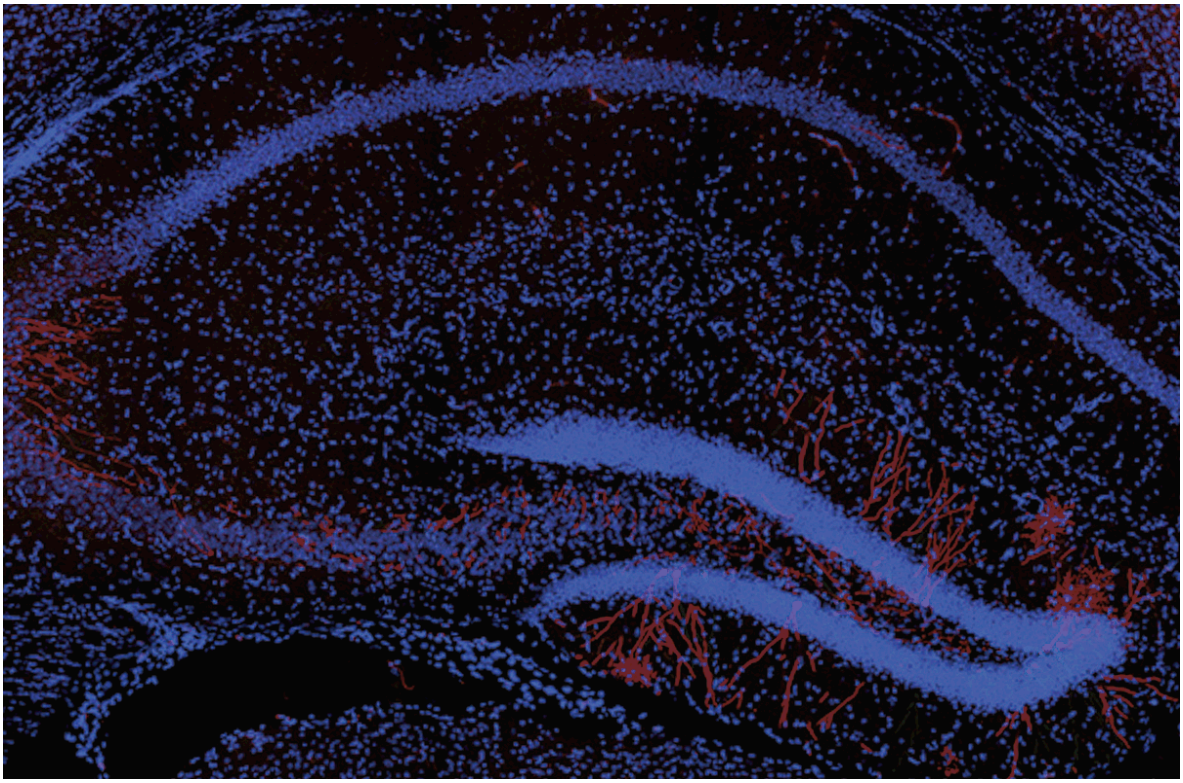
ForNeuroCell



Symposium of the Bavarian Research Network for Adult Neural Stem Cells ForNeuroCell

April 07 – 08, 2011
CARL FRIEDRICH
VON SIEMENS STIFTUNG
Südliches Schlossrondell 23
80638 München

Funded by Bavarian State Ministry
of Sciences, Research and the Arts

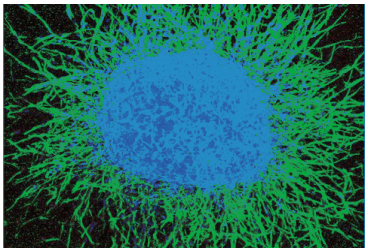
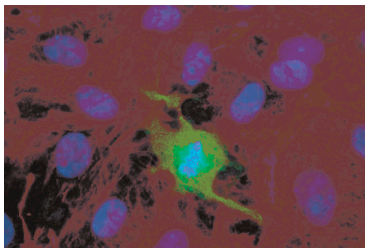
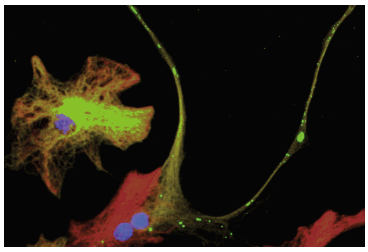
Symposium of the Bavarian Research Network for Adult Neural Stem Cells ForNeuroCell

April 07, 2011

10.30 – 17.30	Closed session of ForNeuroCell
17.00	Registration
17.30 – 19.00	**Poster**
19.00	**Welcome** Juergen Winkler, Speaker ForNeuroCell
19.10	**Lecture** **Creating neuronal diversity in the human neocortex** Arnold Kriegstein, The Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research at UCSF, San Francisco, USA
20.15	**Get together with buffet dinner**

April 08, 2011

Session I	**Adult Neurogenesis: Molecular Mechanisms** Chair: Magdalena Götz, Michael Sendtner
08:30 – 09:10	**Vasculature-guided migration of neuronal precursors in the postnatal brain: From development to disease** Armen Saghatelyan, Département de Psychiatrie, Université Laval, Centre de Recherche Université Laval, Robert-Giffard (CRULRG) Beauport (Québec), Canada
09:10 – 09:40	**Voluntary versus forced metamorphosis of astroglia or pericytes into neurons** Benedikt Berninger, Department of Physiological Genomic, Ludwig Maximilians University, Munich, Germany
09:40 – 10:20	**Genetic control and functional consequences of adult neurogenesis** Gerd Kempermann, CRTD – Center for Regenerative Therapies Dresden, and DZNE, German Center for Neurodegenerative Diseases, Dresden, Germany
10:20 – 11:00	**Coffee break**
11.00 – 11:40	**Hdac inhibitor and neural stem cell transplantation (HINT) method for the treatment of spinal cord injury** Kinichi Nakashima, Laboratory of Molecular Neuroscience, Nara Institute of Science and Technology, Takayama, Ikoma, Japan

Session II

Imaging and Beyond: Watching Structure and Probing Function
Chair: Albrecht Stroh, Jochen Herms

11:40 – 12:20	**Imaging development and plasticity of adult-born neurons in the mouse olfactory bulb** Adi Mizrahi, Department of Neurobiology, The Alexander Silbermann Inst. of Life Sciences, The Hebrew University of Jerusalem, Jerusalem, Israel
12:20 – 13:00	**Defining the neuronal circuitry of fear** Andreas Lüthi, Friedrich Miescher Institut, Basel, Switzerland
13:00 – 15:00	**Lunch-Poster Session**
14:00 – 15:00	Guided tour
Session III	**Translation: From Bench to Bedside** Chair: Wolfgang Wurst, Chichung Lie
15:00 – 15:40	**Immunregulatory properties of mesenchymal stem cells: Lessons from preclinical animal studies** Antonio Uccelli, Department of Neurosciences, Ophthalmology and Genetics, University of Genoa, Genoa, Italy
15:40 – 16:20	**Role of a-synuclein propagation in neuronal and stem cell dysfunction; pathogenesis and therapeutical prospects** Eliezer Masliah, Department of Pathology and Medicine, University of California, San Diego, USA
16:20 – 16:50	**Coffee break**
16:50 – 17:20	**Best Posters: Presentation 3x10min**
17:20 – 17:50	**Adult neurogenesis in Parkinson disease: a potential link to premotor symptoms** Juergen Winkler, Division of Molecular Neurology, Friedrich-Alexander- University Erlangen-Nuremberg, Erlangen, Germany
17:50	Closing remarks

Key Lecture

CREATING NEURONAL DIVERSITY IN THE HUMAN NEOCORTEX

Arnold Kriegstein

Recent insights gained from studies of the developing cerebral cortex are illuminating potential evolutionary steps that contributed to structural and functional features of the human brain. Radial glial cells (RG), long thought to simply guide embryonic nerve cells during migration, have now been identified as neuronal stem cells in the developing brain. RG cells undergo self-renewing, asymmetric divisions to generate neuronal precursors that can further proliferate in the subventricular zone (SVZ) to increase neuronal number. Unlike the developing rodent cortex, the developing human cortex contains a massively expanded SVZ (OSVZ) that is thought to account for the bulk of cortical neurogenesis. We have begun to characterize the types and locations of progenitor cells responsible for human cortical development. We found that large numbers of radial glia-like cells and intermediate progenitor cells populate the human OSVZ. The OSVZ radial glia-like cells have a long basal process but, surprisingly, are non-epithelial since they lack contact with the ventricular surface. Using real-time imaging and clonal analysis, we demonstrate that these cells undergo self-renewing asymmetric divisions to generate neuronal progenitor cells that can further proliferate. We have recently found that progenitor cells resembling oRG cells are present in mouse embryonic neocortex, and arise from asymmetric divisions of radial glia. Time-lapse imaging reveals that the cells undergo self-renewing asymmetric divisions to generate neurons. These results suggest that oRG cells are probably present in all mammals and are not a specialization of a larger brain with increased cortical area. Instead, an evolutionary increase in the number of oRG cells and their transit amplifying daughter cells likely amplified neuronal production and contributed to increased cortical size and complexity in the human brain.

Session I
Talk I

VASCULATURE-GUIDED MIGRATION OF NEURONAL PRECURSORS IN THE POSTNATAL BRAIN: FROM DEVELOPMENT TO DISEASE

Armen Saghatelyan

The adult mammalian forebrain retains the remarkable capacity to produce new neurons throughout its entire life span. Neuronal precursors are generated in the subventricular zone (SVZ) and migrate first tangentially, in chains, along the rostral migratory stream (RMS) and once in the olfactory bulb (OB), turn to migrate radially and individually out of the RMS into the bulbar layers. One of the key question in the field is how can these neuronal precursors migrate such a long distance, through complex brain territories, from the posterior (SVZ) to the most anterior parts of the brain (OB). What keeps neuronal precursors in the migratory pathway and what orients them towards OB?

We demonstrate that adult neuronal precursors are guided towards and into OB by blood vessels that topographically outline the RMS and serve as a physical scaffold for migrating neuroblasts in the adult rodent brain. Blood vessels also provide molecular cues that guide neuronal precursors in the adult RMS. The endothelial cells synthesize brain-derived neurotrophic factor (BDNF) that is released within the extracellular space and fosters migration of neuronal precursors in the adult mammalian forebrain.

We also show that astrocytes orchestrate the formation and structural reorganization of this very particular vasculature scaffold in the early postnatal RMS. Our morphological analysis demonstrates that during the early developmental periods, RMS contains only few blood vessels oriented randomly with regard to the migrating neuroblasts. The first parallel blood vessels appear at the border of RMS where most of astrocytes are located. We reveal that conditioning medium derived from SVZ-RMS but not from cortex or cerebellum astrocytes potentiate formation and growth of new blood vessels and that this effect is dependent on VEGF signalling.

