Format of the Journal Club

The Journal Club aims to acquaint graduate students with key publications in their field of study and to help them catching up with most recent scientific findings. Furthermore, by choosing publications from Tübingen research groups, the lecturers will introduce students to current research questions pursued in their laboratories.

Before the start of the semester, students will be provided with a list of recent publications from the field of cellular and molecular neuroscience, neurogenetics and state-of-the-art methods. Every student has to pick one paper that he/she has to study and to prepare for presentation.

At the beginning of each session, the lecturer will briefly introduce the field of research covered by the papers and will also justify the selection of these particular articles for the journal club. Students who signed up for one specific paper will present the essentials of the study in a concise 15--20 min presentation addressing (i) the rationale of the study, (ii) the methods applied and (iii) the major findings. They also have to prepare questions derived from the study, which will be discussed in class. In order to ensure a lively discussion, it is required that every student has read the paper before each session. Otherwise, he/she will not be able to participate in and contribute to the discussion.

Qualification goals

The Journal Club will improve the students' skills of understanding and debating current research topics in their field. Students will have to critically evaluate the findings published in recent articles: are the results of the study valid, how useful are the results and do the results lead to new research or to new applications. Furthermore, the adequacy of the research design, the controls used and the statistics employed can be discussed. The goal of the seminar is not only content-based but also aims to advance students' discussion and presentation skills.

Course requirements

In addition to presenting one particular publication (see above), students ...

1) ... are required to submit a one-page long essay of one paper of each session (7 essays in total).
   The essays should consist of
   a. a three-sentences long summary,
   b. a short description of the methods employed,
   c. the major findings of the study,
   d. a brief conclusion and outlook.

2) ... should be prepared to explain one or the other figure of the papers presented.

3) ... should be prepared to answer a few basic questions in writing (5 minutes) regarding one or the other publication.
Sarah Helena Nies:
Haploinsufficiency of TBK1 causes familial ALS and fronto-temporal dementia (Freischmidt et al)

Amyotrophic lateral sclerosis (ALS) is a genetically heterogeneous neurodegenerative syndrome hallmarked by adult-onset loss of motor neurons. We performed exome sequencing of 252 familial ALS (fALS) and 827 control individuals. Gene-based rare variant analysis identified an exome-wide significant enrichment of eight loss-of-function (LoF) mutations in TBK1 (encoding TANK-binding kinase 1) in 13 fALS pedigrees. No enrichment of LoF mutations was observed in a targeted mutation screen of 1,010 sporadic ALS and 650 additional control individuals. Linkage analysis in four families gave an aggregate LOD score of 4.6. In vitro experiments confirmed the loss of expression of TBK1 LoF mutant alleles, or loss of interaction of the C-terminal TBK1 coiled-coil domain (CCD2) mutants with the TBK1 adaptor protein optineurin, which has been shown to be involved in ALS pathogenesis. We conclude that haploinsufficiency of TBK1 causes ALS and fronto-temporal dementia.

Maike Nagel:
Identification of Genetic Factors that Modify Clinical Onset of Huntington’s Disease (Genetic Modifiers of Huntington’s Disease Consortium)

As a Mendelian neurodegenerative disorder, the genetic risk of Huntington’s disease (HD) is conferred entirely by an HTT CAG repeat expansion whose length is the primary determinant of the rate of pathogenesis leading to disease onset. To investigate the pathogenic process that precedes disease, we used genome-wide association (GWA) analysis to identify loci harboring genetic variations that alter the age at neurological onset of HD. A chromosome 15 locus displays two independent effects that accelerate or delay onset by 6.1 years and 1.4 years, respectively, whereas a chromosome 8 locus hastens onset by 1.6 years. Association at MLH1 and pathway analysis of the full GWA results support a role for DNA handling and repair mechanisms in altering the course of HD. Our findings demonstrate that HD disease modification in humans occurs in nature and offer a genetic route to identifying in-human validated therapeutic targets in this and other Mendelian disorders.
Julian Hinz:

*Generation of three-dimensional retinal tissue with functional photoreceptors from human iPSCs (Zhong et al)*

Many forms of blindness result from the dysfunction or loss of retinal photoreceptors. Induced pluripotent stem cells (iPSCs) hold great potential for the modelling of these diseases or as potential therapeutic agents. However, to fulfill this promise, a remaining challenge is to induce human iPSC to recreate in vitro key structural and functional features of the native retina, in particular the presence of photoreceptors with outer-segment discs and light sensitivity. Here we report that hiPSC can, in a highly autonomous manner, recapitulate spatiotemporally each of the main steps of retinal development observed in vivo and form three-dimensional retinal cups that contain all major retinal cell types arranged in their proper layers. Moreover, the photoreceptors in our hiPSC-derived retinal tissue achieve advanced maturation, showing the beginning of outer-segment disc formation and photosensitivity. This success brings us one step closer to the anticipated use of hiPSC for disease modelling and open possibilities for future therapies.

