus. The neurotransmitter dopamine is intensively involved in this process and a decreased proliferation of neural precursor cells was described in the SVZ of Parkinson disease (PD) patients as well as in PD animal models. We concentrated on determining (1) the effect of dopaminergic stimulation and (2) the effect of protein aggregation on adult neurogenesis.
To stimulate the dopaminergic system, we chose a widely used dopamine receptor 2/3 ($D_{2/3}$) – agonist (pramipexole, PPX). Our study points towards a potent effect of PPX to increase SVZ proliferation and olfactory bulb neurogenesis in 6-OHDA lesioned rats. One of the most exciting ideas for repair is that one might be able to harness the adult brain's endogenous capacity for cell renewal. Inducing an increase in the number of proliferating cells, it might be possible (1) to direct newborn cells of the adult brain to regions affected by the disease and (2) to locally differentiate into the specific neuronal phenotype that succumb due to the disease. In this respect, we showed that the combined application of the growth factors EGF and FGF-2 increases dopaminergic neurogenesis in the olfactory bulb, promote migration of newly-generated DCX-positive neuroblasts from the SVZ into the adjacent lesioned striatum, however fail there to obtain a dopaminergic phenotype. Both studies indicate that exogenous applied compounds (even orally applied) are capable to induce cellular repair mechanisms.
Our second line of research was examining the hypothesis that neurogenic regions are more susceptible to protein aggregation disorders than other brain regions. We determined neurogenesis in several transgenic mouse models for synucleinopathies: (1) In mice that expresses high levels of wild-type human α-synuclein in the olfactory bulb and the dentate gyrus neuronal integration of adult neural progenitor cells is impaired in the SVZ/ olfactory bulb system as well as in the hippocampus indicating that expression of human α-synuclein impairs adult neurogenesis. (2) A53T α-synuclein transgenic mice showed a more pronounced decrease in olfactory neurogenesis. Here, both a decrease in cell proliferation and an increase in apoptotic profiles within the SVZ were observed. (3) In addition, to determine mechanisms involved in the effects of α-synuclein expression on neurogenesis we studied a mouse ES based model. The reduced neurogenesis in α-synuclein-expressing mES cells was reverted by over-expressing constitutively active NICD. Similarly, in α-synuclein transgenic mice, the alterations in neurogenesis in the hippocampal subgranular zone were accompanied by decreased Notch-1, NICD and Hes-5 expression. Taken together, these results suggest that accumulation of α-synuclein may impair neurogenesis by interfering with the Notch signaling pathway. (4) In a recent study using a conditional tet-regulatable model of PD expressing human α-synuclein under the control of the CAMKIIα promoter we demonstrated, that the α-synuclein mediated impaired neurogenesis is (a) independent of the promoter used, (b) that blocking synuclein expression restores adult hippocampal and olfactory neurogenesis, and more importantly (c) may offer new therapeutic targets to interfere with early non-motor symptoms of PD associated with a reduced hippocampal neurogenesis (i.e. affective and cognitive deficits in PD). These findings suggest that different pathways associated with α-synuclein aggregation processes interfere with the generation and integration of newly generated neurons in neurogenic regions. Consecutively, we hypothesize that impaired neurogenesis in the olfactory bulb and the hippocampus may be the underlying structural substrate for pre-motor symptoms early in the course of PD such as olfactory deficits, depression and cognitive deficits.

Transcription factors controlling maturation in adult hippocampal neurogenesis

Chichung D. Lie
Institute of Developmental Genetics, Research Group Adult Neurogenesis, Helmholtz Zentrum München- German Research Center for Environmental Health, Neuherberg, Germany

The hippocampal dentate gyrus is one out of two regions of the mammalian brain where neural stem cells continuously generate new functional neurons throughout adulthood. Newborn neurons are integrated into the hippocampal circuit in an extensive morphological and electrophysiological maturation process. Recent work has shown that this process is at least in part controlled by neuronal activity. The molecular mechanism, through which these signals regulate maturation is largely unknown. We hypothesize, that neuronal activity results in the activation of specific transcriptional programs, which in turn control the maturation process. In order to characterize such transcriptional programs we have searched for transcription factors, which show specific activity in immature newborn neurons of the adult dentate gyrus. We have found that the phosphorylated (i.e., active) form of the transcription factor CREB is strongly present in immature neurons of the adult dentate gyrus. In order to manipulate CREB-signaling specifically in newborn neurons, we injected retroviruses encoding for a dominant-negative CREB mutant. Inhibition of the CREB-pathway resulted in the loss of neuronal marker expression, decreased neurite outgrowth, and decreasing numbers of newborn neurons, indicating that CREB-activity is necessary for the differentiation, morphological maturation, and survival of new neurons.
We also found that the transcription factor Sox11 is almost exclusively expressed in the adult neurogenic areas. Detailed analysis of the Sox11 expression pattern in the dentate gyrus revealed that Sox11 is transiently expressed in newborn hippocampal neurons. Interestingly, Sox11 expression overlaps completely with the expression of doublecortin, a microtubule associated protein involved in the control of neurite outgrowth. Reporter assays indicate that Sox11 controls the expression of doublecortin. Moreover, overexpression of Sox11 in neural stem cells in vitro is sufficient to induce the expression of doublecortin and b-tubulin III.
Taken together our findings indicate that CREB and Sox11 are part of a transcriptional program regulating the maturation of new hippocampal neurons. Future experiments will investigate if and how neuronal activity regulates CREB-signaling and Sox11-expression in hippocampal neurogenesis and will determine the transcriptional targets of CREB and Sox11 in the maturation of new hippocampal neurons.

Neurogenesis from glia cells – new views on reactive gliosis and neural repair in the adult mammalian brain

Magdalena Götz
Institute of Stem Cell Research, Helmholtz Zentrum München- German Research Center for Environmental Health & Physiological Genomics, Institute of Physiology, Munich University, Germany

Despite neurogenesis from radial glial cells during development and in the adult dentate gyrus and from astrocytes in the adult subependymal zones of the lateral ventricle, the majority of glial cells fail to generate neurons in the adult mouse brain. To overcome this obstacle we have compared the transcriptomes of neurogenic versus non-neurogenic glial cells during development and adulthood. This analysis suggested active BMP-signalling selectively in adult neurogenic, but not other parenchymal astrocytes. Indeed, functional analysis by tamoxifen-inducible deletion of the central signalling mediator Smad4 in adult neural stem cells or infusion of the extracellular BMP-antagonist Noggin revealed a key role of BMP-signalling in neurogenesis from astrocyte-like stem cells in the adult neurogenic niche. BMP-signalling acts by suppressing the alternative pathway of oligodendrogliogenesis via downregulation of the transcription factor Olig2. Notably, Olig2-expressing progenitors dominate outside the neurogenic niches, and I will present novel data about their progeny using fate-mapping by inducible tamoxifen-mediated recombination in the adult mouse brain in vivo. Moreover, Olig2 is up-regulated upon injury and contributes to inhibiting neurogenesis. Deletion of Olig2 and forced expression of neurogenic fate determinants allows eliciting neurogenesis in about half of the transduced glial cells in vivo in the injury site. Moreover, we have recently identified a subpopulation of astrocytes that reactivates stem cell properties upon brain injury, thereby providing an ideal target to elicit endogenous neurogenesis local to the injury site. Taken together, our work on the mechanisms regulating neurogenesis from glial cells has demonstrated that local sources of glial cells can be reverted towards neurogenesis and may serve to repair neurons after injury.

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Functional maturation of the first synapse in olfaction: development and adult neurogenesis

Pierre M. Lledo
Laboratory for Perception and Memory, Pasteur Institute, CNRS Paris, France

Neurogenesis occurs in the adult central nervous systems of a variety of vertebrate and non-vertebrate organisms. The roles that the new neurons play, and the factors that influence their birth and survival, are critical issues that are under intense investigation. Significant progress has been made in understanding the functions of new neurons in the olfactory system, a pathway where neurons are added throughout life in a wide range of species. With the adult mouse olfactory bulb as a model, this presentation will address a series of fundamental questions concerning the role(s) that neurogenesis plays in the normal functioning of adult neuronal circuits: Why does neurogenesis persist in some part of the adult brain but not in other ones? Is it a mere recapitulation of embryogenesis or rather a unique process adapted to the adult forebrain? Why is it restricted, apparently, to only few specific regions in normal conditions? How do these regions balance the need for plasticity with the need to maintain already–functional information processing networks? Is neurogenesis in the adult brain a constant, restorative process, or is it flexible, producing different numbers of neurons to certain regions according to an animal's environmental experience? And are new neurons in the adult brain born to perform a particular task not possible for mature neurons, or are they generated as flexible units to undertake whichever role their target structure is in need of most? Together, the confluence of data from different model systems suggests a complex dialogue between the environment and processes controlling neuronal birth and differentiation. This presentation will touch on these various topics that are of broad interest to neuroscientists not only studying developmental processes, and mechanisms of cognitive functions but also to those seeking for novel strategies aimed at using endogenous neuronal stem cells for brain repair.

Embryonic, adult, and cancer stem cells *in vitro* and *in vivo*

Dennis A. Steindler
The McKnight Brain Institute of the University of Florida, USA

Simultaneous in vitro and in vivo comparisons of the nature and behaviors of embryonic, adult, and cancer stem-like cells provide insights into the roles for these potent cells during normal and abnormal tissue generation. Embryonic stem cell-derived neural precursor cells (ESNPs), adult stem and progenitor cells (e.g. multipotent astrocytic stem cells, or MASCs and adult human neural progenitor cells, or AHNPs), and glioma cancer stem cells are studied under a variety of culture conditions that mimic particular cell-cell and cell-substrate interactions that occur in vivo; growth conditions have been shown to dramatically affect the proliferation and fate choice of these different stem/progenitor cells. ESNPs were found to give rise to different neuronal fates when exposed to different extracellular matrix substrates such as laminin and fibronectin. A novel in vitro system has also shown the ability to induce neurogenesis from postnatal and adult MASCs that mirrors subventricular zone (SVZ) neuropoiesis as it occurs in vivo. AHNPs from the cerebral cortex and hippocampus also exhibit multipotency in vitro and in vivo (following transplantation into immunocompromised mice) when grown in a similar culture system, and live cell imaging, immunophenotypic analysis, and electrophysiological studies all reveal MASCs and AHNPs that exhibit astrotypic characteristics, but can give rise to large numbers of neuroblasts. These cells have many attributes in common with neurogenic radial and astrotypic cells found within distinct neuroproliferative and boundary regions throughout the neuraxis during CNS development. Following the completion of CNS pattern formation and development, when these cells lose their neurogenic abilities (except for the SVZ and hippocampus where they retain stem/progenitor cell capabilities), injuries and disease induce what appear to be fully differentiated astrocytes to assume a neurogenic role whereby they upregulate their expressions of developmentally-regulated proteins, e.g. extracellular matrix molecules, and attempt to recapitulate neurogenic programs. In vitro growth conditions can be created that restore neurogenic programs of these cells, and novel in vitro systems, again relying on cell-substrate interactions, are used to study the proliferation and fate choice behaviors of glioma-initiating, stem-like cells that reveal cell cycle and differentiation behaviors common to both normal and transformed stem/progenitor cells. All of these studies together suggest that there are many similarities in the dynamic behaviors of both normal and cancerous stem/progenitor cells which contribute to cell lineage diversity during normal and abnormal tissue genesis, and there are common roles for stem cell-like astrocytes during brain development, regeneration, and tumorigenesis.