Finally, we demonstrate that BDNF-dependent vasculature-mediated migration of neuronal precursors is re-activated by the injured brain to recruit neuroblasts into the post-stroke striatum. About 90% of neuroblasts that migrate out of SVZ to the post-stroke striatum do so along the blood vessels. Interestingly, while BDNF is not expressed along the blood vessels in the control sham-operated striatum, stroke induces expression of this trophic factor with a pattern similar to that observed in the RMS.

Altogether, these data reveal the role of vasculature in the guidance of neuronal precursors under normal conditions and following injury.

Talk II

VOLUNTARY VERSUS FORCED METAMORPHOSIS OF ASTROGLIA OR PERICYTES INTO NEURONS

Benedikt Berninger

I will discuss neurogenesis from astroglia from two perspectives, namely the physiological process of how astroglia-like stem cells generate neurons in the adult subependymal zone (SEZ) and how parenchymal astroglia or other somatic brain cell types such as pericytes from the cerebral cortex can be instructed towards neurogenesis.

To directly observe cell division and lineage progression of astroglia-like stem cells, we isolated cells from the adult SEZ and cultured them without growth factors at low density. We demonstrate here that SEZ cells in this culture system are primarily neurogenic and progress through stereotypic lineage trees comprising asymmetric stem cell divisions, symmetric transit-amplifying divisions and final symmetric neurogenic divisions.

The fact that in the absence of exogenously supplied growth factors and signals provided by the local niche such neurogenic lineage progression takes place in a highly stereotypic fashion, suggests that it is to a significant degree cell-intrinsic or pre-programmed at the beginning of the lineage.

Contrasting with this physiological metamorphosis of astroglia into neurons, we have previously shown that astroglia from the postnatal cerebral cortex are non-neurogenic, but can be reprogrammed in vitro to generate neurons following forced expression of neurogenic transcription factors, such as Neurog2 or Mash1. Here we extend these studies to another somatic brain cell type, namely paricytes isolated from the adult cerebral cortex of both mouse and man. Importantly, while neurogenic fate determinants alone fail to induce neurogenesis, upon co-expression with Sox2 a striking synergism can be observed allowing for the generation of functional neurons.These studies provide a basis for direct reprogramming of cells endogenous to the brain as a viable alternative to transplantation for brain repair.

Talk III

GENETIC CONTROL AND FUNCTIONAL CONSEQUENCES OF ADULT NEUROGENESIS

Gerd Kempermann

Adult neurogenesis is a complex quantitative trait (QTL) and highly polygenic with individual genes explaining very little of the variance found in populations. We study adult neurogenesis in the BXD panel of recombinant inbred mice as a genetic reference population in order to explore the individual and interactive contribution of groups of genes to the net regulation. To achieve this goal, transcriptome data are integrated into the analysis and the covariance between expression data and the physiological phenotype is used to generate networks of potentially regulatory genes, which can be further explored. In a next step, we combined this approach with an analysis addressing the function of adult neurogenesis. We have developed a modification of the classical water maze test based on the analysis of the strategies used by the mice to address, how new neurons increase cognitive flexibility in the acquisition and reversal period of the test.

Many studies have explored the question what happens to the function of the hippocampus, when adult neurogenesis is ablated but gain-of-function experiments have as yet not been published. Physical exercise and environmental enrichment are two behavioral paradigms with proven effects on adult neurogenesis. They are additive in their effect and despite similar end results, appear to have specific sub-effects on adult neurogenesis. Studying the same phenotypes in the BXD panel revealed large variance in diverse subtraits and revealing a suggestive covariance with the adult neurogenesis traits.

Talk IV

HDAC INHIBITOR AND NEURAL STEM CELL TRANSPLANTATION (HINT) METHOD FOR THE TREATMENT OF SPINAL CORD INJURY

Kinichi Nakashima

Neural stem cells (NSCs) possess the ability to self-renew and to differentiate into the three major cell types found in the central nervous system (CNS): neurons, astrocyte and oligodendrocytes. Recent studies have shown that epigenetic gene regulation events such as DNA methylation and histone modification play important roles in regulating NSC fate specification. In this context, we have previously shown that the histone deacetylase (HDAC) inhibitor and well-known antiepileptic drug, valproic acid (VPA) enhances neuronal differentiation of NSCs. Perhaps because patterns of NSC differentiation are exquisitely controlled during normal embryonic development, restoration of damaged neural networks in the injured adult CNS is severely limited. Here, using a mouse model of spinal cord injury (SCI), we show that administering VPA and transplanting NSCs (Hdac Inhibitor and Neural stem cell Transplantation; HINT) enhances the functional recovery of their hindlimbs. Neuronal differentiation of transplanted NSCs was promoted in VPA-treated mice. Anterograde corticospinal tract tracing revealed that transplant-derived neurons partially reconstructed the broken neuronal circuits, most likely in a ‘relay’ manner. Ablation of the transplanted cells abolished the recovery of hindlimb motor function, indicating that transplanted cells contributed directly to the improvement of motor function. These data raise the possibility that epigenetic regulation in transplanted neural stem cells can be exploited to provide treatment for SCI.

Session II
Talk I

IMAGING DEVELOPMENT AND PLASTICITY OF ADULT-BORN NEURONS IN THE MOUSE OLFACTORY BULB

Adi Mizrahi

The adult mammalian brain maintains a prominent stem cell niche in the subventricular zone supplying new local neurons to the olfactory bulb (OB). We use time lapse in vivo two-photon microscopy to examine the dynamics of dendritogenesis and synaptogenesis of these neurons in mice. Specifically, I will discuss how we follow formation and elimination of clusters of postsynaptic markers (PSD95-GFP) during development and in adulthood. Immature PGNs showed high levels of PSD95 puncta dynamics while mature PGNs are more stable. However, since structural dynamics persist in adulthood we hypothesized that synaptogenesis of fully integrated neurons may account for their plasticity. By combining intrinsic signal imaging and two photon imaging we then followed PSD95 puncta in sensory enriched glomeruli. Sensory input up-regulated dendritogenesis and synaptogenesis during development but stabilized synapses in mature cells. These data provide evidence for distinct mechanisms of activity-based regulation during development and in adulthood. We suggest that the window for plasticity of adult-born neurons spans well into their mature phase. Finally, I will discuss our preliminary data showing two-photon targeted physiology of these neurons on our way to decipher their contribution (if any) to olfactory processing.

Talk II

DEFINING THE NEURONAL CIRCUITRY OF FEAR

Andreas Lüthi

We use an interdisciplinary approach to address the question how amygdala microcircuits mediate the acquisition and extinction of conditioned fear responses. In my talk, I will describe how switches in the activity between distinct types of amygdala neurons mediate context-dependent expression and extinction of fear memories. Moreover, I will present recent data demonstrating that functionally distinct types of amygdala neurons are specifically embedded and precisely connected both within the local circuitry and within larger-scale neuronal networks. Thus, in contrast to previous models suggesting that amygdala neurons are active during states of high fear and inactive during states of low fear, our findings indicate that activity in specific neuronal circuits within the amygdala cause opposite behavioral outcomes and provide a new framework for understanding context-dependent expression and extinction of fear behavior.

Session III

Talk I

IMMUNREGULATORY PROPERTIES OF MESENCHYMAL STEM CELLS: LESSONS FROM PRECLINICAL ANIMAL STUDIES

Antonio Uccelli

Talk II

ROLE OF A-SYNUCLEIN PROPAGATION IN NEURONAL AND STEM CELL DYSFUNCTION; PATHOGENESIS AND THERAPEUTICAL PROSPECTS

Eliezer Masliah

Parkinson's Disease (PD) and Dementia with Lewy bodies (DLB) are common neurogenerative disorders of the aging population characterized by abnormal accumulation of a-synuclein in synapses. Alterations in the balance between factors promoting aggregation, clearance and synthesis of a-synuclein might be centrally involved in the formation of oligomers and the pathogenesis of neurodegeneration. In addition, recent studies suggest that propagation of a-synuclein oligomers from neuron to neuron and neuron to glia in a prion-like fashion might also contribute to neurodegenerative process. a-synuclein can propagate from cell to via exocytosis-endocytosis, trans-synaptic delivery and exosomes. Grafted neuronal precursor cells uptake and propagate a-synuclein oligomers leading to graft dysfunction and neuro-inflammatory responses. While a-synuclein propagate in a synuclein reach environment, deletion of endogenous a-synuclein reduces the dissemination of exogenously applied a-synuclein from neuron to grafted stem cells. These findings demonstrate the cell-to-cell transmission of a-synuclein aggregates and provide critical insights into the mechanism of progression and treatment in PD and other a-synucleonopathies. Therapeutical strategies directed at reducing the formation and propagation of a-synuclein oligomers might be critical in developing new treatments for PD and DLB. Among them considerable effort has been devoted in the last few years at promoting the clearance. This can be achieved by increasing lysosomal activity (autophagy) or degradation with specific synucleinproteases such as neurosin or via utilizing antibodies or small organic molecules that block a-synuclein. *The propagation studies where done with support from NIH and in collaboration with Dr. Seung-Jae Lee at Konkuk University Seoul, Korea.*

Best Posters
Poster 2

RABIES VIRUS MEDIATED TRACING OF SYNAPSES ONTO ADULT GENERATED NEURONS

Deshpande A[1], Conzelmann KK[3], Götz M[1,2], Berninger B[1,2]
[1]Department of Physiological Genomics, Institute of Physiology, Ludwig-Maximilians University, Munich [2]Institute for Stem Cell Research, National Research Center for Environment and Health, Neuherberg, [3]Max von Pettenkofer Institute and Gene Center, Ludwig-Maximilians-University Munich, Munich

In the mammalian brain adult neurogenesis is confined to two neurogenic niches - the subgranular zone of the dentate gyrus (DG) of the hippocampus and the subependymal zone (SEZ) of the lateral ventricle. Although it is known that the newborn neurons integrate into preexistent networks, the precise nature of this functional integration as well as cellular specificity of the pre- and postsynaptic targets is not well understood. Here we aimed at identifying the presynaptic partners of adult generated neurons by modifying a recently developed method of retrograde tracing of monosynaptic connections using an EnvA pseudotyped reporter-expressing rabies virus. In order to specifically target proliferating cells we designed a Moloney based retrovirus that allows for the expression of two key proteins – TVA, required for infection by the EnvA-pseudotyped rabies virus and a glycoprotein, required for transsynaptic spread by the rabies virus. Progenitors transduced with the retrovirus will get infected by the replication competent pseudotyped rabies virus and can subsequently transport the virus to their presynaptic partners upon neuronal maturation and integration into the network. Accordingly, in embryonic cortical cultures, infection with the rabies virus 2-3 weeks after transduction with the retrovirus, we observed double (retro- and rabies virus)-transduced cells, surrounded by a cluster of RV only infected cells. Paired recordings from double (postsynaptic) and single (presynaptic) labeled cells showed that these cells are indeed synaptically connected.