Lisa Schwarz:

*Calcium-induced Calpain Mediates Apoptosis via Caspase-3 in a Mouse Photoreceptor Cell Line (Sharma & Rohrer)*

The *rd* mouse, an accepted animal model for photoreceptor degeneration in retinitis pigmentosa, has a recessive mutation for the gene encoding the β-subunit of the cGMP phosphodiesterase. This mutation results in high levels of cGMP, which leaves an increased number of the cGMP-gated channels in the open state, thus allowing intracellular calcium (Ca²⁺) to rise to toxic levels, and rapid photoreceptor degeneration follows. To delineate the events in *rd* photoreceptor degeneration, we demonstrated an increase in calpain and caspase-3 activity, hypothesizing that Ca²⁺-mediated apoptosis in photoreceptors is mediated by calpain, involving mitochondrial depolarization and caspase-3 activation. To examine this hypothesis further, a murine photoreceptor-derived cell line (661W) was treated with the Ca²⁺ ionophore A23187, cGMP-gated channel agonist 8-bromocGMP, or phosphodiesterase inhibitor isobutylmethylxanthine to mimic the increased Ca²⁺ influx seen in the *rd* photoreceptors. Ca²⁺-induced cell death in 661W cells was found to be mediated by calpain and caspase-3 and could be completely inhibited by the calpain inhibitor SJA6017, implicating both calpain and caspases in the apoptotic process. The apoptotic events correlated in an SJA6017-inhibitable manner with bid cleavage, mitochondrial depolarization, cytochrome c release, and caspase-3 and -9 activation. We concluded that Ca²⁺ influx in the *rd* model of photoreceptor degeneration leads to the activation of the cysteine protease calpain, which executes apoptosis via modulation of caspase-3 activity.
April 29 Julia Schulze-Hentrich

**Yue Zhang:**

*α*-Synuclein Sequesters Dnmt1 from the Nucleus - A NOVEL MECHANISM FOR EPIGENETIC ALTERATIONS IN LEWY BODY DISEASES (Desplats et al)

DNA methylation is a major epigenetic modification that regulates gene expression. Dnmt1, the maintenance DNA methylation enzyme, is abundantly expressed in the adult brain and is mainly located in the nuclear compartment, where it has access to chromatin. Hypomethylation of CpG islands at intron 1 of the SNCA gene has recently been reported to result in overexpression of α-synuclein in Parkinson disease (PD) and related disorders. We therefore investigated the mechanisms underlying altered DNA methylation in PD and dementia with Lewy bodies (DLB). We present evidence of reduction of nuclear Dnmt1 levels in human postmortem brain samples from PD and DLB patients as well as in the brains of α-synuclein transgenic mice models. Furthermore, sequestration of Dnmt1 in the cytoplasm results in global DNA hypomethylation in human and mouse brains, involving CpG islands upstream of SNCA, SEPW1, and PRKAR2A genes. We report that association of Dnmt1 and α-synuclein might mediate aberrant subcellular localization of Dnmt1. Nuclear Dnmt1 levels were partially rescued by overexpression of Dnmt1 in neuronal cell cultures and in α-synuclein transgenic mice brains. Our results underscore a novel mechanism for epigenetic dysregulation in Lewy body diseases, which might underlie the decrease in DNA methylation reported for PD and DLB.

**Betül Uysal:**

Distinctive patterns of DNA methylation associated with Parkinson disease - Identification of concordant epigenetic changes in brain and peripheral blood leukocytes (Masliah et al)

Parkinson disease (PD) is a multifactorial neurodegenerative disorder with high incidence in the elderly, where environmental and genetic factors are involved in etiology. In addition, epigenetic mechanisms, including deregulation of DNA methylation have been recently associated to PD. As accurate diagnosis cannot be achieved pre-mortem, identification of early pathological changes is crucial to enable therapeutic interventions before major neuropathological damage occurs. Here we investigated genome-wide DNA methylation in brain and blood samples from PD patients and observed a distinctive pattern of methylation involving many genes previously associated to PD, therefore supporting the role of epigenetic alterations as a molecular mechanism in neurodegeneration. Importantly, we identified concordant methylation alterations in brain and blood, suggesting that blood might hold promise as a surrogate for brain tissue to detect DNA methylation in PD and as a source for biomarker discovery.
**Artemis Koumoundourou:**
*Otx2 Promotes the Survival of Damaged Adult Retinal Ganglion Cells and Protects against Excitotoxic Loss of Visual Acuity In Vivo (Ibad et al)*

Retinal ganglion cells (RGCs) are the projection neurons from the eye to the brain and their loss results in visual impairment in a number of diseases. Transcription factors with a homeodomain can translocate between cells and, in at least one reported case, can stimulate neuronal survival. Otx2 is a homeoprotein transcription factor expressed in the retina that is taken up by RGCs. We thus hypothesized that Otx2 capture could regulate the survival of adult RGCs. We report that Otx2 stimulates the survival of adult mouse and rat RGCs *in vitro* and protects RGCs against NMDA-induced toxicity *in vivo* in mice. In the latter model, Otx2 also preserves visual acuity.