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Crosstalk between the immune system and neural progenitor cells

Tony Wyss-Coray
Stanford University School of Medicine, Stanford, USA

Immune and injury responses have been implicated recently in the regulation of adult neurogenesis in rodents. The complement system serves a central role in initiating inflammatory processes and linking innate and adaptive immune responses. To determine whether complement may be involved in regenerative processes in the brain we studied the role of complement receptors in neurogenesis. We discovered that complement receptor 2 (CR2), which is a component of the B cell receptor complex and present on astrocytes in the brain, is also expressed by neural progenitor cells (NPCs). Mice lacking CR2 (*Cr2*$^{-/-}$) exhibited increased neurogenesis and had five times as many doublecortin-positive neuronal precursor cells in the dentate gyrus at 16 month of age. Stereotaxic injection of complement C3d, one of the ligands of CR2, into the dentate gyrus inhibited neurogenesis in wildtype but not *Cr2*$^{-/-}$ mice. C3d also inhibited proliferation of primary mouse NPCs derived from wildtype mice and induced phosphorylation of AKT; C3d did not exert these effects *Cr2*$^{-/-}$ NPCs. Our results are consistent with a model where endogenous CR2 signaling inhibits baseline neurogenesis in the hippocampus. C3d produced at inflammatory sites in the brain may inhibit neurogenesis through the activation of CR2 signaling in NPCs.

Regulation of adult neurogenesis: involvement of TGF-beta

Ludwig Aigner
Department of Neurology, University of Regensburg, Regensburg, Germany

Transforming growth factor (TGF)-beta has multiple physiological functions in the adult central nervous system (CNS). It modulates inflammatory responses and controls proliferation of microglia and astrocytes. In the diseased brain, TGF-beta1 expression is up-regulated and, depending on the cellular context, its activity can be beneficial or detrimental regarding regeneration. TGF-beta1 might be involved in the control of neural stem and progenitor cell proliferation during adult neurogenesis, in particular during neurodegeneration. For example, we demonstrated that TGF-beta1 reduces neural stem and progenitor proliferation *in vitro* and *in vivo* after intracerebroventricular infusion. Moreover, in animal models of Huntington's Disease, where neural progenitor proliferation is reduced, TGF-beta signaling is elevated as demonstrated by increased phospho-Smad2 levels. Other members of the TGF family of proteins such as bone morphogenetic proteins (BMPs) induce astroglial differentiation. Central to the TGF-beta and to the BMP signaling are Smad molecules with Smad7 being a common intracellular negative feedback mechanism that inhibits TGF-beta and BMP signaling. To investigate the role of Smad7 in adult neurogenesis, we analyzed neural stem and progenitor cell proliferation and differentiation in Smad7 -/- mice. Unexpectedly, these mice showed enhanced proliferation of adult neural stem/progenitor cells in vivo as well as long-term maintenance of BrdU-labeled cells. In vitro we observed both a higher sphere-forming capacity in mutant cells as well as faster growth and cell cycle progression. Inhibitor studies revealed that these effects are independent of TGF-beta and BMP signaling. The enhanced proliferation might be at least partially mediated by elevated EGF-signaling, as mutant cells showed higher expression and activation levels of the EGF-receptor; and an EGF-receptor inhibitor reduced the proliferation of these cells in a dose-dependent manner. Our data indicate that endogenous Smad7 plays a TGF-beta independent role in regulating neural stem/progenitor cell proliferation.

Multimodal imaging of receptor function and pathway activity in the rodent brain

Markus Rudin
Institute for Biomedical Engineering UZH/ETH Zürich and Institute for Pharmacology and Toxicology, Zürich, Switzerland

Non-invasive imaging techniques have become central for the study of central nervous system (CNS), its pathologies and for monitoring therapeutic interventions. Today a number of specific imaging approaches are available and a further developed at a rapid pace allowing the annotation of CNS structures with functional and molecular information. Functional imaging probes metabolic and hemodynamics consequences associated with neuronal activity, while molecular and cellular imaging applications comprise the visualization and quantification of the receptor distribution, occupancy, and function, or of monitoring the infiltration and migration of labeled cells into the CNS. Focus of the presentation will be the assessment of receptor function using fMRI readouts and the development of molecular imaging tools that target specific signal transduction pathways.

fMRI studies involving pharmacological stimulation/inhibition of CNS receptors assess functional consequences of a ligand-receptor (or drug-receptor) interaction fMRI, thus providing an indirect readout on molecular processes via physiological coupling. The approach complements classical receptor binding studies. These functional readouts offer significant opportunities with regard to the characterization of CNS disorders such as neurodegenerative or psychiatric diseases and might also provide attractive biomarkers for evaluating the efficacy of therapeutic interventions.

Targeted molecular imaging techniques, on the other hand, yield immediate insight in molecular and cellular events in the living organism. Besides direct receptor imaging using a labeled reporter ligand, visualization of the activation of signal transduction pathways constitutes an attractive application. As an application, a reporter gene assay propping the hypoxia-inducible factor pathway will be discussed.

Stem cells, precursors and myelin repair

Robin J.M. Franklin
Cambridge Centre for Brain Repair, University of Cambridge, Cambridge CB3 OES, UK

Remyelination, the process by which new myelin sheaths are restored to demyelinated axons, represents one of the most compelling examples of adult multipotent stem/progenitor cells contributing to regeneration of the injured CNS. This process can occur with remarkable efficiency in both clinical disease, such as multiple sclerosis, and in experimental models, revealing an impressive ability of the adult CNS to repair itself. However, the inconsistency of remyelination in multiple sclerosis, and the loss of axonal integrity that results from its failure, makes enhancement of remyelination an important therapeutic objective. Identifying potential targets will depend on a detailed understanding of the cellular and molecular mechanisms of remyelination. This talk will review 1) the nature of the cell or cells that respond to demyelination and generate new oligodendrocytes, identifying current areas of uncertainty and addressing the role of adult CNS stem and progenitor cells, 2) intrinsic factors regulating precursor differentiation and 4) how an environment favourable to remyelination is generated, and will introduce the concept of a matrix of signalling events critical for the successful completion of remyelination.

Cell and biomaterial-based regenerative strategies for spinal cord repair

Norbert Weidner
Department of Neurology, University of Regensburg, Regensburg, Germany

Tissue loss, axon transection and demyelination represent pathogenetic key features of spinal cord injury. The main rationale for cell-based therapies following spinal cord injury is a) the replacement of degenerated spinal cord parenchyma by an axon growth promoting scaffold and b) the remyelination of de-/dysmyelinated axons. A potential source to meet these requirements are adult neural progenitor cells (NPC). Following transplantation of NPC cell suspensions into the acutely injured rat spinal cord NPC survived, integrated along axons and differentiated into astro- and oligodendroglial lineages. After co-transplantation with primary fibroblasts, NPC were retained within the lesion site. The lesion cavity was completely replaced. Furthermore, axon regeneration into NPC/fibroblasts co-grafts was significantly augmented, most likely mediated through cell-contact associated mechanisms.

Endogenous oligodendrogliogenesis is not sufficient to promote appropriate remyelination. In vitro, oligodendroglial differentiation can be strongly enhanced by co-cultivating mesenchymal stem cells (MSC) with NPC. Therefore, we were interested to see whether MSC co-grafted with NPC will significantly enhance oligodendroglial differentiation of NPC in vivo. First results suggested a shift of NPC differentiation towards an oligodendroglial phenotype. Ongoing studies investigate whether the promotion of oligodendroglial differentiation enhances remyelination within and outside of MSC/NPC co-grafts.

Eliciting axon regrowth and remyelination alone is not sufficient to induce functional recovery after spinal cord injury. Appropriate target reinnervation and functional recovery depend on longitudinally directed regrowth of transected axons. Therefore, alginate-based highly anisotropic capillary hydrogels were introduced into an axon outgrowth assay in vitro and adult rat spinal cord lesions in vivo. In vitro alginate-based scaffolds elicited highly oriented linear axon regrowth and appropriate target neuron reinnervation. After implantation into acute cervical spinal cord lesions in adult rats, alginate-based anisotropic capillary hydrogels induced directed axon regeneration across the artificial scaffold, thus, representing a promising strategy to induce directed nerve regrowth following spinal cord injury.

Poster

Isolation of multipotent astroglia from the injured brain and the adult stem cell niche

Pratibha Tripathi, Dilek Colak and Magdalena Gotz

Institute of Stem Cell Research, Helmholtz Zentrum München- National Research Institute for Environment and Health, Neuherberg/Munich, Germany

Adult neural stem cells, as the source of life-long neurogenesis, reside in the subependymal zone (SEZ) lining the lateral wall of the lateral ventricles and in the dentate gyrus of the hippocampus. In both neurogenic regions, a subset of glial fibrillary acidic protein (GFAP) expressing astrocytes functions as stem cells, but it is not known how to distinguish these stem cell astrocytes from other astrocyte populations. We have identified a subpopulation of adult SEZ astrocytes that expresses CD133 and the PDGFα receptor. Using fluorescence-activated cell sorting to isolate this distinct population of astrocytes from hGFAP/eGFP mice, we show that the SEZ astrocyte population that expresses CD133/PDGFα receptor is able to form self-renewing neurospheres. Strikingly, we found a similar population of dedifferentiated astrocytes in non-neurogenic brain regions following stab wound injury in the cerebral cortex. Moreover, these astrocytes isolated from the injured cortex, also reveal the stem cell properties of self-renewal and multipotency. Transcriptional profiling of these multipotent astrocytes will be presented. Taken together, our work allows, for the first time, the identification and characterization of the astrocyte subtypes that act as neural stem cells.