To identify presynaptic partners of adult-generated neurons in vivo, we performed stereotaxic injections of the retrovirus encoding TVA and the glycoprotein into the dentate gyrus of adult C57B/L6 mice, followed by a second injection of the EnvA-pseudotyped rabies virus. At earlier time points we observed both, double labeled granule neurons as well as cells in the hilar region of the dentate gyrus which were labeled with rabies virus only. Neurochemical marker expression, location of the cell body as well as the pattern of their axonal and dendritic arborisations suggest that these cells comprise distinct types of local interneurons. These data are consistent with the idea that GABAergic interneurons are amongst the first to form synapses onto newly generated granule cells. At later time points following rabies virus injection, we also observed distinct labeling of pyramidal neurons in the entorhinal cortex, the primary excitatory input to granule neurons via the perforant path.

We performed similar studies in the olfactory bulb where we injected the retrovirus into the SEZ to label progenitors, followed (4 days later) by the rabies virus injection into the rostral migratory stream to hit migrating cells expressing the retroviral transgenes. Upon their arrival in the olfactory bulb we observed a heterogeneous population of cells infected with rabies virus alone in the olfactory bulb itself and interestingly, also pyramidal neurons in the anterior olfactory nucleus (AON) which are known to form projections onto bulbar granule cells. This data shows that some of the earliest excitatory synaptic input onto new-born neurons in the olfactory bulb originates in the AON.

Poster 11

SC1 IS A CRITICAL MEDIATOR OF DIFFERENTIATION OF NEURAL STEM CELLS IN DEVELOPING MOUSE BRAIN

Götz R, Orel N, Sendtner M
Institute for Clinical Neurobiology, Universitätsklinikum Würzburg, Würzburg

Prdm4 or Schwann cell factor 1 (SC1) belongs to the family of Prdm proteins which contain zinc finger motifs and a protein segment called the positive regulatory (PR) domain. We have shown before that SC1 is a direct binding partner of the p75 neurotrophin receptor that can translocate into the nucleus after receptor activation and affect the expression of distinct cyclin genes in PC12 cells and other cell lines. Overexpression of SC1 in mouse embryonic neural stem cells inhibits mitosis and induces apoptosis. Whereas neither neurotrophic factor signalling nor absence of the p75 neurotrophin receptor protected neural stem cells from apoptosis caused by SC1 overexpression, the absence of Bax did. The conditional elimination (using a “floxed” SC1 allele) of the SC1 gene in cultured embryonic forebrain neural stem cells did not affect their proliferation or alter the cell cycle profile in serum-free medium in the presence of epidermal growth factor and basic fibroblast growth factor. Differentiation of cultured neural stem cells into neurons as well as astrocytes was severely impaired in the absence of SC1. In conclusion, our data suggest that SC1 may act in neural stem cells as a key regulator of neuronal and astrocytic differentiation and survival by mediating neurotrophin effects via p75 neurotrophin receptor signalling.

Poster 18

ROLE OF SOXC PROTEINS IN ADULT HIPPOCAMPAL NEUROGENESIS

Mu L[1]*, Berti L[1]*, Maserdotti G[2]*, Covic M[1], Michaelidis T[1,3], Merz K[1], Herold S[1], Haslinger A[1], Wegner M[4], Sock E[4], Lefebvre V[5], Götz M[2,6], Aigner L[7], Couillard Despres S[7], Berninger B[2], Lie DC[1]

[1]Research Group Adult Neurogenesis and Neural Stem Cells, Institute of Developmental Genetics, Helmholtz Zentrum München, German Research Center for Environmental Health, Munich-Neuherberg [2]Department of Physiological Genomics, Institute of Physiology, Ludwig-Maximilians University Munich, Munich [3]Foundation for Research & Technology-Hellas, Biomedical Research Institute, University of Ioannina, Science and Technology School, 45110 Ioannina [4]Institute for Biochemistry, Emil-Fischer-Zentrum, University Erlangen-Nürnberg, Erlangen, Germany [5]Lerner Institute, Cleveland Clinic, Cleveland, Ohio [6]Institute for Stem Cell Research, National Research Center for Environment and Health, Munich-Neuherberg [7]Institute for Molecular Regenerative Medicine Paracelsus Medical University, Salzburg

* these authors contributed equally

In the adult mammalian brain, neural stem and progenitor cells generate neurons throughout adulthood in two specific regions, the subgranular zone of the hippocampal dentate gyrus and the subventricular zone of the lateral ventricle. Neuronal fate determination, subtype specification and differentiation are controlled by the interaction of different transcription factors. In our study, we found Sox4 and Sox11, members of the group C Sox transcription factor family, to be prominently expressed in doublecortin (DCX)-expressing neuronal committed precursors and immature neurons in the neurogenic areas of the adult brain. Our in vitro studies indicate that overexpression of Sox4 and Sox11 upregulate DCX mRNA levels and promote the generation immature neurons from adult neural stem cells, whereas loss of Sox4 and Sox11 decreases DCX mRNA level and reduces the generation of immature neurons. In vivo absence of Sox11 and Sox4 impaired the development of immature neurons, and overexpression of Sox11 delayed the further differentiation of immature neurons. Finally, we provide evidence that SoxC transcription factors bind to DCX and also can active DCX promoter in vitro. These data suggest that transcription factors of the SoxC family have essential roles in the transcriptional regulation of neuronal differentiation in adult neurogenesis.

Talk III

ADULT NEUROGENESIS IN PARKINSON DISEASE: A POTENTIAL LINK TO PREMOTOR SYMPTOMS

Juergen Winkler

Parkinson's disease (PD) is characterized by a progressive loss of distinct, relatively well-defined neuronal populations in different neurotransmitter projection systems. The clinical course is slow and current strategies are not able to halt or even reverse the progression of the disease. The pathological hallmark in PD is alpha-synuclein aggregates observed within Lewy-bodies and Lewy-neurites. The most prominent biochemical deficit is the dopaminergic dysfunction within the nigro-striatal projection resulting from neuronal loss within the substantial nigra pars compacta. Although the prototypical motor symptoms in PD are bradykinesia, rigor, tremor and postural instability, the recognition that non-motor symptoms occur in the pre-motor phase have revolutionized our understanding in PD. Olfactory loss and neuropsychiatric deficits such as depression and anxiety are the most prevalent symptoms in the pre-motor phase of PD. Based on the spatial and temporal pattern of alpha-synuclein pathology in the lower brainstem nuclei and the olfactory bulb, one exciting idea is that PD-associated mechanisms such as alpha-synuclein aggregation or the dopaminergic deficit may interfere with cellular and synaptic plasticity in both neurogenic regions at a very early stage of the disease. The interaction between adult hippocampal as well as subventricular zone (SVZ)/ olfactory bulb neurogenesis and PD associated neurodegenerative mechanisms revealed that both proliferation and/ or survival of newly generated neural cells are severely affected in different preclinical *in vivo* models. In addition, previous post-mortem studies revealed that adult neurogenesis is impaired in the SVZ/ olfactory bulb and the hippocampus of PD patients. Taken these experimental and clinical findings together, impaired adult neurogenesis may be related to the most prevalent and important non-motor symptoms, but more importantly, it offers the opportunity to develop novel strategies to restore some of these symptoms.

Notes

Poster 1

CONTINUOUS LIVE IMAGING OF ADULT NEURAL STEM CELL DIVISION AND LINEAGE PROGRESSION IN VITRO

Ortega F[1], Costa MR[3], Brill MS[1], Beckervordersandforth R[2], Petrone C[2], Schroeder T[2], Götz M[1, 2], Berninger B[1, 2]
[1] Department of Physiological Genomics, Institute of Physiology LMU, [2] Institute of Stem Cell Research, HelmholtzZentrum München, [3] Instituto do Cérebro, Universidade Federal do Rio Grande do Norte

Still very little is known about the intrinsic specification of adult neural stem cells (NSCs) and to which extent they depend on their local niche. To observe adult NSC cell division and lineage progression independent of the normal niche environment, we isolated cells from the adult subependymal zone (SEZ) and cultured them without growth factors at low density. We demonstrate here that SEZ cells in this culture system are highly neurogenic and isolated adult NSCs progress through stereotypic lineage trees comprising asymmetric stem cell divisions, symmetric transit-amplifying divisions and final symmetric neurogenic divisions. Stem cells, identified by their astro/radial glial identity and their slow-dividing nature, were observed to generate fast dividing astro/radial glia. Strikingly, these fast dividing astro/radial glia eventually divided asymmetrically with one daughter cell generating neuroblasts after several rounds of symmetric amplifying divisions, while the other daughter cell gave rise to 1 or 2 cells of astroglial identity. These astroglial cells typically ceased to proliferate, raising the question whether they have become permanently postmitotic or entered quiescence. Upon challenge with mitogenic growth factors such as EGF/FGF2 we found that these astroglial cells can indeed re-enter cell cycle suggesting that they may retain stem cell character.

Poster 2

RABIES VIRUS MEDIATED TRACING OF SYNAPSES ONTO ADULT GENERATED NEURONS

Deshpande A[1], Conzelmann KK[3], Götz M[1,2], Berninger B[1,2]
[1]Department of Physiological Genomics, Institute of Physiology, Ludwig-Maximilians University, Munich [2]Institute for Stem Cell Research, National Research Center for Environment and Health, Neuherberg, [3]Max von Pettenkofer Institute and Gene Center, Ludwig-Maximilians-University Munich, Munich

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Poster 3

THE ROLE OF THE TRANSCRIPTION FACTOR OTX2 IN CHOROID PLEXUS SPECIFICATION ALSO REVEALS A ROLE OF SIGNALS CONVEYED BY THE CEREBROSPINAL FLUID DURING DEVELOPMENT

Johansson PA, Irmler M, Beckers J, Acampora D, Simeone A and Götz M
Helmholtz Center Munich, German Research Center for Environmental Health, Institute for Stem Cell Research, Neuherberg/Munich

It has long been suggested that cerebrospinal fluid (CSF) might contain important signalling molecules influences brain development, however direct evidence has so far been lacking. The choroid plexuses (CP), which secrete CSF, differentiate between embryonic day (E) 10 and 14 in mouse embryos and acquire both barrier, secretory and transport capacities shortly after their formation (Johansson et al 2006). However, little is still known about factors regulating CP development and thereby influencing CSF composition. Here we set out to elucidate these functions by manipulation the transcription factor Otx2, which we have suggested to define CP territory by reciprocal inhibition with the neuroepithelial determinant Emx2 (von Frowein et al 2006). Thus the aim of this study was to investigate the effect of genetic Otx2 deletion on CP development and its possible subsequent effects on CSF composition and brain development.