**Ksenija Martinovic:**
*Engrailed homeoprotein recruits the adenosine A1 receptor to potentiate ephrin A5 function in retinal growth cones (Stettler et al)*

Engrailed 1 and engrailed 2 homeoprotein transcription factors (collectively Engrailed) display graded expression in the chick optic tectum where they participate in retino-tectal patterning. *In vitro*, extracellular Engrailed guides retinal ganglion cell (RGC) axons and synergises with ephrin A5 to provoke the collapse of temporal growth cones. *In vivo* disruption of endogenous extracellular Engrailed leads to misrouting of RGC axons. Here we characterise the signalling pathway of extracellular Engrailed. Our results show that Engrailed/ephrin A5 synergy in growth cone collapse involves adenosine A1 receptor activation after Engrailed-dependent ATP synthesis, followed by ATP secretion and hydrolysis to adenosine. This is, to our knowledge, the first evidence for a role of the adenosine A1 receptor in axon guidance. Based on these results, together with higher expression of the adenosine A1 receptor in temporal than nasal growth cones, we propose a computational model that illustrates how the interaction between Engrailed, ephrin A5 and adenosine could increase the precision of the retinal projection map.
**Astrid Alsema:**
*Activation of hypoxia signaling induces phenotypic transformation of glioma cells: implications for bevacizumab antiangiogenic therapy (Hui Xu et al)*

Glioblastoma (GBM) is the most common and deadly primary brain tumor in adults. Bevacizumab, a humanized monoclonal antibody against vascular endothelial growth factor (VEGF), can attenuate tumor-associated edema and improve patient symptoms but based on magnetic resonance imaging, is associated with nonenhancing tumor progression and possibly gliosarcoma differentiation. To gain insight into these findings, we investigated the role of hypoxia and epithelial-mesenchymal transition (EMT)-associated proteins in GBM. Tumor markers of hypoxia and EMT were upregulated in bevacizumab-treated tumors from GBM patients compared to untreated counterparts. Exposure of glioma cells to 1% oxygen tension increased cell proliferation, expression of EMT-associated proteins and enhanced cell migration in vitro. These phenotypic changes were significantly attenuated by pharmacologic knockdown of hypoxia-inducible Factor 1α (HIF1α) or HIF2α, indicating that HIFs represent a therapeutic target for mesenchymal GBM cells. These findings provide insights into potential development of novel therapeutic targeting of angiogenesisspecific pathways in GBM.

**Sven Aschenbroich:**
*Inhibition of histamine receptor 3 suppresses glioblastoma tumor growth, invasion, and epithelial-to-mesenchymal transition (Jia-Ji Lin et al)*

Histamine receptor 3 (H3R) is expressed in various tumors and correlated with malignancy and tumor proliferation. However, the role of H3R in tumor invasion and epithelial to mesenchymal transition (EMT) remains unknown. Here, we explored the H3R in the highly invasive glioblastoma (GBM) and U87MG cells. We found that H3R mRNA and protein levels were up-regulated in the GBM and glioma cell lines compared to normal brain tissue and astrocytes. In U87MG cell line, inhibition of H3R by siRNA or the antagonist ciproxifan (CPX) suppressed proliferation, invasiveness, and the expression of EMT activators (Snail, Slug and Twist). In addition, expression of epithelial markers (E-cadherin and ZO-1) was up-regulated and expression of mesenchymal markers (vimentin and N-cadherin) was down-regulated in vitro and in vivo in a xenograft model. In addition, we also showed that inhibition of H3R by siRNA or CPX inactivated the PI3K/Akt and MEK/ERK signaling pathways, while inhibition of Akt or ERK activity with antagonists or siRNAs suppressed H3R agonist (R)-(α)-(−)-methylhistamine dihydrobromide (RAMH) mediated invasion and reorganization of cadherin-household. In conclusion, overexpression of H3R is associated with glioma progression. Inhibition of H3R leads to suppressed invasion and EMT of GBM by inactivating the PI3K/Akt and MEK/ERK pathways in gliomas.
**June 10 Patrizia Rizzu**

**Marco Siekmann:**

*PERK inhibition prevents tau-mediated neurodegeneration in a mouse model of frontotemporal dementia (Radford et al)*