Characterization of Adult Human Hippocampal Stem/Precursor cells

FA Siebzehnrubl[1,2], E Pauli[3], R Coras[1], D Mueller[1], B Rings[1], B Berninger[4], and I Blumcke[1]

[1] Dept Neuropathology, University Hospital Erlangen, Germany
[2] Dept Neuroscience, McKnight Brain Institute, University of Florida, Gainesville, FL, USA
[3] Dept Neurology, University Hospital Erlangen, Germany
[4] Dept Physiology, LMU Munich, Germany

Adult neural stem cells offer the fascinating perspective of restoring selective neuronal cell loss in neurodegenerative diseases, such as Parkinson's. Generation of sufficient amounts of transplantable neuronal precursor cells or differentiated progenies in vitro will be a key step towards such targeted reconstructive therapy option. Here, we systematically investigated the ability to isolate, propagate and differentiate stem/progenitor cells of the adult human hippocampus obtained from epilepsy surgery. As an important prerequisite, two issues need careful attention: (1) To investigate whether human epilepsy tissue harbors similar regenerative capacity than normal rodent brain. (2) The propensity to induce neuronal differentiation by in vitro protocols adopted from animal tissue, i.e. using HDAC inhibitors (Siebzehnrübl et al., 2007). Thirteen surgical specimens were available for our study. Neural progenitors were expanded as monolayer cultures adherent to laminin/poly-ornithine coated plates in N5 medium supplemented with EGF, FGF2 and LIF. Neuronal differentiation was induced by growth factor withdrawal. Cultures were then treated with a combination of Shh, SAHA and FGF8 for 48 hours, followed by forskolin and NGF. After 14 days of differentiation, cellular phenotypes were assessed by fluorescence immunostaining for neuronal markers (Map2, NeuN, ßIII Tubulin). Spontaneous neuronal differentiation was observed in 1-3% of cells in all experiments (n=8 patients). Intriguingly, addition of SAHA and forskolin increased this number to 60-71% in three patients, whereas no similar induction potential could be recognized in all other specimens. There was no correlation with the patient's age at surgery or duration of the epileptic disorder/age at onset, respectively. However, the propensity for neuronal differentiation was higher in those specimens without hippocampal cell loss, in particular of the dentate gyrus, i.e. Mesial Temporal Sclerosis. Indeed, preliminary analysis in 4 patients showed a strong correlation between the propensity to learn and recall memory (using WADA testing) with in vitro neuronal induction. With respect to previous animal models, our preliminary data experimentally confirm the impact of hippocampal neurogenesis for learning in humans. Furthermore, these results indicate that any success to further propagate and induce specific neuronal phenotypes in humans, i.e. dopaminergic neurons, will critically depend on available tissue samples and underlying disease pathomechanisms.

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Spontaneous in vitro transformation of adult neural precursors into stem-like cancer cells

F.A. Siebzehnrübl [1*], R.Coras [1], D. Müller [1], B. Rings[1], I. Jeske [1], H.B. Huttner [2], D. Corbeil [3], J. Kaesbauer [4], T. Appl [5], S. von Hörsten [5] and I. Blümcke [1]

[1] Department of Neuropathology, University of Erlangen-Nuremberg
[2] Department of Neurology, University of Erlangen-Nuremberg
[3] Tissue Engineering Laboratories (Biotec), Technical University of Dresden
[4] Center for Human Genetics, Regensburg
[5] Experimental Therapy, Franz-Penzoldt-Center, University of Erlangen-Nuremberg

[*] Present address: University of Florida, McKnight Brain Institute, Dept Neuroscience Gainesville, FL, USA

Recent studies have found that cellular self-renewal capacity in brain cancer is heterogeneous, with only stem-like cells having this property. A link between adult stem cells and cancer stem cells remains, however, to be shown. Here, we describe the emergence of cancer stem-like cells from in vitro cultured adult brain stem cells. Adult rat subventricular zone stem cells transformed into tumorigenic cell lines after expansion in vitro. These cell lines maintained characteristic features of stem-like cells expressing Nestin, Musashi1 and CD133, but continued to proliferate upon growth factor withdrawal. Karyotyping detected multiple acquired chromosomal aberrations and syngeneic transplantation into the brain of adult rats resulted in malignant tumor formation. Tumors revealed streak necrosis and displayed a neural as well as undifferentiated phenotype. Deficient downregulation of platelet-derived growth factor receptor alpha was identified as candidate mechanism for tumor cell proliferation and its knock-down by siRNA resulted in a reduction of cell growth. Our data point to adult brain precursor cells to be transformed in malignancies. Furthermore, in vitro expansion of adult neural stem cells, which will be mandatory for therapeutic strategies in neurological disorders, also harbors the risk for amplifying precursor cells with acquired genetic abnormalities and induction of malignant tumors after transplantation.

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Reprogramming of adult astroglia into functional neurons by neurogenic fate determinants in vitro

Rodrigo Sánchez[1], Roland H. Goldbrunner[2], Magdalena Götz[1], Benedikt Berninger[1]

1 Department of Physiological Genomics, Institute of Physiology, LMU München,
2 Department of Neurosurgery, LMU München, Germany

We have previously shown that astroglia from the mouse postnatal neocortex can be reprogrammed in vitro by neurogenic fate determinants such as neurogenin-2 (Ngn2), Mash1 or Pax6 to generate functional neurons (Berninger et al., 2007). Here we addressed the question whether this approach is also viable for adult astroglia. Indeed glial cells in the adult cerebral cortex can be induced towards neurogenesis in vivo after injury (Buffo et al., 2005), but most of these neurons are immature and analysis of their functional characteristics is difficult in vivo. Moreover, the glial cell type from which these neurons arise has not yet been determined. Therefore we established here an in vitro model of adult rat astroglia. Glial cultures were prepared from rat neocortex between postnatal days 7 to adulthood and expanded in medium containing serum and EGF/FGF2. At this stage more than 95 % of the cells were positive for astroglial markers. These cells were then transduced with retroviral vectors encoding for Mash1 or Ngn2 and cultured in serum free medium without EGF/FGF2 for differentiation. Mash1, and to a lesser degree, Ngn2 induced a neuronal phenotype in the transduced astroglia at all ages examined. Indeed upon Mash1 transduction of astroglia isolated from P56 cortices 73 ± 8 % were found to express TuJ1. Moreover, these cells fire TTX-sensitive action potentials indicating that also adult astroglia can be reprogrammed into functional neurons by virtue of a single transcription factor. As endogenous astroglia may eventually serve as a source for the replacement of degenerated neurons, we have now extended our studies to astroglia isolated from the temporal lobe from epilepsy patients. Of note, human astroglia up-regulated TuJ1 upon transduction with Mash1 suggesting a partial reprogramming towards a neuronal phenotype.

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Expression of the transcription factor Sox11 in the adult hippocampal neurogenic lineage

Anja Badde, Merve Güvenlioglu, D. Chichung Lie

Institute of Developmental Genetics, Research Group Adult Neurogenesis, Helmholtz Zentrum München- German Research Center for Environmental Health, Neuherberg, Germany

The generation of neurons from neural stem cells (NSCs) persists in two discrete niches of the adult brain: the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG) and the subventricular zone (SVZ) of the lateral ventricle. During development, neuronal fate determination, subtype specification and differentiation are controlled by the interaction of different transcription factors. The transcriptional cascade which controls the generation of neurons from neural stem cells in the adult brain is largely unknown. In this study we examined the expression of developmental neurogenic transcription factors in the adult neurogenic niche. We found that Sox11, a member of the SRY-related HMG box (SOX) family of transcription factors, is highly expressed in the neurogenic lineage of the adult dentate gyrus and the SVZ/OB system. In addition, the temporo-spatial expression pattern suggests that this transcription factor controls early stages of neurogenesis. Indeed, in vitro gain-of-function studies indicated that Sox11 is involved in the regulation of the expression of early neuronal markers in adult neural stem cells. Future experiments will determine whether Sox11 is essential for the generation of new neurons in the adult hippocampus and which target genes are controlled by Sox11 in this process.

Examining the role of Notch-signalling pathway in the regulation of Sox2

O. Ehm, L. Berti, A. Badde, D. C. Lie

Institute of Developmental Genetics, Research Group Adult Neurogenesis, Helmholtz Zentrum München- German Research Center for Environmental Health, Neuherberg, Germany

During adulthood neural stem cells continuously give rise to new neurons in the dentate gyrus of the hippocampus. The maintenance and differentiation of neural stem cells have to be tightly balanced in order to sustain hippocampal neurogenesis throughout lifetime. Previous studies have indicated that the transcription factor Sox2 is necessary for the maintenance of neural stem cells during adulthood. We have now confirmed that Sox2 is expressed in the neural stem cell population of the adult hippocampus using a BrdU-pulse chase paradigm and analysis for co-labelling of Sox2 positive cells with stem cell markers and markers for the glial and neuronal lineage.
The Notch-signalling pathway has been implicated in stem cell maintenance in several stem cell systems. Immunohistochemical analysis revealed that Notch-signalling is active in most of the Sox2-positive cells in the SGZ. Moreover, we found evidence that Delta a ligand for the notch receptor is expressed in the hippocampal neurogenic niche. Stimulation of the notch pathway increases the activity of a Sox2-reporter-construct. In addition, overexpression of the intra-cellular domain of the Notch receptor or activation of the receptor with recombinant Jagged1 protein increases the RNA levels of Sox2. Conversely, inhibition of Notch-signalling by a γ-secretase inhibitor decreases Sox2 RNA levels. These studies suggest that Notch-signalling contributes to the regulation of Sox2 expression in adult neural stem cells in vitro.
Future studies will determine whether Sox2 is a direct target of the Notch-signalling pathway and whether Notch-signalling is necessary for the maintenance of Sox2 expression in adult neural stem cells in vivo.

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Molecular and cellular responses of adult neural progenitors to transforming growth factor-beta.

B. Lehner and L. Aigner

Department of Neurology, University of Regensburg, Regensburg, Germany

Transforming growth factor (TGF)-ß1 is a multifunctional cytokine regulating a number of physiological and patho-physiological processes in the adult brain. Its expression is elevated during neurodegeneration, where its activities have been attributed to immune-modulation and neuroprotection.
Neurodegeneration is associated with reduced levels of neurogenesis. The molecular cues inhibiting neurogenesis under neurodegenerative pathologies are currently not known. We have postulated that TGF-ß1 might be one of the crucial factors involved in limiting neurogenesis in the diseased brain. Thus, we recently could demonstrate that TGF-ß1 inhibits neural stem cell proliferation and neurogenesis in vitro using neurosphere cultures and in vivo after intracerebroventricular infusion of the factor (Wachs et al., 2006). The effect of TGF-ß1 on neural stem cell proliferation was long-lasting (up to eight weeks), although it was reversible. Since TGF-ß1 has been demonstrated to be cytostatic for a number of cell types, we hypothesized that TGF-ß1 affects the cell cycle of adult neural stem and progenitor cells (NSCs).
Here, we analyzed the cell cycle of NSCs by using Ki-67/PI FACS analysis. Moreover, we performed DNA Array expression analysis of cell cycle and cell proliferation regulating genes. We demonstrate that TGF-ß1 i) shifts NSCs into G0 of the cell cycle and ii) regulates the expression of a number of cell cycle (e.g.Ccnb1, tp53, p57, Ccng1, Ccnd2) and cell proliferation (e.g.Dab2, Hes 1, Qscn 6, Jag 1, TGF-ßR2) associated genes.
These data support the role of TGF-ß1 as an inhibitor of adult neurogenesis by arresting NSCs in G0 of the cell cycle and modulating the expression of a lot of genes.

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Investigating the role of PI3K/mTOR signalling in adult neural stem cells

Alexandra Karl*, Elena Chanina, D. Chichung Lie

Institute of Developmental Genetics, Research Group Adult Neurogenesis, Helmholtz Zentrum München- German Research Center for Environmental Health, Neuherberg, Germany
* International Master's Program in Experimental and Clinical Neurosciences, University of Regensburg, Germany

The phophoinositide-3-kinase (PI3K)/mammalian target of rapamycin (mTOR) signalling-pathway is an important regulator of proliferation and differentiation of embryonic stem cells. Its role in the regulation of adult neural stem cells and adult neurogenesis is only poorly understood. Here, we investigated the effect of pharmacological blockade of PI3K and mTOR by Ly 294002 and rapamycin, respectively, on the proliferation and differentiation of adult neural stem cells. Adult hippocampal stem cells treated with Ly 294002 for 6 and 24 hours showed significant a decrease in proliferation, whereas treatment with rapamycin even for prolonged periods of time had no effect on the proliferation rate of these cells. The effect of mTOR signalling on differentiation was analyzed by culturing adult hippocampal stem cells with rapamycin for 3-4 days under differentiating conditions. Treatment with rapamycin resulted in a significant reduction in the percentage of neural stem cells which differentiated into neurons. In contrast, no effect on astrogliogenesis was observed. These in vitro observations raise the possibility that the mTOR pathway may be specifically required for the neuronal differentiation and maturation in adult neurogenesis. Based on these findings we are currently performing studies to investigate the role of the mTOR pathway in differentiation and maturation in adult neurogenesis in vivo.