Conditional deletion of floxed Otx2 alleles using the roof-plate specific Gdf7-Cre mouse line (recombination from E10,5) resulted in loss of Otx2 only in the 4th ventricular CP (see also Currle et al 2005). While the CP markers transthyretin and aquaporin-1 were expressed in the 4th ventricular CP at E12 indicating its proper specification, it was severely hypomorphic at later stages due to increased cell death. Interestingly, selective defects in 4th ventricular CP also resulted in markedly increased protein concentration in the CSF and a decrease in the size of the forebrain ventricles at E14 and E16. Intriguingly we also observed a significant decrease in proliferation in the far distant cerebral cortex at E16. Transcriptome analysis of the mutant CP reveals significant alterations in candidate growth factors which will be presented. Thus, selective deletion of Otx2 in the 4th ventricle confirmed a role of Otx2 in CP differentiation and long range effects in brain development.

To further determine the role of Otx2 at other sites of CP development, we took advantage of the Otx2::CreERT2 mouse line to delete Otx2. Tamoxifen-mediated induction at E9 resulted in an almost complete absence of the forebrain choroid plexi with no remnants of transthyretin indicating the complete lack of CP specification after early deletion of Otx2.

Taken together, these data demonstrate a key role for Otx2 in CP specification, development and function. Further, hypotrophy of the 4th ventricle CP resulted in changes in CSF composition and in the development of non-choroidal tissues far distant from the site of deletion supporting the concept of long range influences of the CSF on brain development.

Poster 4

THE BRAHMA RELATED GENE 1 (BRG1)-CONTAINING CHROMATIN REMODELLING COMPLEX INTERACTS WITH THE TRANSCRIPTION FACTOR PAX6 TO MAINTAIN THE NEUROGENIC POTENTIAL OF ADULT NEURAL PROGENITORS

Ninkovic J[1,2], Jawerka M[2], Beilschmidt L[1], Ashery-Padan R[3], Götz M [1,2]
[1]Helmholtz Zentrum München, Institute of Stem Cell Research, Ingolstädter Landstraße 1, D-85764 Neuherberg, [2]Institute of Physiology, Physiological Genomics, Ludwig-Maximilians University Munich, Schillerstr.46, Munich, [3]Human Genetics Faculty of Medicine, Tel Aviv University, Ramat Aviv

Neural stem cells (NSC) persist in the adult subependymal zone life-long and have the capacity to produce both olfactory bulb (OB) interneurons and oligodendrocytes of the corpus callosum. The intrinsic fate determinants Pax6 or Olig2 influence this fate decision and are regulated by extrinsic signals, such as BMP inhibiting Olig2 expression (Colak et al., 2008; Jablonska, et al, 2010). However, the molecular mechanisms of how Pax6 endows cells with a neurogenic fate are still elusive. Here we show that Brg1, an ATP-dependent chromatin remodelling factor, is required for the neurogenic fate maintenance. In the absence of Brg1 function, adult-generated neuroblasts convert to glial cells expressing the hallmarks of glial progenitors such are NG-2 and Olig2. We further showed that Brg1 directly interacts with Pax6 in the migrating neuroblasts at the late stage of their differentiation. Similar to Brg1 loss-of-function, the loss of Pax6 in neuroblasts, even when already migrating along the rostral migratory stream (RMS) towards the OB, results in the fate conversion of these neuroblasts towards an NG2+ glia fate. Interestingly, Pax6 expression is not altered in the Brg1 mutant and Pax6 can not mediate neurogenesis in the absence of Brg1. These data suggest that Brg1 and Pax6 form a functional complex necessary for the maintenance of neuroblast fate in the adult RMS. We shall further present data demonstrating a transient role of this complex in neuronal differentiation, as this complex is no longer needed in mature OB neurons, such as the dopaminergic periglomerular neurons that do not alter their identify upon loss of either Pax6 or Brg1. In summary, our data reveal the functional complex of the transcription factor Pax6 with the Brg1-containin chromatin remodelling machinery as a key mechanism to maintain neuronal fate in migrating neuroblasts, thereby identifying for the first time a mechanism necessary for the active maintenance of commitment in adult neurogenesis.

Poster 5

ROLE OF FOXO TRANSCRIPTION FACTORS IN ADULT NEURAL STEM CELL MAINTENANCE

Khan MA[1], Schäffner I[1], Schwarz T[1], DePinho RA[2], Götz M[3], Aigner L[4], Lie DC[1]
[1]Research Group Adult Neurogenesis and Neural Stem Cells, Institute of Developmental Genetics, Helmholtz Zentrum München, German Research Center for Environmental Health, Munich-Neuherberg, [2]Dana-Farber Cancer Institute, Boston, [3]Department of Physiological Genomics, Institute of Physiology, Ludwig-Maximilians University Munich, Munich, [4]Institute for Molecular Regenerative Medicine Paracelsus Medical University, Salzburg

The relative quiescence of neural stem cells (NSC) in the adult neurogenic regions ensures stem cell longevity (i.e., long-term maintenance of self-renewal ability) by prevent ing exhaustion of the limits of proliferation capacity. Recent studies have identified a number of pathways including PI3K-AKT, Smad, Notch and Wnt controlling embryonic and tissue stem cell self-renewal, maintenance and regenerative responses.

FoxO transcription factors play an important role in diverse physiologic processes, including induction of cell cycle arrest, stress resistance and DNA repair. Four members of the FoxO subfamily, FoxO1, FoxO3, FoxO4 and FoxO6 are important downstream targets of the evolutionarily conserved PI3K-AKT pathway. Using a conditional knockout strategy we are investigating the effects of stem cell specific loss of FOXOs on neurogenesis in vitro and in vivo. In vitro, loss of FOXOs results in strongly increased stem cell proliferation and self-renewal. Interestingly, the profound in vitro effects of loss of Foxo on stem cell proliferation and self-renewal are not observed in the in vivo siutation, indicating the loss of FOXO is compensated. Ongoing work is investigating the pathways in adult neural stem cell proliferation and self-renewal downstream of FOXO. In addition, we are investigating the molecular mechanisms that compensate for the loss of Foxo in neural stem cells in vivo.

Poster 6

FROM STEM CELLS TO COGNITION: DISINHIBITON OF TGF-BETA SIGNALLING BY SMAD7 KNOCKOUT IN NEURAL STEM CELLS CONTRIBUTES TO HIPPOCAMPAL NEUROGENESIS ASSOCIATED LEARNING AND MEMORY.

Menon A[2], Kandasamy M[1], Chirasani S[2], Couillard-Despres S[1], Felder S[2], Kleiter I[2], Aigner L[1]
[1]Institute of Molecular Regenerative Medicine, Paracelsus Medical University, Salzburg, [2]Department of Neurology, University of Regensburg

Adult neurogenesis has been seen to be regulated by a plethora of internal and external stimuli. Recent studies have demonstrated that the dysregulation of the TGF-beta signalling cascade, as seen during various neurological and psychiatric disorders, has an impact on this ongoing process of neurogenesis. TGF-beta has been known to be involved in development, cell proliferation, cell differentiation and also is known for its neuro protective effect.

In this study we aim to understand the role of SMAD7 which acts as a feedback inhibitor of TGF-beta signalling cascade, on hippocampal neurogenesis and its impact on cognition. To study the role of SMAD7, we generated a transgenic mouse model where the SMAD7 can be conditionally knocked out in Nestin expressing neural stem/precursor cells via a tamoxifen - inducible CRE recombinase system.

We demonstrate that knocking out of SMAD7 gene results in reduced proliferation of the stem/precursor cells in the sub granular zone of the dentate gyrus in the hippocampus at 3 months of age. However the newly generated cells in the recombined mice showed better survival compared to the wild type. When tested in morris water maze, the recombined mice also showed better learning capacity and enhanced memory. The enhancement in hippocampal dependant cognition in the recombined mice was correlated with an increase in the newly generated immature neuronal population and a higher percentage of expression of mature neuronal marker. To address how ageing influences the process of neurogenesis under the impact of SMAD7 deletion, we analysed one year old mice that were recombined at 3 months of age and found a similar pattern of reduction in cell proliferation and a better survival potential of the newly generated cells. Surprisingly the recombined animals proved to be resilient to the ageing impact on learning. As these 1 year old mice still retained enhanced learning ability as was seen during 3 months of age, but contrary to their 3 months old counterparts the retainment of enhanced memory was not observed.The differentiation pattern in these aged recombined mice showed a significant increase of DCX positive cells, specifically the mature DCX population. However the percentage of newly generated mature neurons remained unchanged. Our study shows that the disinhibition of TGF-beta signalling cascade by SMAD7 knock out has an impact on hippocampal neurogenesis and its associated cognitive function.

Poster 7

RBPJK-DEPENDENT SIGNALLING IS ESSENTIAL FOR MAINTENANCE OF ADULT NEURAL STEM CELLS

Schwarz TJ[1], Ehm O[1], Vogt A[2], Büttner F[3] , Göritz C[4], Covic M[1], Schäffner I[1], Karaca E[1], Kempkes B[5], Kremmer E[6], Frisén J[4], Sarioglu H[7], Glöckner J[2,7], Lie DC[1]
[1]Research Group Adult Neurogenesis and Neural Stem Cells, Institute of Developmental Genetics, [2]Division of Experimental Ophthalmology, University of Tübingen, Tübingen, [3]Department for Bioinformatics and System Biology, [4]Department of Cell and Molecular Biology, Medical Nobel Institute, Karolinska Institute, SE-171 77 Stockholm, [5]Department of Gene Vectors, [6]Institute of Molecular Immunology, [7]Department of Protein Science, Helmholtz-Center Munich, German Research Center for Environmental Health, Munich-Neuherberg

The ability of neural stem cells aNSCs to sustain neurogenesis throughout life depends on the balance of stem cell maintenance and differentiation.
We found that the Notch/RBPJκ signalling pathway is highly active in aNSCs of the dentate gyrus (DG). Conditional inactivation of RBPJκ in aNSCs in vivo resulted in increased neuronal differentiation of aNSCs in the adult DG at an early time-point and depletion of the Sox2 positive neural stem cell pool and suppression of hippocampal neurogenesis at a later time-point. Moreover, RBPJκ deficient neural stem cells displayed impaired self renewal in vitro and loss of expression of the transcription factor Sox2. ChIP analysis revealed the presence of RBPJκ and the intracellular domain of the Notch receptor (NICD) on the Sox2 promoter. We also found that RBPJk interacts with the PI3 kinase pathway. For further understanding how the Notch/RBPJk pathway might interact with different signaling pathways to regulate aNSCs maintenance we investigate the protein interactom of RBPJ by mass spectrometry. First results show an enrichment of proteins in the interactom of RBPJk with RNA and DNA binding properties.