Abstract The PERK-eIF2α branch of the Unfolded Protein Response (UPR) mediates the transient shutdown of translation in response to rising levels of misfolded proteins in the endoplasmic reticulum. PERK and eIF2α activation are increasingly recognised in postmortem analyses of patients with neurodegenerative disorders, including Alzheimer’s disease, the tauopathies and prion disorders. These are all characterised by the accumulation of misfolded disease-specific proteins in the brain in association with specific patterns of neuronal loss, but the role of UPR activation in their pathogenesis is unclear. In prion-diseased mice, overactivation of PERK-eIF2α-P signalling results in the sustained reduction in global protein synthesis, leading to synaptic failure, neuronal loss and clinical disease. Critically, restoring vital neuronal protein synthesis rates by inhibiting the PERK-eIF2α pathway, both genetically and pharmacologically, prevents prion neurodegeneration downstream of misfolded prion protein accumulation. Here we show that PERK-eIF2α-mediated translational failure is a key process leading to neuronal loss in a mouse model of frontotemporal dementia, where the misfolded protein is a form of mutant tau. rTg4510 mice, which overexpress the P301L tau mutation, show dysregulated PERK signaling and sustained repression of protein synthesis by 6 months of age, associated with onset of neurodegeneration. Treatment with the PERK inhibitor, GSK2606414, from this time point in mutant tau-expressing mice restores protein synthesis rates, protecting against further neuronal loss, reducing brain atrophy and abrogating the appearance of clinical signs. Further, we show that PERK-eIF2α activation also contributes to the pathological phosphorylation of tau in rTg4510 mice, and that levels of phospho-tau are lowered by PERK inhibitor treatment, providing a second mechanism of protection. The data support UPR-mediated translational failure as a generic pathogenic mechanism in protein-misfolding disorders, including tauopathies, that can be successfully targeted for prevention of neurodegeneration.

**Behnaz Shahsavari:**

*Cytoplasmic protein aggregates interfere with nucleocytoplasmic transport of protein and RNA (Woerner et al)*

Amyloid-like protein aggregation is associated with neurodegeneration and other pathologies. The nature of the toxic aggregate species and their mechanism of action remain elusive. Here, we analyzed the compartment specificity of aggregate toxicity using artificial β-sheet proteins, as well as fragments of mutant huntingtin and TAR DNA binding protein–43 (TDP-43). Aggregation in the cytoplasm interfered with nucleocytoplasmic protein and RNA transport. In contrast, the same proteins did not inhibit transport when forming inclusions in the nucleus at or around the nucleolus. Protein aggregation in the cytoplasm, but not the nucleus, caused the sequestration and mislocalization of proteins containing disordered and low-complexity sequences, including multiple factors of the nuclear import and export machinery. Thus, impairment of nucleocytoplasmic transport may contribute to the cellular pathology of various aggregate deposition diseases.
Nikolas Maragkos:
*A one-hit model of cell death in inherited neuronal degenerations* (G. Clarke et al)

In genetic disorders associated with premature neuronal death, symptoms may not appear for years or decades. This delay in clinical onset is often assumed to reflect the occurrence of age-dependent cumulative damage. For example, it has been suggested that oxidative stress disrupts metabolism in neurological degenerative disorders by the cumulative damage of essential macromolecules. A prediction of the cumulative damage hypothesis is that the probability of cell death will increase over time. Here we show in contrast that the kinetics of neuronal death in 12 models of photoreceptor degeneration, hippocampal neurons undergoing excitotoxic cell death, a mouse model of cerebellar degeneration and Parkinson’s and Huntington’s diseases are all exponential and better explained by mathematical models in which the risk of cell death remains constant or decreases exponentially with age. These kinetics argue against the cumulative damage hypothesis; instead, the time of death of any neuron is random. Our findings are most simply accommodated by a ‘onehit’ biochemical model in which mutation imposes a mutant steady state on the neuron and a single event randomly initiates cell death. This model appears to be common to many forms of neurodegeneration and has implications for therapeutic strategies.

A short N-terminal domain of HDAC4 preserves photoreceptors and restores visual function in retinitis pigmentosa (Guo et al)

Retinitis pigmentosa is a leading cause of inherited blindness, with no effective treatment currently available. Mutations primarily in genes expressed in rod photoreceptors lead to early rod death, followed by a slower phase of cone photoreceptor death. Rd1 mice provide an invaluable animal model to evaluate therapies for the disease. We previously reported that overexpression of histone deacetylase 4 (HDAC4) prolongs rod survival in rd1 mice. Here we report a key role of a short N-terminal domain of HDAC4 in photoreceptor protection. Expression of this domain suppresses multiple cell death pathways in photoreceptor degeneration, and preserves even more rd1 rods than the full-length HDAC4 protein. Expression of a short N-terminal domain of HDAC4 as a transgene in mice carrying the rd1 mutation also prolongs the survival of cone photoreceptors, and partially restores visual function. Our results may facilitate the design of a small protein therapy for some forms of retinitis pigmentosa.