Creb signalling in the maturation of newborn neurons in the adult dentate gyrus

Ravi Jagasia, Sabine Herold, and D. Chichung Lie

Institute of Developmental Genetics, Research Group Adult Neurogenesis, Helmholtz Zentrum München- German Research Center for Environmental Health, Neuherberg, Germany

Neural stem cells continuously generate new functional neurons in the hippocampal dentate gyrus throughout adulthood. Hippocampal network activity regulates the maturation and integration of new hippocampal neurons. The intracellular signalling pathways involved in these processes are largely unknown. Here we investigate the role of CREB in the development of hippocampal neurons in the adult mouse. We demonstrate that cyclic AMP response element binding protein (CREB) is strongly active in newly generated immature neurons. To manipulate CREB signalling in vivo we have generated retroviruses expressing constitutively active and dominant negative forms of CREB. Modulating CREB activity has profound affects on hippocampal adult neurogenesis in vivo. Loss of CREB-signalling leads to loss of expression of immature neuronal markers, aberrant dendritic growth and loss of newborn neurons in the adult hippocampus. Interestingly, increased CREB activation leads to increased maturation and as well loss of newborn neurons compared to wild-type cells. We conclude that CREB signalling is tightly regulated and involved in the maturation and integration of adult generated dentate granule neurons. We are now investigating using a retroviral strategy which pathways are upstream of CREB signalling in hippocampal neurogenesis, including both GABA- and NMDA-mediated neuronal

Smad4-mediated BMP-signalling directs adult neural stem cells towards neurogenesis by inhibiting the generation of oligodendrocytes

Dilek Colak[1], Tetsuji Mori[1,2], Alexander Pfeifer[3], Chuxia Deng[4] and Magdalena Götz[1,5]

[1]Helmholtz Zentrum München – National Research Center for Environment and Health, Institute for Stem Cell Research, Neuherberg/Munich, Germany
[2] present address: Department of Anatomy and Cell Science, Kansai Medical University, Moriguchi, Osaka, Japan
[3] Institute for Pharmacology and Toxicology , Medical University, Bonn, Germany
[4]Genetics of Development and Disease Branch, NIDDK, National Institutes of Health, Bethesda, Maryland, USA
[5] Physiological Genomics, University of Munich, Munich, Germany

Members of the transforming growth factor (TGF)b superfamily influence stem cell fate and self-renewal in various organs, but their role in the adult neural stem cell niche is not well understood. Besides expression of several Bmp ligands (2,4,6,7), the feed-back inhibitor Noggin, BMP specific type II receptor (BMPRII) and the common signalling mediator Smad4 in one of the adult neural stem cell niches, the subependymal zone (SEZ), phosphorylated Smads 1, 5 and 8 were detected in label-retaining GFAP-positive neural stem cells. Moreover, phosphorylated Smad1,5 and 8 were detected in Dlx2-positive, but not Olig2-positive transit-amplifying precursors and was also absent in doublecortin-positive neuroblasts. To examine the role of BMP-signalling in this adult neurogenic niche, we conditionally deleted Smad4, the common mediator of TGFβ and BMP signalling, and infused the BMP-specific extracellular inhibitor Noggin. Both manipulations resulted in a similar phenotype with a severe decrease in neurogenesis, related to up-regulation of Olig2 in Dlx2-positive transit-amplifying precursors. The latter migrate to the corpus callosum and differentiate into oligodendrocytes, at the expense of neurogenesis in the OB. These results suggest suggesting that Smad4-dependent BMP signalling seems allows neurogenesis in the adult neurogenic niche by suppressing the oligodendroglial lineage.

Smad7 regulates adult neural stem/progenitor proliferation in a TGF-beta independent manner.

Monika Krampert[1,2], Frank-Peter Wachs[1], Mahesh Kandasamy[1], Carl-Henrik Heldin[2], Rainer Heuchel[2] and Ludwig Aigner[1]

[1] Department od Neurology, University Regensburg, Regensburg, Germany
[2] Ludwig Institute for Cancer Research, Uppsala, Sweden

Members of the transforming growth factor family modulate the proliferation, differentiation and survival of many cell types. Recently it was shown that TGF-beta suppresses the proliferation of adult neural progenitor cells both in vivo and in neurosphere cultures. We therefore investigated neurogenesis in mice deficient for the TGF-beta inhibitor Smad7. Unexpectedly, these mice showed enhanced proliferation of adult neural stem/progenitor cells in vivo as well as longterm maintenance of BrdU-labeled cells. However, this was not followed by an increase in neurogenesis. In vitro we observed both a higher sphere-forming capacity in mutant cells as well as faster growth and cell cycle progression. Inhibitor studies revealed that these effects are independent of TGF-beta and BMP signaling. The enhanced proliferation might be at least partially mediated by elevated EGF-signaling, as mutant cells showed higher expression and activation levels of the EGF-receptor; and an EGF-receptor inhibitor reduced the proliferation of these cells in a dose-dependent manner. Our data indicate that endogenous Smad7 plays a TGF-beta independent role in regulating neural stem/progenitor cell proliferation.

Deletion of TrkB in adult progenitors results in defect hippocampal neurogenesis and increased anxiety-like behavior

Matteo Bergami[1], Roberto Rimondini[2], Spartaco Santi[3], Robert Blum[4], Magdalena Götz[4] and Marco Canossa[2,5]

[1]Department of Human and General Physiology, University of Bologna, Bologna, Italy; [2]Department of Pharmacology, University of Bologna, Bologna, Italy; [3]CNR-IGM (IOR) Bologna, Italy; [4]Department of Physiological Genomics, Institute of Physiology, Ludwig-Maximilians University Munich, Munich, Germany; [5]Italian Institute of Technology, Genova.

New neurons in the adult dentate gyrus are widely held to incorporate into hippocampal circuitry via a stereotypical sequence of morphological and physiological transitions, yet the molecular control over this process remains unclear. We studied the role of BDNF/TrkB signaling in adult neurogenesis by deleting the full-length TrkB via Cre expression within adult progenitors in $TrkB^{lox/lox}$ mice. By four weeks after deletion, the growth of dendrites and spines is reduced in adult-born neurons demonstrating that TrkB is required to create the basic organization of synaptic connections. Later, when new neurons normally display facilitated synaptic plasticity and become preferentially recruited into functional networks, lack of TrkB results in impaired neurogenesis-dependent long-term potentiation and cell survival becomes compromised. Despite the restricted deletion of TrkB in recently generated neurons, a remarkably increased anxiety-like behavior was observed in mice carrying the mutation, demonstrating the key role of adult neurogenesis in the regulation of emotion.

Specific requirement of HDAC2 for survival of neurons generated in the adult brain

Melanie Jawerka[1], Dilek Colak[1], Carmen Spiller[2], Sabine Lagger[3], Wolfgang Wurst[4], Martin Göttlicher[2,5] and Magdalena Götz[1,6]

GSF-National Research Institute for Environment and Health, Institute for Stem Cell Research[1], Institute for Toxicology[2], Institute for Developmental Genetics[4] Neuherberg/Munich, Germany;
Max F. Perutz Laboratories, Medical University of Vienna, Institute of Medical Biochemistry[3], Vienna, Austria
Institute of Toxicology and Environmental Hygiene[5], Technical University Munich; Physiological Genomics[6], University of Munich, Munich, Germany

The histone deacetylase (HDAC) 2 is known to play a role in transcriptional regulation by modifying the N-terminal ends of the histone tails resulting in decreased transcriptional activity. To understand the role of epigenetic mechanisms involved in neural stem cells in development and adulthood, we first examined HDAC2 protein localisation in the developing and adult brain. HDAC2 protein was present at high levels in postmitotic neurons in the developing and adult brain. However, neuronal precursors in the developing cerebral cortex were hardly HDAC2-immunoreactive, while HDAC2-immunoreactivity increased in glial precursors of the prospective white matter at postnatal stages. In pronounced contrast to the absence of HDAC2 in neuronal precursors during development, we observed HDAC2 protein in double-cortin-positive precursors in the adult dentate gyrus (DG) and the adult subependymal zone (aSEZ), the two regions of adult neurogenesis.
We were able to identify, that HDAC2 plays a key role of HDAC2 in adult, but not embryonic neurogenesis. While brain development and adult stem cell fate are largely normal in mice lacking the catalytic activity of HDAC2, the proliferation of adult neural progenitors is increased and neurons derived from both zones of adult neurogenesis die at a specific maturation stage in the absence of functional HDAC2. Retroviral injections further suggest that neuronal differentiation is impaired in the DG. In vitro cultures of neural stem cells from adult SEZ also showed a decrease in neuronal maturation while proliferation and self renewal capacity is not altered in HDAC2-/- cultures. Mixed cultures derived from wildtype and HDAC2 deficient mice and transplantation experiments further underline the cell-autonomous effect of HDAC2 during the maturation of adult generated neurons.
These data therefore identify for the first time a specific role of HDAC2 in the nervous system revealing a remarkable difference in the molecular mechanisms governing neuronal maturation in the embryonic and adult brain. Thus, the function of HDAC2 opens new avenues towards understanding the molecular machinery regulating the specific requirements for maturation and integration of new neurons into the adult brain.

Keywords: Histone deacetylase, chromatin modification, neural stem cells, epigenetics

Mitochondrial Dynamics in adult hippocampal neurogenesis

Kathrin Steib, D. Chichung Lie, Ravi Jagasia

Institute of Developmental Genetics, Research Group Adult Neurogenesis, Helmholtz Zentrum München- German Research Center for Environmental Health, Neuherberg, Germany

Adult hippocampal neurogenesis is thought to be crucial for learning and memory. Decreased performance of aged rodents in behavioural tests for hippocampal function is paralleled by a decrease in the number of newly generated hippocampal neurons. Impairment of mitochondrial function is a major factor for aging in many tissues including the brain. We aim to investigate the question of how mitochondrial dynamics and function affect hippocampal adult neurogenesis. Mitochondrial morphology is dictated by the balance of two opposing processes, mitochondrial fusion and fission. Very little is known about the functional role of these processes under steady-state conditions, but data suggests that they play crucial roles in neuronal function, including viability and synaptogenesis. We have begun to elucidate the role of mitochondrial fusion and fission in the process of adult hippocampal neurogenesis in vitro and in vivo. To this end we have generated lentiviruses and retroviruses, which encode for mitochondrial Dsred to visualize mitochondria in adult neural stem cells and their progeny. In addition, we have generated viruses expressing transgenes (drp-1(wt), and drp-1(dn)), which allow the disruption of mitochondrial fusion and fission. Our present data indicate that altering mitochondrial morphology affects developmental stages of adult hippocampal neurogenesis, including neuronal and astrocytic differentiation, and dendritic maturation . These studies suggest a novel link between mitochondrial function and neural stem cell biology.