Poster 8

IMPACT OF ALPHA SYNUCLEIN ON ADULT NEURAL STEM AND PRECURSOR CELL BIOLOGY IN VITRO

Schroedel T[1], Vogelgsang J[1], Schneider J[1], Masliah E[2], Klucken K[1], Winkler J[1,2]
[1]Division of Molecular Neurology, Friedrich-Alexander-University Erlangen-Nürnberg, [2]Department of Neurosciences, University of California San Diego, CA

Parkinson's disease (PD) is a progressive neurodegenerative movement disorder. The major motor symptoms of PD are related to the severe loss of dopaminergic (DA) neurons in the substantia nigra depleting striatal dopamine levels. At present no causal therapy exists able to halt progression, or even more important to reverse the disease course. One possible therapeutical approach may be exogenous cell replacement strategies depending on the generation of a substantial amount of well characterized, nontransformed cells in vitro. Furthermore, survival of transplanted cells and integration into pre-existing, but diseased neural networks are a crucial prerequisite for the efficacy of cell-based strategies.

Aggregation of alpha synuclein (aSyn) plays an important role in the disease progression of PD. Synuclein associated pathology was also detected in fetal nigral cells several years after grafting into PD patients' brains. Furthermore, decreased adult neurogenesis was observed in transgenic mice expressing human aSyn under the PDGFß promoter and in PD patients. However, it is still unclear, how aSyn interferes with the generation and differentiation of newly generated or grafted cells. Therefore we aim to study the impact of aSyn on adult neural stem and precursor cells (aNSCs) in vitro.

aNSCs were isolated from adult neurogenic regions, such as the subventricular zone, the olfactory bulb and the hippocampal dentate gyrus of transgenic mice expressing human aSyn under the PDGFß promoter. After establishing neurosphere cultures, human aSyn expression was detected at the RNA and protein level in aNSCs under proliferation conditions (EGF and FGF-2). Immunofluorescence staining revealed numerous round intracytoplasmic accumulations immunoreactive for human aSyn. Nevertheless, proliferation of aNSCs was not altered compared to cells derived from non transgenic animals. This is consistent with previous in vivo findings that proliferation of aNSCs is not affected in the SVZ and hippocampus of these transgenic mice.

As a next step we plan to analyse the intracellular distribution, ultrastructural composition and metabolism of aSyn in proliferating aNSCs. Moreover we will explore, if and how aSyn interferes with the differentiation of aNSCs as this would be another important requirement for a successful cell replacement strategy.

Poster 9

Analysis of the stage specific transcriptome in adult hippocampal neurogenesis

Englberger E[1], Covic M[1], Jagasia R[2], Merz K[1], Benet-Pages A[3], Strom T[3], Bender A[4], Robson P[5], CD Lie[1]

[1] Helmholtz Zentrum München, Germany, Institute of Developmental Genetics, Adult Neural Stem Cells and Neurogenesis Group, [2] Roche, Basel, Switzerland, [3] Helmholtz Zentrum München, Germany, Institute of Human Genetics, [4] LMU München, Germany, Dept. of Neurology, [5] Stem Cell & Developmental Biology, Genome Institute of Singapore

In adult hippocampal neurogenesis, neural stem cells in the subgranular zone of the hippocampal dentate gyrus give rise to new functional dentate granule neurons throughout adulthood. The development of newborn neurons in the adult rodent brain has previously been characterized in great detail with regard to marker expression, morphology, and electrophysiological properties. An important missing piece towards the understanding of the mechanisms regulating adult neural stem cell differentiation into functional neurons, however, is the knowledge of the regulatory networks that control distinct stages of adult neurogenesis (proliferation, fate determination, maturation and integration into the network).

The goal of this project is to characterize the transcriptome of neural stem cells and their progeny in their natural niche at defined developmental stages. To this end, we are exploring the possibility to combine retroviral birthdating of newly generated cells and isolation of labelled cells with transcriptome analysis. Here, we will present our recent progress on the development of this technique.

The identification of gene expression profiles during specific stages of adult hippocampal neurogenesis will generate new knowledge and open new avenues towards the understanding of the regulation of stem cells and their progeny in the adult brain.

Poster 10

PROSPECTIVE ISOLATION OF ADULT MOUSE NEURAL STEM CELLS REVEALS NEW INSIGHTS INTO THEIR MOLECULAR IDENTITY AND FACTORS REGULATING NEURAL STEM CELL FATE AND RENEWAL

Bayam E[1], Beckervordersandforth-Bonk R[1], Tripathi P[1], Ninkovic J[1], GötzM [1,2]
[1] Helmholtz Zentrum München Institute of Stem Cell Research Ingolstädter Landstraße 1, Neuherberg, [2] Institute of Physiology, University of Munich, Schillerstraße 46, München

Adult neural stem cells (aNSCs) in the subependymal zone (SEZ) of the lateral ventricle have been suggested as a special subset of astrocytes or tanycytes sharing hallmarks with radial glia. However, a global view on their molecular signature is still missing as aNSCs could so far not be prospectively isolated with sufficient purity. Recently we described a novel Fluorescence Activated Cell Sorting (FACS) based approach that allows to isolate all aNSCs with 70% purity. We also present a novel fate-mapping approach that underlines the NSC identity of these cells in vivo. Isolation of mRNA from this highly enriched aNSC fraction and from other cells within and outside the neurogenic niche allowed reliable transcriptome analysis with the predicted differential expression levels confirmed by RT qPCR, in situ hybridizations and immunostainings. These data allowed several key insights into molecular signature of stem cells, ependymal cells and parenchymal astrocytes as well as identification of new candidate genes for neurogenic niche and fate determinants. In the meeting expression analysis of these new candidate genes as well as data from functional experiments will be presented.

Poster 11

SC1 IS A CRITICAL MEDIATOR OF DIFFERENTIATION OF NEURAL STEM CELLS IN DEVELOPING MOUSE BRAIN

Götz R, Orel N, Sendtner M
Institute for Clinical Neurobiology, Universitätsklinikum Würzburg, Würzburg

Prdm4 or Schwann cell factor 1 (SC1) belongs to the family of Prdm proteins which contain zinc finger motifs and a protein segment called the positive regulatory (PR) domain. We have shown before that SC1 is a direct binding partner of the p75 neurotrophin receptor that can translocate into the nucleus after receptor activation and affect the expression of distinct cyclin genes in PC12 cells and other cell lines. Overexpression of SC1 in mouse embryonic neural stem cells inhibits mitosis and induces apoptosis. Whereas neither neurotrophic factor signalling nor absence of the p75 neurotrophin receptor protected neural stem cells from apoptosis caused by SC1 overexpression, the absence of Bax did. The conditional elimination (using a “floxed” SC1 allele) of the SC1 gene in cultured embryonic forebrain neural stem cells did not affect their proliferation or alter the cell cycle profile in serum-free medium in the presence of epidermal growth factor and basic fibroblast growth factor. Differentiation of cultured neural stem cells into neurons as well as astrocytes was severely impaired in the absence of SC1. In conclusion, our data suggest that SC1 may act in neural stem cells as a key regulator of neuronal and astrocytic differentiation and survival by mediating neurotrophin effects via p75 neurotrophin receptor signalling.

Poster 12

LOCALIZED P75^{NTR} EXPRESSION SPECIFIES AXONS DURING NEURONAL POLARIZATION

Bergami M[1], Zuccaro E[2], Vignoli B[2], Santi S[3], Cancedda L[2], Canossa M[2]
[1]Dept. of Physiological Genomics, Ludwig-Maximilians-Universität-München, [2]Dept. of Neuroscience, Italian Institute of Technology-Genoa, [3]CNR-ITOI, Istituto Ortopedico Rizzoli-Bologna

Axonal specification is a critical step in neuronal development achieved by selective trafficking and segregation of key cytoplasmic components into undifferentiated neurites. Hence, the initial accumulation of components into one neurite may result as predictive for their role in axonal differentiation. Here, we report that the asymmetric distribution of the pan-neurotrophin p75^{NTR} receptor in developing neurons is required to specify the axonal identity of naive neurites both in vivo and in vitro. We demonstrate that p75 is the neurotrophin receptor which mediates the effects of neurotrophins on axon specification. Neocortical neurons either lacking (knock-down) or ectopically expressing the p75^{NTR} receptor fail to initiate the axon. Similarly, newly generated neurons in the adult hippocampus also develop without extending axons. We show that these phenotypes are the result of a neurotrophin-dependent direct interaction between p75^{NTR} and the polarity protein mPar-3, which also loses its polarized distribution once we interfere with p75^{NTR} expression. Taken together, our data define a new role of p75^{NTR} at early stages of neuronal development for the specification of axons.

Poster 13

DEFINING THE ROLE OF SUBCELLULAR MITOCHONDRIAL LOCALIZATION IN SYNAPTIC INTEGRATION OF NEWBORN NEURONS IN THE ADULT MOUSE BRAIN

Steib K, Ebert B, Jagasia R, Lie DC
Research Group Adult Neurogenesis and Neural Stem Cells, Institute of Developmental Genetics, Helmholtz Zentrum München, German Research Center for Environmental Health, Munich-Neuherberg

Adult neurogenesis, i.e., the generation of new functional neurons in the adult mammalian brain, contributes to hippocampal and olfactory function. A key step in adult neurogenesis is the functional integration of new neurons into existing circuits. How functional integration is achieved and how specific synapses are formed onto newly generated neurons is only poorly understood. There is evidence for a role of mitochondria in neuronal development.

Our present data indicate that manipulation of mitochondrial fusion and fission inhibits and promotes the morphological maturation of newly generated granule neurons, respectively. One interesting observation in these studies was that such manipulation of mitochondrial dynamics also appeared to affect the subcellular distribution of mitochondria. In future, we aim to study the contribution of mitochondrial transport and mitochondrial accumulation to active sites as a central cell biological mechanism for synapse formation and stabilization. We will pursue this question using a number of different techniques including retrovirus mediated gene transfer, live cell imaging, and electrophysiology. These studies will not only promote our understanding of neurogenesis-dependent plasticity, but may also provide the basis for therapeutic strategies that aim at harnessing stem cells for the treatment of neurological diseases.

Poster 14

A NEW MOUSE MODEL OF 'DOUBLE-CORTEX' - THE ROLE OF RHOA IN CORTICAL DEVELOPMENT

Cappello S[1]*, Böhringer C[1]*, Brakebusch C[3,4], Götz M[1,5]
[1] Institute of Stem Cell Research, Helmholtz Center Munich, Neuherberg/München, [3] Biomedical Institute, University of Copenhagen, 2200 Copenhagen, 4 BRIC, University of Copenhagen, 2200 Copenhagen, [5] Institute of Physiology, University of Munich, München
* These authors contributed equally

The events that organize brain structure during development include neurogenesis, cell migration as well as axon projections and guidance. A hallmark of the mammalian neocortex is the arrangement of functionally distinct neurons in six horizontal layers which possess distinct properties in different sensory or motor areas. The importance of this arrangement is clearly revealed when it is disturbed, as is the case in subcortical/periventricular band heterotopia with neurons located below the white matter. Genetic analysis of human brain malformations have highlighted a number of cytoskeletal-associated proteins underlying these functions. Notably however, mutations of the genes so far identified in human patients fail to mimic this phenotype in mouse models.

Here we conditionally inactivated the small GTPase RhoA selectively in the developing cortex by the use of the Emx1::Cre mouse line. Cre expression starts around E9.5 and RhoA protein is no longer detectable in the cerebral cortex by E12. Homozygous mutant mice grow to adulthood and exhibit a pronounced subcortical/periventricular band heteropia or 'doublecortex' with a normally layered cortex on top of the white matter and a prominent but unlayered cortex below the white matter. Birthdating and transcription factor analysis demonstrates that the layered cortex contains the correct neuronal subtypes of all layers in their normal sequence, even though Cux2+ layer 2/3 neurons are reduced in number. Conversely, the 'doublecortex' underlying the white matter is mostly composed of late born, Cux2+ neurons that would normally settle in layers 2/3. Interestingly, both cortices contain a normal density of GABAergic neurons.

Developmental analysis demonstrated that soon after loss of RhoA the apical organisation of adherens junctions is lost and cells, including radial glia, become scattered and misoriented. Transplantation experiments suggest a non-cellautonomous origin of the doublecortex dysplasia, while cell-autonomous RhoA deletion also exerts altered neuronal migration and cell motility.

Taken together, this mouse model provides a unique tool to examine the function of an adult cerebral cortex with severe subcortical/periventricular band heterotopia as well as elucidate the cell intrinsic role of RhoA in cortical progenitors.

Poster 15

IMPACT OF SOX PROTEINS ON ADULT OLIGODENDROGENESIS IN THE NORMAL AND LESIONED SPINAL CORD

Liu L, Bischof M, Wegner M
Institute for Biochemics, Emil-Fischer-Center, University Erlangen-Nürnberg, Erlangen

NG2-positive cells are a ubiquitous population of proliferative progenitors in the adult mammalian CNS. They are highly related to embryonic oligodendrocyte precursors and give rise to myelinating oligodendrocytes throughout life. The transcription factors of the Sox protein family are important regulators of embryonic oligodendrocyte precursors and oligodendrocyte generation. We propose to determine which Sox proteins are expressed in adult NG2-positive cells and to use conditional gene deletion strategies in the mouse to analyse how Sox proteins influence the properties of this precursor cell population in the normal and lesioned spinal cord. So far, we found that Sox genes are similarly expressed in adult NG2-positive cells and embryonic oligodendrocyte precursors, with Sox2, Sox3, Sox5, Sox6, Sox8 and Sox10 being present in most NG2-positive cells. Only Sox9 occurrence differed between both precursor cell populations. For conditional deletion of Sox genes in adult NG2-positive cells we developed a tamoxifen-dependent strategy. This strategy will use either Pdgfra::CreERT2 or U2::CreERT2 transgenes.

Poster 16

MULTIPLE ROLES OF SOXD PROTEINS IN OLIGODENDROCYTE DEVELOPMENT

Zimmermann Y, Schillinger A, Liu L, Baroti V, Lommes P, Wegner M, Stolt CC
Institute for Biochemics, Emil-Fischer-Center, University Erlangen-Nürnberg, Erlangen

Transcription factors of the SoxD protein family have previously been shown to prevent precocious specification and terminal differentiation of oligodendrocyte progenitor cells in the developing spinal cord. Using mice with specific deletion of the SoxD proteins Sox5 and Sox6 in the central nervous system, we now show that SoxD proteins additionally influence migration of oligodendrocyte progenitors in the spinal cord as well as in the forebrain. In mutant mice, emigration of oligodendrocyte progenitors from the ventricular zone and colonization of the mantle zone are significantly delayed probably because of reduced expression of PDGF receptor alpha and decreased responsiveness towards PDGF as a main migratory cue. In addition to this cell-autonomous effect on PDGF receptor alpha expression, SoxD proteins furthermore promote oligodendroglial migration by keeping the cells in an undifferentiated state and preventing a premature loss of their migratory capacity. This indirect effect becomes particularly important during late embryonic and early postnatal phases of oligodendroglial development. Comparison with oligodendroglial development in other mouse mutants furthermore suggests that SoxD proteins exert their influence on oligodendroglial migration by modulating the activity of the SoxE proteins Sox9 and Sox10.

Poster 17

MODELS OF ATYPICAL PARKINSON'S DISEASE: IMPLICATION ON ADULT NEURO- AND GLIOGENESIS

May VEL[1], Klucken K[1], Winner B[2], Wegner M[3], Masliah E[4], Winkler J[1,4]
[1]Division of Molecular Neurology, Friedrich-Alexander-University Erlangen-Nürnberg, [2]Junior Research Group III, Interdisciplinary Center for Clinical Research, Nikolaus-Fiebiger Center for Molecular Medicine, Friedrich-Alexander-University Erlangen-Nürnberg, [3]Institut für Biochemie, Emil-Fischer-Zentrum, Universität Erlangen-Nürnberg, [4]Department of Neurosciences, University of California, San Diego, CA

One of the pathological hallmarks of sporadic/typical Parkinson disease (PD) is the accumulation and aggregation of alpha-synuclein (αSyn) in neurons, whereas in Multiple System Atrophy (MSA), an atypical form of PD, αSyn aggregates are present in oligodendrocytes, too. MSA is an adult-onset progressive, neurodegenerative disease that presents with autonomic dysfuntion, accompanied by Parkinsonian motor symptoms, corticospinal tract dysfuntion and cerebellar ataxia. Cellular pathology reveals gliosis and myelin degeneration early during disease progression. Furthermore, cell loss was observed at later stages in oligodendroglial and neuronal nuclei. The glial cytoplasmic inclusions of αSyn (GCI) are widely distributed in the pons, medulla, putamen, cerebellum, the substantia nigra (pars compacta) and preganglionic autonomic structures and occur earlier than αSyn inclusions in neuronal nuclei and cytoplasmata.

In order to understand the role of αSyn in newly generated cells in the forebrain we examined neuro- and gliogenesis in transgenic mice expressing human wild-type αSyn under the promoter for myelin basic protein (MBP). In this model the severity of the neurological deficits, the age of onset and neuropathological alterations are associated with the level of transgene expression.

Regions in the adult forebrain with the capacity to generate new neurons (= adult neurogenesis), the subventricular zone and the hippocampus, showed a distinct pattern of impaired cellular plasticity. We detected a reduced proliferation in the SVZ and in addition, a reduced survival of newly generated cells in the olfactory bulb as well as in the granular zone of the gyrus dentatus. No changes of adult neurogenesis were detectable in the in the olfactory bulb.

In contrast to the neurogenic regions, we detected an increased survival of bromodesoxyuridine (BrdU) labelled cells in non-neurogenic regions such as the motor cortex, the piriform cortex, the striatum and the corpus callosum. Future phenotypical characterization of these $BrdU^+$ cells will reveal whether an increased number of neural and/or glial precursors are present in non-neurogenic regions in adult MBP transgenic animals. We are currently using the markers NG2, Sox2 and GalC to elucidate the role/level of intrinsic compensatory mechanisms and glial plasticity potentially contributing to (oligodendro-) gliosis seen in MSA.

Poster 18

ROLE OF SOXC PROTEINS IN ADULT HIPPOCAMPAL NEUROGENESIS

Mu L[1]*, Berti L[1]*, Maserdotti G[2]*, Covic M[1], Michaelidis T[1,3], Merz K[1], Herold S[1], Haslinger A[1], Wegner M[4], Sock E[4], Lefebvre V[5], Götz M[2,6], Aigner L[7], Couillard Despres S[7], Berninger B[2], Lie DC[1]

[1]Research Group Adult Neurogenesis and Neural Stem Cells, Institute of Developmental Genetics, Helmholtz Zentrum München, German Research Center for Environmental Health, Munich-Neuherberg [2]Department of Physiological Genomics, Institute of Physiology, Ludwig-Maximilians University Munich, Munich [3]Foundation for Research & Technology-Hellas, Biomedical Research Institute, University of Ioannina, Science and Technology School, 45110 Ioannina [4]Institute for Biochemistry, Emil-Fischer-Zentrum, University Erlangen-Nürnberg, Erlangen, Germany [5]Lerner Institute, Cleveland Clinic, Cleveland, Ohio [6]Institute for Stem Cell Research, National Research Center for Environment and Health, Munich-Neuherberg [7]Institute for Molecular Regenerative Medicine Paracelsus Medical University, Salzburg

* these authors contributed equally

In the adult mammalian brain, neural stem and progenitor cells generate neurons throughout adulthood in two specific regions, the subgranular zone of the hippocampal dentate gyrus and the subventricular zone of the lateral ventricle. Neuronal fate determination, subtype specification and differentiation are controlled by the interaction of different transcription factors. In our study, we found Sox4 and Sox11, members of the group C Sox transcription factor family, to be prominently expressed in doublecortin (DCX)-expressing neuronal committed precursors and immature neurons in the neurogenic areas of the adult brain. Our in vitro studies indicate that overexpression of Sox4 and Sox11 upregulate DCX mRNA levels and promote the generation immature neurons from adult neural stem cells, whereas loss of Sox4 and Sox11 decreases DCX mRNA level and reduces the generation of immature neurons. In vivo absence of Sox11 and Sox4 impaired the development of immature neurons, and overexpression of Sox11 delayed the further differentiation of immature neurons. Finally, we provide evidence that SoxC transcription factors bind to DCX and also can active DCX promoter in vitro. These data suggest that transcription factors of the SoxC family have essential roles in the transcriptional regulation of neuronal differentiation in adult neurogenesis.