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Telomerase expression and telomere length in progenitor and stem cell populations of the adult zebrafish brain.

Susanne Sprungala, Dr. Laure Bally-Cuif

Institute of Developmental Genetics, Group: Zebrafish Neurogenetic, Helmholtz Zentrum München-National Research Center for Environmental Health, Neuherberg, Germany

Adult neural stem cells can be found life-long in the vertebrate adult brain, but their number decreases with age. The mechanisms of stem cell maintenance and depletion remain incompletely understood. Because telomerase activity is involved in the maintenance of dividing cell populations, avoiding their telomere attrition and their senescence, it might be implicated in the regulation of neural stem cell populations. Therefore, we aim at studying the expression and role of telomerase in progenitor and stem cell populations of the adult brain, in the teleost zebrafish. Indeed, the brain of zebrafish has recently been established as a model to study adult neural stem cells (Adolf et al., Grandel et al., Chapouton et al.). There, several neurogenic areas throughout the brain contain progenitors in abundance as well as cells displaying stem cell characteristics : e.g. interspersed cells bordering the telencephalic ventricle (Adolf et al) and a condensed cell population in the posterior midbrain expressing the transcription factor Her5 (Chapouton et al.).
Our aim is firstly to determine i) the length of telomeres and ii) the expression of telomerase in distinct cell populations of the neurogenic areas, focussing our attention on the telencephalon mostly. We analyse: a) the quickly dividing population, expressing the proliferation marker PCNA, b) the self-renewing cells, remaining in the cycle three months after BrdU incorporation, c) the non-dividing radial glial population, which might represent a quiescent or a postmitotic differentiated population, d) the postmitotic parencymal population and e) the *her5*-expressing stem cell like population of the midbrain. Preliminary data show that the quickly dividing and self-renewing populations have significantly longer telomeres than their neighbouring parenchymal postmitotic populations. Accordingly, quickly dividing cells express strongly the RNA encoding for the telomerase catalytic subunit Tert. Thus, both long lasting and quickly dividing cells maintain long telomeres.
In the future we wish to address the requirement for telomerase in maintaining adult neural stem cells, using a telomerase zebrafish mutant. Sustained telomerase expression might be the reason for the abundance of neurogenesis in the adult fish brain.

Generation of new dopaminergic neurons in the adult mouse brain

S. Herold and D. C. Lie

Institute of Developmental Genetics, Research Group Adult Neurogenesis, Helmholtz Zentrum München- German Research Center for Environmental Health, Neuherberg, Germany

The generation of neurons from neural stem cells (NSCs) persists in the adult subventricular zone (SVZ) of the lateral ventricle. In the SVZ, NSCs give rise to neuroblasts, which migrate along the rostral migratory stream (RMS) and integrate as mature interneurons in the olfactory bulb (OB). A subset of these newborn cells displays a Tyrosine-Hydroxylase (TH) -positive dopaminergic phenotype. The goal of this work is to understand the molecular signals that regulate the generation of dopaminergic olfactory bulb interneurons. Analysis of a reporter mouse for the Wnt/b-catenin pathway provided evidence that the pathway is active in the SVZ/OB system. In vitro gain-of-function studies indicated that Wnt/b-catenin signalling is sufficient to enhance the generation of TH-expressing neurons from adult neural stem cells. Previous studies have suggested that cAMP-signaling participates in the regulation of the TH-gene. Indeed we observed that stimulation of cAMP-signaling in the context of Wnt-signaling promoted the generation of TH-expressing neurons. Interestingly, stimulation of cAMP-signaling in the absence of Wnt-signaling did not enhance the generation of TH-expressing neurons. These data indicate that Wnt-signaling and cAMP may cooperate in the generation of dopaminergic neurons from adult neural stem cells. Future experiments will be directed at understanding the significance of Wnt-signaling and cAMP-signaling in the generation of new dopaminergic olfactory bulb interneurons in vivo.

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A novel role of Pax6 in postmitotic neurons - molecular dissection of Pax6 function in adult neurogenesis

Jovica Ninkovic[1], Nicole Haubst[1], Ruth Ashery-Padan[3] and Magdalena Götz[1,2]

1 Institute for Stem Cell Research, Helmholtz Center Munich-German Research Center for Environmental Health, Neuherberg/Munich; Germany
2 Physiological Genomics, University of Munich, Munich, Germany
3 Human Genetics Faculty of Medicine, Tel Aviv University, Ramat Aviv, Israel.

Periglomerular (PG) and granular cell layer neurons in the olfactory bulb (OB) are generated throughout the lifespan of higher vertebrates. The transcription factor Pax6 is expressed in maturing neuroblasts and is downregulated in postmitotic OB neurons after they acquire their neurotransmitter identity, except in PG neurons adopting a dopaminergic phenotype. We have previously shown that Pax6 is crucial for the generation of all neuroblasts and promotes differentiation of dopaminergic PG neurons (Hack et al., 2005). Here, we dissected these functions in time and at the molecular level.

To examine the role of Pax6 in postmitotic dopaminergic PG neurons, Cre under control of the DAT-promoter was used to delete Pax6 (Ashery-Padan et al., 2000) when neurons start to acquire their neurochemical specification. Strikingly, the proportion of TH positive periglomerular cells was significantly reduced after deletion of Pax6, and no Cre/TH-positive neurons could be detected any longer. Genetic fate mapping analysis further revealed that Pax6 is required for the survival of dopaminergic neurons, thus revealing for the first time a role of Pax6 in postmitotic neurons.

Given the distinct roles of Pax6 in dividing neuronal precursors and differentiating postmitotic neuronal subtypes, we examined whether these effects may be exterted by distinct isoforms of Pax6 utilizing its different DNA-binding domains. Retroviral vectors containing three different Pax6 constructs, the full length Pax6 comprising the two DNA-binding domains, the paired domain with (5a) and without (canonical) the exon 5a as well as the homeodomain and a third construct lacking the paired domain (PD-less) were used. Application of these retroviral vectors in adult neurosphere cells in vitro or injection into the subependymal zone or rostral migratory stream in vivo revealed that both forms of the paired domain directed SEZ precursors towards neuronal lineages and promoted the number of dopaminergic periglomerular neurons. In contrast, overexpression of the PD-less Pax6 isoform inhibited neurogenesis and slightly decreased the proportion of newly generated dopaminergic neurons. Thus, the paired domain is crucial to exert both functions of Pax6 in adult neurogenesis while the PD-less homeodomain counteracts these. Thus the regulation of Pax6 isoforms in the OB seems to be crucial to regulate the number and identity of adult generated neuron

A Dlx2- and Pax6-dependent transcriptional code for periglomerular neuron specification in the adult olfactory bulb

Monika S. Brill[1, 2], Marina Snapyan[3], Hilde Wohlfrom[1, 2], Jovica Ninkovic[2], Melanie Jawerka[1, 2], Grant S. Mastick[4], Armen Saghatelyan[3], Ruth Ashery-Padan[5], Benedikt Berninger[1, 2] & Magdalena Götz[1, 2, 6]

[1]Department of Physiological Genomics, Institute of Physiology, Ludwig-Maximilians University Munich, Munich, Germany
[2]Institute for Stem Cell Research, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany
[3]Centre de recherche Université Laval Robert-Giffard, Unité de neurobiologie cellulaire, Québec, Canada
[4]Biology Department, University of Nevada, Reno, Biology Department, Reno,
[5]Sackler Faculty of Medicine, Department of Human Molecular Genetics and Biochemistry,Tel Aviv University, Tel Aviv, Israel

Distinct olfactory bulb (OB) interneurons are thought to become specified depending on from which of the different subregions lining the lateral ventricle wall they originate, but the role of region-specific transcription factors (TFs) in the generation of OB interneurons diversity is still poorly understood. Despite the crucial roles of the Dlx family of TFs for patterning and neurogenesis in the ventral telencephalon during embryonic development, their role in adult neurogenesis has not yet been addressed. Here we show that in the adult brain Dlx 1 and Dlx2 are expressed in progenitors of the lateral, but not the dorsal subependymal zone (SEZ), thus exhibiting a striking regional specificity. Using retroviral vectors in order to examine the function of Dlx2 in a cell-autonomous fashion, we demonstrate that this TF is necessary for neurogenesis of virtually all OB interneurons arising from the lateral SEZ. Beyond its function in generic neurogenesis, Dlx2 also plays a crucial role in neuronal subtype specification in the OB promoting specification of adult-born periglomerular neurons (PGNs) towards a dopaminergic fate. Strikingly, Dlx2 requires interaction with Pax6, as Pax6 deletion blocks Dlx2-mediated PGN specification. Thus, Dlx2 wields a dual function by first instructing generic neurogenesis from adult precursors and subsequently specifying PGN subtypes in conjunction with Pax6.

Function of the Frizzled Wnt receptors in mDA neuron development

Sebastian Stübner[1], Thomas Fischer[1], Wolfgang Wurst[1,2], Nilima Prakash[1]

[1] IDG, Helmholtz Zentrum München – German Research Center for Environmental Health, Neuherberg/ Munich, Germany;
[2] Max Plank Institute of Psychiatry, Munich, Germany

Dopaminergic neurons located in the mammalian ventral midbrain (mDA neurons) are involved in the control of motor, affective and cognitive brain functions. Degeneration of these neurons leads to Parkinson's disease (PD), and their dysfunction is thought to underlie the pathogenesis of schizophrenia, addiction and clinical depression. Studying the normal generation of these neurons is therefore also of high clinical relevance, in particular for the development of potential regenerative therapies for PD. We have recently demonstrated a crucial role of Wnt1-mediated signalling for the generation of mDA neurons in vivo. The secreted glycoprotein Wnt1 is expressed in the ventral midbrain in close vicinity to the developing mDA neurons, and ectopic expression of Wnt1 results in the ectopic induction of mDA neurons within the anterior hindbrain. Wnt ligands bind to the seven transmembrane domain receptors of the Frizzled family, which then transduce the signal by at least three distinct pathways: the canonical Wnt/ß-catenin pathway, the planar cell polarity (PCP) pathway, and the Ca^{2+} pathway. As a first attempt to unravel the signal transduction pathway downstream of Wnt1 in mDA neuron development, we performed a detailed analysis of the expression of the 10 known murine Fzd receptor genes in the neural tube of E9.5, E10.5 and E12.5 mouse embryos. These are the stages when mDA progenitors can first be distinguished by the expression of Aldh1a1/Raldh1 and Nr4a2/Nurr1 (E9.5-10.5), or when differentiating mDA neurons are readily identifiable in the ventral midbrain by the expression of tyrosine hydroxylase (Th; E12.5), the key enzyme in dopamine biosynthesis. Our analysis suggested the Fzd3 and Fzd6 receptors as the strongest candidates to transduce the Wnt1 signal in the ventral mesencephalon. We therefore proceeded to characterise the function of these two Fzd receptors in the generation of mDA neurons in vivo by analysing the development of these neurons in the $Fzd3^{-/-}$ and $Fzd6^{-/-}$ single mutant mice. We have used in situ hybridisation (ISH) and immunohistochemistry (IHC) to detect different mDA neuronal markers (Nr4a2/Nurr1, Th, Pitx3 and the dopamine transporter, Dat) at three important time-points in mDA neuron development: induction (E10.5), differentiation (12.5) and maintenance (E17.5). Our analysis of the $Fzd3^{-/-}$ and $Fzd6^{-/-}$ mice, however, did not reveal any changes in the expression patterns of these markers at all stages analysed, indicating that neither Fzd3 nor Fzd6 alone are necessary for the generation of mDA neurons. Furthermore, patterning of the midbrain was not affected in these mutants, as assessed by the normal expression of Wnt1 and Fgf8 at the mid-/hindbrain boundary. Due to the highly dynamic and overlapping spatiotemporal expression pattern of the murine Fzd genes, it is very likely that Fzd3 and Fzd6 are functionally redundant. We are currently exploring this possibility by analysing the development of mDA neurons in the $Fzd3^{-/-}$; $Fzd6^{-/-}$ double mutant mice.