Poster 19

miRNA BASED REGULATION OF TRANSCRIPTION FACTOR SOX11 DURING ADULT NEUROGENESIS

Rehfeld F[1], Cernilogar F[1], Sock E[2], Wegner M[2], Lie DC[1]
[1]Research Group Adult Neurogenesis and Neural Stem Cells, [2] Institute for Biochemistry, Emil-Fischer-Zentrum, University Erlangen-Nürnberg, Erlangen

The process of neurogenesis in the adult mammalian brain has been target of extensive research since its discovery and experimental proof in the late 1990s. Nevertheless regulation of this highly dynamic process remains elusive. Previous studies could show that transcription factor Sox11 is expressed exclusively in the neurogenic niches of the adult mammalian brain. Its expression is overlapping with the early neuronal marker doublecortin and can only be found in neuronal precursors and immature neurons. Overexpression of Sox11 leads to an increase in the population of doublecortin positive immature neurons. This suggests a functional, stage specific role of Sox11 in the process of adult neurogenesis. How the expression of Sox11 during neurogenesis is regulated remains unknown. Here we provide first evidence that miRNAs can repress Sox11. In particular the evolutionary conserved miRNA let-7 was shown to inhibit Sox11 expression by binding its 3'UTR. The obtained data suggests a functional role of the let-7 mediated Sox11 repression during neuronal maturation.

Poster 20

THE ROLE OF CANONICAL WNT SIGNALLING IN THE DEVELOPMENT OF MESO-DIENCEPHALIC DOPAMINERGIC NEURONS IN VIVO

Götz S[1], Stübner S[1], Zimmermann C[1], Peng C[1], Trümbach D[1], Wurst W[1,2], Prakash N[1]
[1]Helmholtz Centre Munich, German Research Center for Environmental Health, and Technical University Munich, Institute of Developmental Genetics, Neuherberg/ Munich, [2]Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE) Standort München, Max Planck Institute of Psychiatry, Munich

Meso-diencephalic dopaminergic (mdDA) neurons control voluntary movement, cognition and reward. Degeneration or dysfunction of these neurons leads to neurological and psychiatric diseases, among them Prakinson's disease and schizophrenia. Potential therapeutic cell replacement strategies of mdDA neuron-related disorders include in vitro differentiation of ES/iPS cells and in-situ reprogramming of other cell types (e.g. astrocytes). These approaches require a comprehensive understanding of genetic networks controlling the development of mdDA neurons for an efficient generation of mdDA neurons.

In previous studies we could show that Wnt1, an activating ligand of the canonical Wnt signaling pathway, regulates a genetic network which is required for the correct patterning of the murine ventral midbrain (VM) and the establishment of the mdDA progenitor domain.

However, these and other studies also indicated a later role of the canonical Wnt1/□-catenin pathway in the differentiation of mdDA neurons. Our next analyses were aimed at identifying the intracellular Wnt signal transduction pathway in mdDA neuron development. Since the activity of the Fzd receptors bound by the Wnt1 ligand in this process appears to be highly redundant (Stuebner et al., 2010), we have subsequently focussed on the identification of the nuclear effectors and target genes of Wnt1 in mdDA neuron generation. A detailed expression analysis of the four known nuclear effectors of the TCF/LEF family, Tcf7, Tcf7l1, Tcf7l2 and Lef1, suggested Lef1 as the potential transcription factor involved in the activation of Wnt1 target genes in the developing mdDA domain. However, the analysis of Lef1 KO embryos did not reveal a reduction of mdDA neurons during development. This result suggested that other, probably yet unknown intracellular/nuclear effectors may mediate the Wnt1 effect on mdDA neuron development. Furthermore, from these analyses it remained unclear whether all or only a subpopulation of the mdDA precursors respond to the Wnt1 signal in the murine VM. We therefore took advantage of the BATgal transgenic reporter mice, which express β-galactosidase only in the presence of nuclear β-catenin due to Wnt pathway activation (Maretto et al., 2003). We identified a Wnt-responsive (□-Gal+) TH+ and Pitx3+ cell subpopulation within the VM domain, suggesting that "canonical" Wnt/□-catenin signalling is indeed active in differentiating mdDA precursors/neurons. In addition, we detected a Wnt-responsive subpopulation within the oculomotor nucleus (Isl1+) and the red nucleus (Brn3a+). In order to identify the Wnt1 target genes and potentially unknown nuclear effectors in these Wnt-responsive cells, we are now performing a comparative transcriptome analysis of the Wnt-responsive versus Wnt-non-responsive mdDA precursors/neurons.

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Poster 21

THE CANONICAL WNT/B-CATENIN PATHWAY IN THE IN VITRO DIFFERENTIATION OF MURINE ES AND IPS CELLS INTO MESODIENCEPHALIC DOPAMINERGIC NEURONS

Meier F[1], Zhang J[1], Fukusumi Y[1], Pertek A[1], Kühn R[1], Wurst W[1,2], Prakash N[1]
[1] Institute of Developmental Genetics, Helmholtz Centre Munich, German Research Centre for Environmental Health (GmbH) and Technical University Munich, Neuherberg, Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE) Standort München, Max-Planck-Institute of Psychiatry, Munich

The expected increase of neurodegenerative brain disorders in our ageing society has fostered the study of the underlying molecular mechanisms in vitro and the development of stem cell-based regenerative therapies using patient-derived pluripotent stem cells in recent years. A major caveat of these approaches, however, still is the incomplete or only partial differentiation of these stem cells into the desired neural subtype in vitro. Parkinson´s Disease (PD) is characterised by the progressive loss of mesodiencephalic dopaminergic (mdDA) neurons in the human ventral midbrain. These neurons are required for the control and modulation of voluntary movement and other cognitive and affective behaviours; their loss therefore leads to the very disabling and typical motor and non-motor symptoms of PD. The replenishment of the degenerating mdDA neurons by healthy ones is therefore still one of the major therapeutic strategies for PD.

We and others have shown that the “canonical” Wnt1/□-catenin signalling pathway plays a crucial role in the specification and differentiation of mdDA precursors and neurons in the murine ventral midbrain (VM) in vivo. Most published differentiation protocols for the generation of mdDA neurons in vitro have a very low efficiency (usually in the range of 5-10% mdDA neurons of the total cell population). Activation of the “canonical” Wnt signalling pathway, however, has been omitted from most if not all of these previously published differentiation protocols. We have therefore set out to modify already known protocols and establish new ones for the differentiation of murine embryonic stem (mES) cells into mdDA neurons in vitro, with a particular focus on the participation of the Wnt1 signalling pathway in this process. Our results indeed show that the proportion of Th and Pitx3 double positive mdDA neurons is increased by the application of Wnt1 in a modified version of the so called “5 stage” mES cell differentiation protocol by Lee et al. (2000). Furthermore, the morphology of these Wnt1-treated in vitro differentiated mdDA neurons resembles more their in vivo counterparts as compared to the published differentiation paradigms.

We have also generated a induced pluripotent stem (iPS) cell line from Pitx3$^{+/GFP}$ mice, which can be used for the direct monitoring of mdDA neuron differentiation in the culture dish and for the purification of these cells by fluorescence-activated cell sorting (FACS) for transplantation purposes. We are currently characterizing this Pitx3$^{+/GFP}$ iPS cell line in detail. We will also use this cell line for exploring the participation of additional signalling pathways in Wnt1-mediated mdDA neuron differentiation in vitro, to further optimize the numbers and quality of mdDA neurons obtained in this experimental paradigm.

Poster 22

THE ROLE OF CANONICAL WNT-SIGNALING IN ADULT MIDBRAIN DOPAMINERGIC DIFFERENTIATION IN VIVO

Zhang J[a], Meier F[a], Prakash N[a], Vogt Weisenhorn DM[a,b], Wurst W[a,b]
[a]Institute of Developmental Genetics, Helmholtz Centre Munich, German Research Centre for Environmental Health (GmbH) and Technical University Munich, Neuherberg [b]Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE) Standort München, Max-Planck-Institute of Psychiatry, Munich

Wnts include 19 members in mammals and play a critical role in neural development as well as adult neurogenesis. However, the identity of the Wnts and of their Frizzled (Fzd) receptors expressed in the adult rodent brain remained unknown. We therefore studied the expression patterns of the 19 Wnt ligands and 10 Fzd receptors in the adult mouse brain by in situ hybridization and RT-PCR. Wnt1, one of the best known Wnt ligands activating the canonical Wnt/beta-catenin signaling pathway and thereby promoting beta-catenin–dependent gene expression, plays a pivotal role for midbrain dopaminergic (mDA) neuron development in the mouse embryo. However, we could not detect Wnt1 expression in the midbrain of adult wild-type mice, correlating with the absence of dopaminergic neurogenesis in the adult ventral midbrain. We therefore assessed the effect of Wnt1 over-expression in the adult ventral midbrain of $En1^{+/Wnt1}$ knock-in mice. Wnt1 over-expression stimulated the proliferation of neural precursors (NPs) in the adult ventral midbrain, which differentiated mostly into oligodendroglia and only a few neurons, but did not generate GFAP+ astrocytes or TH+ mDA neurons. Furthermore, Wnt1 over-expression significantly up-regulated the transcription of FGF20, an important growth factor promoting mDA neuron survival, and of Lmx1a, a critical transcription factor in the differentiation of mDA neurons, suggesting that Wnt1 over-expression in the adult ventral midbrain could have a neuroprotective function. We are now assessing how the constitutive activation of the canonical Wnt/beta-catenin signaling pathway in doublecortin (Dcx) positive neuronal precursors of the adult forebrain subventricular zone (SVZ) and ventral midbrain affects the proliferation and migration of these cells, and their differentiation into neurons in general and into mDA neurons in particular. To this end, we are using the DCX-CreERT2 transgenic mice generated in the previous funding period, crossed to the $Catnb^{+/lox(ex3)}$ conditional mice for constitutive beta-catenin activation in Dcx+ cells.

Poster 23

ADVERSIVE STRIATAL MICROENVIRONMENT: DETRIMENTAL FOR DIFFERENTIATION AND SURVIVAL FOR RECRUITED SVZ NEURAL PRECURSOR CELLS IN AN ACUTE MODEL OF PARKINSON'S DISEASE?

Schlachetzki JCM[1], Winner B[2], Lie DC[3], Winkler J[1,4]
[1] Division of Molecular Neurology, Friedrich-Alexander-University Erlangen-Nürnberg, [2] Junior Research Group III, Interdisciplinary Center for Clinical Research, Nikolaus-Fiebiger Center for Molecular Medicine, Friedrich-Alexander-University Erlangen-Nürnberg, [3] Research Group Adult Neurogenesis and Neural Stem Cells, Institute of Developmental Genetics, Helmholtz Zentrum München, German Research Center for Environmental Health, Munich-Neuherberg, [4] Department of Neurosciences, University of California San Diego, CA

Reduced neurogenesis is observed in the subventricular zone (SVZ) of patients with Parkinson's disease (PD) and in the acute rodent 6-hydroxydopamine (6-OHDA) model (Hoglinger et al., 2004; Winner et al., 2006). We have recently reported that combined epidermal growth factor (EGF) and fibroblast growth factor 2 (FGF-2) infusion results in increased cell proliferation in the subventricular zone in the acute unilateral 6-OHDA rodent lesion model (Winner et al., 2008). These growth factors also promoted migration of these newly generated neuroblasts into the adjacent striatum. However, EGF and FGF-2 failed to induce survival and dopaminergic differentiation of these cells in the striatum.