Expression and functional analysis of midbrain dopaminergic neuron development in the chicken embryo

Ruth Klafke[1], Andrea Wizenmann[2], Wolfgang Wurst[1] and Nilima Prakash[1]

[1]Helmholtz Zentrum Munich- Research Center for Environmenatl Health, Instutite for Developmental Genetics, Munich- Neuherberg
[2]University of Tuebingen, institute of Anatomy, Tuebingen

Dopamine (DA)-synthesizing neurons constitute a prominent population in the mammalian brain as they are involved in the control and modulation of motor, cognitive, rewarding and neuroendocrine functions. The best studied DA population are the mesencephalic DA (mesDA) neurons, since the degeneration of these neurons leads to Parkinson´s Disease in humans. The mesDA neurons of the mouse arise from the ventral midline of the midbrain and caudal forebrain during embryonic development, and different factors control their induction, specification, proliferation and differentiation. To elucidate conserved pathways for the generation of the mesDA population in higher vertebrates, we performed a spatiotemporal expression mapping for the chicken orthologues of the mouse mesDA marker genes Aldh1a1, Nr4a2 and Pitx3 during early chicken embryonic development. We found striking differences in the expression patterns of Aldh1a1 and Pitx3 between the two species, suggesting that the corresponding proteins acquired distinct functions in birds and mammals during evolution. In addition, we provide evidence that a subpopulation of the mesDA precursors are born in the diencephalon of the chicken embryo, probably reflecting the phylogenetic origin of this neuronal population.
The secreted glycoprotein Wnt1 regulates a genetic network that is required for the early establishment of the mesDA progenitor domain and later for the terminal differentiation of mesDA neurons during mouse embryonic development. Wnt signaling may also be required for the development of mesDA neurons in the chicken embryo, and we therefore performed overexpression studies in this species. Indeed, the ectopic expression of Wnt9a led to an induction of several mesDA marker genes in the ventral fore- and midbrain of the chicken embryo. This suggests that the Wnt-controlled induction of mesDA neurons is a conserved mechanism between chicken and mouse.

A30P alpha-synuclein expression regulates adult olfactory bulb neurogenesis

Franz Marxreiter[1] #, Silke Nuber[2] #, Mahesh Kandasamy[1], Jochen Klucken[1], Robert Aigner[1], Ralf Burgmayer[1], Sébastien Couillard-Déspres[1], Olaf Riess[2], Jürgen Winkler[1,3], and Beate Winner[1,4]

[1] Department of Neurology, University of Regensburg, Regensburg, Germany
[2] Department of Medical Genetics, University of Tübingen, Tübingen, Germany
[3] Department of Neurosciences, University of California, San Diego, La Jolla, CA, USA
[4] The Salk Institute for Biological Studies, Laboratory of Genetics, La Jolla, CA, USA,
# Franz Marxreiter and Silke Nuber contributed equally to this work

Hyposmia is an important and early premotor symptom in Parkinson's disease (PD). In PD brains, alpha-synuclein pathology is present in the olfactory bulb (OB). In addition, the OB is an active region of adult neurogenesis, where newly generated neurons physiologically integrate. Our recent work indicated that olfactory neurogenesis is decreased in adult transgenic models of synucleinopathies due to a decreased survival of newly generated neurons. While accumulation of wildtype alpha-synuclein is one of the pathogenic hallmarks of non-genetic forms of PD, the A30P alpha-synuclein mutation results in an earlier disease onset. Here, we study the regulation of adult neurogenesis in the subventricular zone (SVZ)/OB system in a tetracycline suppressive (tet-off) transgenic model, expressing human mutant A30P alpha-synuclein (A30P) under the control of the Calcium/ Calmodulindependent Kinase 2 alpha (CaMKII) promoter. In A30P transgenic mice alpha-synuclein was present in OB neuroblasts. Without changes in proliferation in the SVZ, significantly fewer newly generated neurons were observed in the OB granule cell and glomerular layer of A30P transgenic mice compared to controls, most likely due to an increased cell death. By tetracycline dependant blocking of A30P alpha-synuclein expression OB neurogenesis was restored to control levels. Our results indicate that (1) alpha-synuclein expression can be regulated in the OB and (2) alpha-synuclein influences the survival and integration of newly generated GABAergic and dopaminergic interneurons.

Dopamine receptor activation via pramipexole enhances olfactory neurogenesis in a Parkinson model

Beate Winner[1,2], Paula Desplats[3], Christian Hagl[1], Robert Aigner[1], Sonja Ploetz[1], Joern Laemke[1], Alexandra Karl[1], Jochen Klucken[1], Ludwig Aigner[1], Eliezer Masliah[3], Erich Buerger[4], Jürgen Winkler[1, 3]

[1]Department of Neurology, University of Regensburg, Regensburg, Germany
[2] Laboratory of Genetics, The Salk Institute for Biological Studies, La Jolla, CA, USA
[3] Department of Neurosciences, University of California San Diego, La Jolla, CA, USA
[4] Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany

Decreased proliferation of neural precursors was described in the SVZ of Parkinson disease (PD) patients as well as in PD animal models. The neurotransmitter dopamine modulates cell proliferation in the embryonic lateral ganglion eminence and dopamine receptors are found in the adult subventricular zone (SVZ).
The aim of this study was to decipher the potential of oral application of the D2/D3 receptor agonist pramipexole (PPX) to modulate adult neurogenesis in a PD model. We demonstrate that PPX increases proliferation in adult rat SVZ derived neurosphere cultures. 6-Hydroxydopamine (6-OHDA) lesioned adult F-344 Fisher rats received either PPX (1,0 mg/kg) or PBS orally twice daily and bromodeoxyuridine (BrdU) for 10 days and were perfused immediately after treatment or 4 weeks after PPX withdrawal. Stereological analysis revealed a significant augmentation in SVZ proliferation in PPX treated animals. Consecutively, enhanced neuronal differentiation and more newly generated neurons were present in the olfactory bulb granule cell layer 4 weeks after PPX withdrawal. In addition, dopaminergic neurogenesis was increased in the glomerular layer of the olfactory bulb after PPX treatment. Neither increased neurogenesis in the hippocampal dentate gyrus, nor newly generated cells were present in the striatum or substantia nigra, however an increased striatal gliogenesis was noted. Motor activity and exploratory behavior as assessed by using an open field paradigm significantly increased in the PPX treated groups.
Oral PPX treatment selectively increases adult neurogenesis in the SVZ-olfactory bulb system and gliogenesis in the striatum. Deciphering the neurogenic potential of D2/D3 mediated signaling pathways may lead to new avenues to induce endogenous neural repair in the adult CNS.

Olfactory bulb dysfunction in Parkinson's disease

J. Klucken[1], J. Brix[1], J. Kalis[1], C. Mößnang[2], N. Mallog[1], U. Bogdahn[1], G. Schuierer[3], M. Greenlee[2], J. Winkler[1], B. Winner[1]

1: Department of Neurology, University of Regensburg, Regensburg, Germany
2: Department of Experimental Psychology, University of Regensburg, Regensburg, Germany
3: Institute for Neuroradiology, University of Regensburg, Regensburg, Germany

In Parkinson's disease (PD) accumulation of α-synuclein aggregates – Lewy bodies and neurites – occur early in the olfactory bulb. Intriguingly, smelling deficits appear in 75-90% of the patients years before first motor symptoms are present. Thus, hyposmia may serve as a important "pre-motor" biomarker in PD identifying patients at a very early stage of the disease, potentially still without a significant dopaminergic cell loss in the substantia nigra. It is yet unclear how α-synuclein aggregates mediated pathomechanism influence olfactory bulb function; however an influence on olfactory bulb neurogenesis has been suggested recently.
We tested the smell function in PD patients and age matched controls using a set of smelling test and show that in contrast to their self-assessment the smell function is significantly impaired in PD patients. Interestingly, the deficits did not correlate to motor fluctuations, autonomic, or psychosis suggesting that smelling deficit represents an independent and underestimated non-motor symptom in PD.
In order to study the underlying structural and functional substrate for the smelling deficit in PD, we are currently studying changes in the volume and function of the olfactory bulb using morphometric and functional MRI techniques. Using a specific set of imaging algorithm we were able to measure the size of the olfactory bulb. Preliminary data show a decreased size of the olfactory bulb in PD patients. Furthermore, we applied different odorants to PD patients paralleled to functional MRI (fMRI) analysis. The fMRI design was validated by comparing the activated brain regions in healthy controls. We currently examine PD patients and age matched controls in order to identify different activation patterns in regions associated with olfactory processing.
Taken together our findings show a reduced olfactory function in PD patients, correlating with a decreased size of the olfactory bulb, and we are currently examining the functional consequences in PD patients. Based on these data we will establish screening tests to identify patients at early disease stages, where neuroprotective or regenerative therapies are more promising than at later stages, where most of the neurons and circuits are already compromised.

Alpha-synuclein oligomerisation: toxic or physiologic?

J. Klucken, R. Aigner, S. Plötz, U. Bogdahn, L. Aigner, J. Winkler

Department of Neurology, University of Regensburg, Regensburg, Germany

Parkinson disease is characterized by the loss of dopaminergic neurons in the substantia nigra and the deposition of eosinophilic, fibrillar, proteinaceous aggregates known as Lewy bodies (LBs). Aggregated alpha-synuclein is the major component of LBs. LBs are found in other so called synucleinopathies, such as Dementia with LBs as well. Recently, oligomeric forms of alpha-synuclein have been described to be the major source for its toxicity rather than the aggregated forms. We were able to visualize oligomeric α-synuclein within cellular structures using a fluorescence lifetime microscopy (FLIM) assay.
Oligomerisation of alpha-synuclein has been linked to the lipid membrane binding ability of its lipophilic N-terminus. Furthermore, interaction with synaptic vesicles has been shown to depend on the membrane binding, particularly for binding to specific membrane microdomains known as "rafts". Rafts are regions within the membrane with a specific lipid composition making them more rigid then other membrane regions. They are crucial for a range of cellular processes, including cell-cell contact and receptor clustering for neurotransmitter or growth factor receptors. In addition, rafts are directly involved in synaptic vesicle fusion with membranes.
So far not many reports contribute to the physiological function of α-synuclein. However, an association to synaptic vesicle metabolism has been suggested. The known α-synuclein mutations leading to familiar PD are located at the lipid binding domain. Thus, interfering with its membrane binding ability might directly influence synaptic vesicle cycling or cellular reactions in response to growth factors or neurotransmitters, resulting in cellular dysfunction and ultimatively in cellular loss.
To further decipher the physiological or toxic role of alpha-synuclein oligomerisation we characterized the cellular distribution of alpha-synuclein oligomers in primary neuron cultures. We show that the distribution of alpha-synuclein oligomers along dendrites shows similarity with the distribution of synaptic buttons suggesting that the raft associated synaptic binding of alpha-synuclein to synaptic vesicles includes oligomer formation. Thus, oligomerisation of alpha-synuclein might not be exclusively toxic but also be necessary for its physiological function.

DJ-1 deficient mice show reduced numbers of midbrain dopaminergic neurons and cognitive impairments

Pham TT[1], Röthig A[1], Giesert F[1], Floss T[1], Kallnik M[1], Weindl K[1], Hoelter S[1], Ahting U[2], Prokisch H[2], Becker L[3], Klopstock T[4], Beyer K[5], Goerner K[5,6], Kahle PJ[5,7], Vogt Weisenhorn DM[1,8], and Wurst W[1,8,9]

[1]Institute of Developmental Genetics,
[2]Institute of Human Genetics,
[3]Institute of Experimental Genetics, Helmholtz Zentrum München-National Research Center for Environment and Health, Neuherberg, Germany
[4]Department of Neurology, Klinikum Grosshadern, Ludwig Maximilians University of Munich, Munich Germany
[5]Department of Metabolic Biochemistry, Adolf Butenandt Institute, Ludwig Maximilians University of Munich, Germany
[6]present address: Advalytix AG, Brunnthal, Germany
[7]Laboratory of Functional Neurogenetics, Department of Neurodegeneration, Hertie Institute for Clinical Brain Research, University Clinics Tübingen, Germany
[8]Max-Planck-Institute of Psychiatry, Molecular Neurogenetics Munich, Germany
[9]Technical University of Munich, Germany

Loss of function of DJ-1 (PARK7) is associated with autosomal recessive early-onset Parkinson´s disease (PD), one of the major age-related neurological diseases. In this study, we extended former studies on DJ-1 knock-out mice by analysing the dopaminergic system not only in young (2 to 6 months old), but also in old DJ-1 deficient mice (18 to 24 months old), generated using the genetrap technology. Although we did not detect any age-related dopaminergic neurodegeneration in the DJ-1 mutant mice, due to the large number of animals analysed, a highly significant reduction (about 10%) in number of dopaminergic neurons throughout the entire lifespan became apparent. Furthermore, since DJ-1 has been implicated in mitochondrial function in vitro we analysed the effect of DJ-1 deficiency onto the mitochondrial respiratory chain in vivo. Interestingly, we found already in young animals a significant up-regulation of mitochondrial respiratory enzyme activities. On the behavioural level, the DJ-1 deficient mice showed a slight impairment in locomotion. Specifically, we detected a significant impairment in cognitive behaviour both young and old DJ-1 knock-out animals - which is known as a major non-motor/pre-clinical symptom of PD. Hence, the DJ-1 deficient mice may represent a valuable animal model to elucidate molecular mechanisms underlying early phases of PD.

Comparative expression analysis of Lrrk1 and 2 in the developing and adult mouse brain

Giesert F, Pham TT, Röthig A, Vogt Weisenhorn DM, Wurst W.

GSF-National Research Center for Environment and Health, Institute of Developmental Genetics, Ingolstadter Landstrasse 1, 85764 Neuherberg, Germany.

Missense mutations in the leucine-rich repeat kinase 2 gene (*Lrrk2)* are linked to autosomal dominant forms of Parkinson's disease. Until now, the role of *Lrrk2* in the aetiology of this neurodegenerative disease remains elusive. To learn more about the physiological role of *Lrrk2* during embryonic development and in the adult brain we examined the distribution of *Lrrk2* mRNA in the mouse embryo and the adult mouse brain. In order to get insights into a possible redundant function of its paralog *Lrrk1* we also examined the expression of this gene.
During development both genes are expressed ubiquitously in the whole embryo at moderate level from mid-gestation onwards and increase until birth. Hotspots of embryonic *Lrrk2* expression are the developing kidney, incisory teeth and nasal bones whereas *Lrrk1* is more prominent in the palate and nose epithelium, in the developing lung and the urogenital ridge. The developing CNS displays no expression signal. However, strong expression of *Lrrk2* in the choroid plexus from E12.5 till adulthood can be detected. Postnatal, *Lrrk2* becomes ubiquitously expressed in the brain; thereafter expression of *Lrrk2* gets stronger specifically in the striatum and the piriform cortex till it reaches adult levels at P21. Expression of *Lrrk2* is highly restricted to neurons and in the cortex and striatum it is expressed in subpopulations of dopamine receptor D1 and D2 immunopositive cells. Also a subpopulation of the medium spiny stellate cells in the striatum marked by DARP32 does strongly express *Lrrk2*. In contrast to *Lrrk2*, *Lrrk1* can not be detected in the adult brain except in the mitral cell layer of the olfactory bulb. Due to the non-overlapping expression pattern, specifically in the adult brain, a redundant function of *Lrrk2* and its paralog *Lrrk1* in the pathogenic process of Parkinson´s disease is not likely.

TGF-beta1 Signaling in the Healthy and Diseased Brain: A Focus on Neurogenic Regions of Transgenic HD models.

M.Kandasamy, F.Rivera, B.Lehner, S.Couillard-Despres, R.Aigner, M.Caioni, S.Plötz, U. Bogdahn, S.von Hörsten, J.Winkler, L.Aigner.

Department of Neurology, University of Regensburg, Regensburg, Germany.

Transforming growth factor (TGF)-beta1 has multiple physiological functions in the adult central nervous system (CNS). It modulates inflammatory responses and controls proliferation of microglia and astrocytes. In the diseased brain, TGF-beta1 expression is up-regulated and, depending on the cellular context, its activity can be beneficial or detrimental regarding regeneration. Recent data suggest that TGF-beta1 might be involved in the control of neural stem and progenitor cell proliferation during adult neurogenesis, in particular during neurodegeneration. Unfortunately, very little is known on TGF-beta signaling in neurogenic regions. Here, we provide a comprehensive and systematic analysis of the expression patterns of TGF-beta signaling components in the adult rodent CNS with focus on the neurogenic regions of healthy and transgenic Huntington's disease (HD) brains.
We demonstrate the anatomical distribution of the TGF-beta receptors RI and RII and of the downstream signaling element phospho-Smad 2 using Western blot analysis and immunehistochemistry. Moreover, we analyzed TGF-beta1 signaling in vitro using neural progenitor cell cultures derived from adult hippocampus or from SVZ. In contrast to the healthy brain, where TGF-beta signaling seems to be confined to differentiating and mature neurons, high levels of TGF-beta signaling were detected in the neurogenic regions animal models of HD. This correlates with reduced rates of cell proliferation and neurogenesis in the HD brains.
We conclude that TGF-beta1 signaling might be an important molecular feature involved in maintenance of neuronal integrity in the healthy brain, but limit neural progenitor proliferation and neurogenesis under disease conditions.

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Impaired hippocampal neurogenesis in R6/2 mice model of Huntington's disease cannot be rescued by physical activity

Z. Kohl, K. Mahesh, B. Winner, R. Aigner, C. Gross, S. Couillard-Despres, U. Bogdahn, L. Aigner, J. Winkler

Department of Neurology, University of Regensburg, Universitaetsstr. 84, 93053 Regensburg, Germany

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder linked to an expanded CAG-triplet nucleotide repeat stretch in the huntingtin gene and is characterized by hyperkinesia, behavioral and emotional changes and cognitive decline. The mutation leads to intracellular protein aggregation, especially in neurons of distinct brain areas. The generation of new neurons in neurogenic regions, such as the subventricular zone of the lateral ventricle and the dentate gyrus of the hippocampus, is affected by these aggregation processes. Similar to other neurodegenerative diseases, a reduction of neurogenesis has been determined in different models of HD. In particular, in the R6/2 transgenic mouse model of HD hippocampal neurogenesis is reduced.
Since physical activity stimulates adult hippocampal neurogenesis, we examined whether running is capable to rescue the impaired hippocampal neurogenesis in R6/2 mice. Proliferation of hippocampal cells measured by proliferating cell nuclear antigen (PCNA) marker was reduced in R6/2 animals by 64% compared to wild type mice. Accordingly, newly generated neurons labeled with doublecortin (DCX) were diminished by 60% in the hippocampus of R6/2 mice. Furthermore, the number of newly generated mature neurons was decreased by 76%.
Within the hippocampus of wild type animals, a four-week running period resulted in a doubling of PCNA-, DCX-, and bromo-deoxyuridine (BrdU)-labeled cells. However, physical exercise failed to stimulate proliferation and survival of newly generated neurons in R6/2 transgenic mouse model of HD.
These findings suggest that mutant huntingtin alters the hippocampal microenvironment thus resulting in an impaired neurogenesis. Importantly, this adverse microenvironment impeded neurogenesis upregulation such as induced by physical exercise. Future studies need to decipher the molecular pathways involved in repressing the generation of new neurons after physical activity in huntingtin transgenic rodents.

Intrinsic cell replacement in the brain and cervical spinal cord after thoracic contusion spinal cord injury in adult rats

K. Pfeifer, B. Kierdorf, B. Winner, N. Weidner
Dept. of Neurology, University of Regensburg, Regensburg, Germany

Despite the failure of transected axons to regenerate following contusion spinal cord injury in adult rats, significant spontaneous functional improvement is observed over time. Certain areas of the central nervous system (CNS) retain the capacity to generate new neurons and glia from an endogenous pool of progenitor cells. Recruitment of neural precursors into CNS lesion sites has been observed. The aim of the present study was to investigate whether endogenous cell replacement (neurogenesis or gliogenesis) in the brain (subventricular zone, corpus callosum, motor cortex) or spinal cord regions remote from the lesion site might represent the structural correlate for the spontaneous functional improvement. Adult Fischer 344 rats (n=8) received severe contusion injuries (200kDyne) of the midthoracic spinal cord using the IH impactor. Uninjured rats served as control (n=10). From post-operative day 4 through 14, both groups received i. p. injections of bromodeoxyuridine (BrdU). Over the course of 6 weeks, almost complete spontaneous locomotor improvement as determined by semiquantitative modified BBB scoring was observed. Six weeks post-operatively, survival of newly generated cells, defined by the number of nuclei immunoreactive for BrdU, remained unaltered in the subventricular zone, the corpus callosum and the motorcortex. Only in the cervical spinal cord (level C3) quantification of BrdU positive nuclei revealed an almost threefold increase in the number of newly generated cells in lesioned animals. These preliminary data suggest that there is no relevant cell replacement in cortical regions, which have been shown to undergo selective neuronal cell death as a sequel of spinal cord injury. In the spinal cord remote from the injury site, newborn cells most likely represent gliogenesis rather than neurogenesis, which could account at least partially for the observed spontaneous functional improvement. Ongoing studies investigate the exact differentiation pattern of BrdU positive cells in these regions. Furthermore, cell proliferation and differentiation in the brainstem, which contains relevant neuronal populations controlling locomotor function, will be investigated.