Here we propose that 6-OHDA injections perturb differentiation and maturation of newly generated neuroblasts into mature, dopaminergic neurons by inducing an adversive striatal environment. To better understand the intrastriatal microenvironment, we analyzed the status of microglial cells in regard to their number and activation state using a time course paradigm post-lesioning. After unilateral injection of 6-OHDA into the medial forebrain bundle, 12-week old Wistar rats were sacrificed 1 (n=8), 2 (n=5), and 6 (n=5) weeks after injection and further processed for immunohistochemical analysis. Striatal deafferentation was already observed 1 week post-lesioning and was progressive until 6 weeks after injection. To characterize microglial activity, sections were stained for Iba-1 positive cells. Over the period of 6 weeks, we observed a progressive increase in the number of Iba-1 positive cells in the striatum. Moreover, microglial phenotype changed from a "resting", surveying character to an activated, phagocytic state in 6-OHDA lesioned animals. No change in microglial cell number was observed in the SVZ. Microglial activity was further analyzed using the phagocytic marker ED-1.

Here, we provide evidence that the number and biological status of microglial cells is enhanced in an acute model of PD. We hypothesize that modulation of microglial activity in the striatum may be a potential target to successfully promote neuronal differentiation and survival of newly generated neuroblasts.

Poster 24

AUTOPHAGY MODULATION ALTERS ALPHA-SYNUCLEIN AGGREGATION AND TOXICITY

Poehler AM, McLean PJ, Rockenstein E, Hyman BT, Masliah E, Winkler J, Klucken J
Division of Molecular Neurology, University Hospital Erlangen, MassGeneral Institute for Neurodegenerative Disease, Department of Neurology, Massachusetts General Hospital and Harvard Medical School, Boston, Department of Neurosciences, University of California San Diego

Neuronal aggregation of the protein alpha-synuclein (aSyn) is the pathological hallmark in the brain of patients with synucleinopathies, such as Parkinson's disease and Dementia with Lewy bodies (DLB). Impaired aSyn proteasomal or lysosomal degradation results in its aggregation and neuronal toxicity. Exploring the autophagosomal lysosomal degradation pathway (ALP) in brain tissue from DLB patients, a mouse model of synucleinopathy, and in cell culture revealed that ALP is significantly altered. As a therapeutic approach we used ALP modulating compounds in vitro and in vivo. Interestingly, we observed increased toxicity of alpha-synuclein by ALP inhibition that was paralleled by a reduced aggregate formation. Two major conclusions can be drawn: first, aggregate formation reflects a detoxifying intracellular mechanism in synucleinopathy; and second, the ALP regulates not only alpha-synuclein degradation, but also modulates its aggregation, both representing a neuroprotective mechanism. Future therapeutic approaches will focus on specific ALP modulation to establish a preventive therapy in synucleinopathies.

Poster 25

IMAGING AND FUNCTIONAL CHARACTERIZATION OF STEM CELLS BY OPTOGENETIC AND MAGNETIC TOOLS

Kressel J[1,2], Berninger B[3], Coras R[4], Blümcke I[4], Zoubaa S[5], Schlegel J[5], Ntziachristos V[2], Zimmer C[1], Stroh A[6]
[1] Department of Neuroradiology, TUM, [2] Institute for Biological and Medical Imaging, Helmholtz Zentrum München, [3] Department of Physiological Genomics, Institute of Physiology, LMU, [4] Department of Neuropathology, University Hospital Erlangen, [5] Division of Neuropathology, Institute of Pathology, TUM, [6] Institute of Neuroscience, TUM

Major diseases commonly cited as likely targets for stem cell therapy include central nervous system disorders. However, to which extend newly integrated stem-cell-derived neurons actually contribute to the functional restoration of degenerated neural circuits remains unclear. Secondary effects as the stimulation of endogenous repair mechanisms might dominate. Up to now, there is no specific functional control over the cells once transplanted. We present here a combined approach to functionally control transplanted cells and use multimodal imaging to detect changes in network activity together with the location of transplanted cells.

For non-invasive detection and long-term tracking by high-field MRI, various stem cell populations (human/murine/embryonic/adult) were efficiently magnetically labeled without any significant effects on vitality rates, retaining their label for many weeks, even months.

For non-invasive optogenetic control of neuronal activity, stem cells were lentivirally transduced using the light-sensitive cation channel Channelrhodopsin-2 (ChR2). ChR2-positive stem cells maintained the undifferentiated state as robustly as non-transduced control cells. ChR2-positive cells can be neuronally differentiated, expressing markers like NeuN, Map2 and N-CAM1.

Adult stem cells from the mouse subventricular zone were neuronally predifferentiated using a viral cotransduction with Neurogenin2-DsRed and ChR2. We show for the first time, that light illumination leads to action potential initiation of adult stem-cell derived neurons, allowing millisecond-scale minimally-invasive optical control of transplanted stem cell-derived neurons, even after transplanted cells are integrated and embedded within host circuitry.

As next step, we apply Ca^{2+} imaging using fluorescent dyes to detect functional integration upon specific optogenetic stimulation of adult stem cell derived neurons.

Poster 26

IN VIVO 2-PHOTON ANALYSIS OF ADULT NEUROGENESIS IN ALZHEIMER´S DISEASE

Filser S[1], Neuner J[1], Michalakis S[2], Biel M[2], Lie DC[3], Herms J[1]
[1]Center for Neuropathology and Prion Research, Ludwig-Maximilians University Munich, Munich, [2] Center for Integrated Protein Science CIPSM and Department of Pharmacy - Center for Drug Research, Ludwig-Maximilians University Munich, Munich, [3] Research Group Adult Neurogenesis and Neural Stem Cells, Institute of Developmental Genetics, Helmholtz Center Munich, German Research Center for Environmental Health, Munich-Neuherberg

Throughout life interneurons of the olfactory bulb (OB) are continuously replaced by cells that arise from the subventricular zone in a process known as adult neurogenesis. By integrating into the olfactory network these adult born neurons lead to a synaptic “rewiring” of the olfactory bulb and thus contribute to the fine tuning of olfactory function. Interestingly, olfactory deficits can already be diagnosed early in the pathogenesis of Alzheimer´s Disease (AD), a neurodegenerative disease that is characterized by a progressive loss of neurons and synapses. For this reason we speculate that in AD adult born neurons fail to integrate successfully into the olfactory network due to a loss of stable synaptic connections. We thus established a novel preparation that enables long term in vivo 2-photon imaging in the mouse OB through a chronic cranial window to analyze the maturation and synaptogenesis of adult born neurons. In addition, we used immunohistochemistry to analyze the pathology and spine densities of adult born neurons in brain slices of transgenic animal models of AD.

Poster 27

LONG-TERM IN VIVO IMAGING OF THE MATURATION AND INTEGRATION OF ADULT-BORN NEURONS IN THE OLFACTORY BULB OF MOUSE MODELS OF PARKINSON'S DISEASE

Neuner J[1], Filser S[1], Michalakis S[2], Biel M[2], Lie DC[3], Herms J[1]
[1] Center for Neuropathology and Prion Research, Ludwig-Maximilians University Munich, Munich, [2] Center for Integrated Protein Science CIPSM and Department of Pharmacy - Center for Drug Research, Ludwig-Maximilians University Munich, Munich, [3] Research Group Adult Neurogenesis and Neural Stem Cells, Institute of Developmental Genetics, Helmholtz Center Munich, German Research Center for Environmental Health, Munich-Neuherberg

In the olfactory bulb (OB), newborn neurons are continuously added to the pre-existing neuronal network, even in the mature brain, throughout life. Once functionally integrated, these adult-born neurons contribute to olfactory function. Patients of Parkinson's disease (PD) repeatedly report on olfactory deficits in early stages of the disease. Moreover, the characteristic Lewy pathology is found in the OB of PD patients. As we assume that these olfactory deficits may result from impairments in the maturation and integration of especially dopaminergic adult-born neurons, we study the kinetics of dendritogenesis and synaptogenesis of these neurons in transgenic mouse models of PD. Technically, we combine the use of in vivo 2-photon laser-scanning microscopy with the stereotactic injection of lentiviral fluorescent constructs for the labeling of newborn neurons at their birth site, the subventricular zone. This complex approach enables us to directly follow the fate of these neurons once they have reached their target site, the OB, from early maturation stage on up to several weeks. Long-term kinetic in vivo studies are crucial in the search for early therapeutic targets for PD.

Poster 28

STRUCTURAL AND FUNCTIONAL ALTERATIONS IN THE OLFACTORY SYSTEM ASSOCIATED WITH PARKINSON DISEASE

Klucken J, Kahlis J, Mallog N, Moessnang C, Winner B, Schuierer G, Greenlee M, Winkler J
Division of Molecular Neurology, Nikolaus-Fiebiger-Center for Molecular Medicine, University Hospital Erlangen, Institute for Experimental Psychology, Institute of Neuroradiology, University of Regensburg

Olfactory impairment is an important premotor symptom in Parkinson disease (PD). PD patients are often not aware of their smelling deficit clinically assessed by a simple olfactory test. Neuropathological findings show early degeneration of the olfactory bulb (OB) in PD, potentially preceding the conversion into the motor-stage of the disease. Thus, impaired olfaction and associated neuronal structures might serve as an important biomarker to identify subjects with an increased risk to develop PD. We tested the hypothesis that impaired olfaction in PD is based on functional impairment of signal transduction based on degenerated primary olfactory structures (e.g. olfactory bulb and tract) resulting in reduced neuronal activity in associated cortical areas. Using structural MRI we detected a reduced size of the olfactory bulb in PD patients. However, using event-related MR imaging during an odor detection paradigm using air dilution olfactometry we observed a hyperactivation of higher ordered olfactory structures suggesting a compensatory plasticity. Furthermore, primary olfactory structures showed preserved discriminatory ability in contrast to a loss of selectivity in secondary olfactory structures. In summary, we identified distinct activation patterns within the disturbed olfactory network in PD consisting both of dysfunctional disinhibition and compensatory up-regulation of neural activity at different levels of olfactory information processing. Our results indicate that impaired olfactory network function is a dynamic process during the course of PD based on structural pathology and compensatory functional changes. Structural and functional analysis of olfaction in elderly population has the potential to serve as a crucial/important diagnostic biomarker in different disease stages, in particular for early detection of PD.

Notes