The Oligodendroglial Program of Adult Neural Progenitors: Differential Effects of CNTF and Mesenchymal Stem Cell Derived Factors

Francisco J. Rivera, Mahesh Kandasamy, Massimiliano Caioni, Rosario Sanchez, Sebastien Couillard-Despres, Norbert Weidner, Ulrich Bogdahn, Ludwig Aigner

Department of Neurology, University of Regensburg, Regensburg, Germany

The oligodendrogenic program of progenitor cells in the adult central nervous system follows a sequential process consisting of progenitor proliferation, fate choice and oligodendroglial determination, differentiation and survival. Previously, we described a soluble activity that is derived from mesenchymal stem cells (MSCs) and instructs adult neural progenitor cells to the oligodendroglial program. Here, we hypothesized that ciliary neurotrophic factor (CNTF) might be a candidate for this activity, since i) CNTF is expressed by MSCs and ii) CNTF can promote oligodendrogenesis during development. Experiments using CNTF-blocking antibodies excluded CNTF to be the MSC derived oligodendroglial activity. Along the course of the study, however, we found differential effects by CNTF and by the MSC-derived activity on neural progenitors. While the MSC-derived activity induced oligodendrogenesis at the expense of astrogenesis and it promoted oligdendroglial differentiation, the effect of CNTF was restricted to the latter one. This was reflected at the levels of cell fate determinants such as Olig1, Olig2, Id2, and the oligodendroglia-maturation transcription factor GTX / Nkx 6.2. While CNTF did not change gene expression of Olig1, Olig2 and Id2, it induced the oligodendrogliamaturation transcription factor GTX / Nkx 6.2. This work describes differential effects of CNTF and MSC derived activity on the oligodendrogenic program. Moreover, since MSCs target the entire oligodendroglial program, the findings strengthen the potential impact of a MSC based approach for the treatment of demyelinating disorders such as multiple sclerosis or of spinal cord injury.

Presenting authors

Aigner Ludwig
Department of Neurology,
University of Regensburg
Universitätsstr.84,
93053 Regensburg
Email: ludwig.aigner@klinik.uni-regensburg.de

Badde Anja
Institute of Developmental Genetics,
Research Group Adult Neurogenesis
Helmholtz Zentrum München
Ingolstädter Landstr. 1
85764 Neuherberg
Email: anja.badde@helmholtz-muenchen.de

Bergami Matteo
Department of Human and General Physiology,
University of Bologna
via San Donato 19/2
I-40126 Bologna, Italy
Email: matteo.bergami@helmholtz-muenchen.de

Björklund Anders
Wallenberg Neuroscience Center,
Lund University, BMC A11,
S-22184 Lund, Sweden
Email: Anders.Bjorklund@med.lu.se

Brill Monika S.
Institute for Stem Cell Research,
Helmholtz Center Munich
IngolstädterLandstr.1
85674 Neuherberg/Munich
Email:monika.brill@helmholtz-muenchen.de

Colak Dilek
Institute for Stem Cell Research,
Helmholtz Center Munich
IngolstädterLandstr.1
85674 Neuherberg/Munich
Email: dilek.colak@helmholtz-muenchen.de

Ehm Oliver
Institute of Developmental Genetics
Research Group Adult Neurogenesis
Helmholtz Zentrum München
Ingolstädter Landstr. 1
85764 Neuherberg
Email: oliver.ehm@helmholtz-muenchen.de

Franklin Robin J.M.
Department of Veterinary Medicine & Cambridge
Centre for Brain Repair,
Madingley Rd., Cambridge CB3 0ES
Email: rjf1000@cam.ac.uk

Gage Fred H.
Laboratory of Genetics LOG-G
The Salk Institute for Biological Studies
10010 N. Torrey Pines Rd.
La Jolla, CA 92037 , USA
Email: gage@salk.edu

Giesert Florian
Institute of Developmental Genetics
Helmholtz Zentrum München
Ingolstädter Landstr. 1
85764 Neuherberg
Email: florian.giesert@helmholtz-muenchen.de

Götz Magdalena
Institute of Stem Cell Research
Helmholtz Zentrum München,
Ingolstädter Landstr.1
85764 Neuherberg, Germany
&
Physiological Genomics, Institute of Physiology,
LMU Munich
Pettenkoferstr. 12,
80336 Munich, Germany
Email: magdalena.goetz@helmholtz-muenchen.de

Herold Sabine
Institute of Developmental Genetics
Research Group Adult Neurogenesis
Helmholtz Zentrum München
Ingolstädter Landstr. 1
85764 Neuherberg
Email: sabine.herold@helmholtz-muenchen.de

Höglinger Günter
Experimental Neurology
Biomedical Research Center (BMFZ)
Philipps University
Hans Meerwein Str
D-35043 Marburg, Germany
Email: Guenter.Hoeglinger@med.uni-marburg.de

Jagasia Ravi
Institute of Developmental Genetics,
Research Group Adult Neurogenesis
Helmholtz Zentrum München
Ingolstädter Landstr. 1
85764 Neuherberg
Email: ravi.jagasia@helmholtz-muenchen.de

Jawerka Melanie
Institute for Stem Cell Research,
Helmholtz Center Munich
85674 Neuherberg/Munich
Email:melanie.jawerka@helmholtz-muenchen.de

Kandasamy Mahesh
Department of Neurology
University of Regensburg
Universitätsstr.84
93053 Regensburg
Email:mahesh.kandasamy@klinik.uni-regensburg.de

Karl Alexandra
International Master's Program in Experimental and
Clinical Neurosciences, University of Regensburg,
Universitätsstr.84
93053 Regensburg, Germany

Klafke Ruth
Institute of Developmental Genetics
Helmholtz Zentrum München
Ingolstädter Landstr. 1
85764 Neuherberg
Email: ruth.klafke@helmholtz-muenchen.de

Klucken Jochen
Department of Neurology
University of Regensburg
Universitätsstr.84,
93053 Regensburg
Email: jochen.klucken@klinik.uni-regensburg.de

Kohl Zacharias
Department of Neurology
University of Regensburg
Universitätsstr.84,
93053 Regensburg
Email: zacharias.kohl@klinik.uni-regensburg.de

Krampert Monika
Department of Neurology
University of Regensburg
Universitätsstr.84,
93053 Regensburg
Email: monika.krampert@klinik.uni-regensburg.de

Lehner Bernadette
Department of Neurology
University of Regensburg
Universitätsstr.84,
93053 Regensburg
Email: bernadette.lehner@klinik.uni-regensburg.de

Lie Chichung D.
Institute of Developmental Genetics,
Research Group Adult Neurogenesis
Helmholtz Zentrum München
Ingolstädter Landstr. 1
85764 Neuherberg
Email: chichung.lie@helmholtz-muenche.de

Lledo Piere M.
Laboratory for Perception and Memory, Pasteur
Institute, CNRS URA 2182, 25 rue du Dr. Roux,
75724 Paris Cedex 15, France
Email: pmlledo@pasteur.fr

Marxreiter Franz
Department of Neurology
University of Regensburg
Universitätsstr.84,
93053 Regensburg
Email: franz.marxreiter@googlemail.com

Ninkovic Jovica
Institute for Stem Cell Research,
Helmholtz Center Munich
IngolstädterLandstr.1,
85674 Neuherberg/Munich
Email:ninkovic@helmholtz-muenchen.de

Pfeifer Kathrin
Department of Neurology
University of Regensburg
Universitätsstr.84,
93053 Regensburg
Email: kathrin.pfeifer@klinik.uni-regensburg.de

Pham TT
Institute of Developmental Genetics
Helmholtz Zentrum München
Ingolstädter Landstr. 1
85764 Neuherberg
Email: pham.trang@helmholtz-muenchen.de

Rivera Francisco J.
Department of Neurology
University of Regensburg
Universitätsstr.84,
93053 Regensburg
Email: francisco.rivera@klinik.uni-regensburg.de

Rudin Markus
Institute for Biomedical Engineering UZH/
ETH Zürich and Institute for Pharmacology and
Toxicology, UZH,
Wolfgang-Pauli Strasse 10
8093 Zürich, Switzerlandrudin@biomed.ee.ethz.ch

Sanchez Rodrigo
Department of Physiological Genomics,
Institute of Physiology, LMU Munich
Pettenkoferstr. 12
80336 Munich
Email: rodrigo.sanchez@lrz.uni-muenchen.de

Siebzehnrübl FA
University of Florida
McKnight Brain Institute, Dept Neuroscience
PO Box 100244
Gainesville, FL 32610-0244, USA Email:
florians@mbi.ufl.edu

Sprungala Susanne
Institute of Developmental Genetics
Helmholtz Zentrum München
Ingolstädter Landstr. 1
85764 Neuherberg
Email: susanne.sprungala@helmholtz-muenchen.de

Kathrin Steib
Institute of Developmental Genetics
Research Group Adult Neurogenesis
Helmholtz Zentrum München
Ingolstädter Landstr. 1
Email: kathrin.steib@helmholtz-muenchen.de

Steindler Dennis A.
McKnight Brain Institute
The University of Florida
100 S. Newell Drive
P.O. Box 100015
Gainesville, FL 32610-0015, USA
Email: steindler@mbi.ufl.edu

Stübner Sebastioan
Institute of Developmental Genetics
Helmholtz Zentrum München
Ingolstädter Landstr. 1
85764 Neuherberg
Email: sebastian.stuebner@helmholtz-muenchen.de

Tripathi Pratiba
Institute for Stem Cell Research,
Helmholtz Center Munich
IngolstädterLandstr.1
85674 Neuherberg/Munich
Email: pratiba.tripathi @helmholtz-muenchen.de

Weidner Norbert
Department of Neurology
University of Regensburg
Universitätsstr.84,
93053 Regensburg
Email: norbert.weidner@klinik.uni-regensburg.de

Winkler Jürgen
Department of Neurology
University of Regensburg
Universitätsstr.84,
93053 Regensburg
Email: juergen.winkler@klinik.uni-regensburg.de

Winner Beate
Salk Institute. Laboratory of Genetics,LOG-G
10010 North Torrey Pines Road
La Jolla, CA 92037-1099, USA
Email: beate.winner@klinik.uni-regensburg.de
winner@salk.edu

Wurst Wolfgang
Institute of Developmental Genetics
Helmholtz Zentrum München
Ingolstädter Landstr. 1
85764 Neuherberg
& Max Planck Institute of Psychiatry, Munich,
& Technical University of Munich
Email: wurst@helmholtz-muenchen.de

Wyss-Coray Tony
Department of Neurology and Neurological Sciences
Stanford University, School of Medicine
300 Pasteur Drive, Rm-A343
Stanford, CA 94305-5235, USA
e-mail: twc@stanford.edu