6\textsuperscript{th} Research Day in medicine
6\textsuperscript{e} Journée de recherche en médecine
6. Forschungstag Medizin

University of Fribourg
Department of Medicine

Biomaterials, medicine and beyond
Wednesday March 15, 2017

Adolphe Merkle Institute
Auditorium (building C, 3\textsuperscript{rd} floor)

Sponsored by: NCCR Bio-inspired materials

Organized for MD, Postdocs and PhD students of the HFR and the University of Fribourg (Medicine, Biology, Biochemistry, Adolphe Merkle Institute and Sport medicine)
Program

09:15 – 09:45 Registration & Coffee, entrance hall, AMI

09:55 – 10:00 Welcome talk, Auditorium 3rd floor, AMI
Dr. Marie-Noëlle Giraud, Cardiology, DepMed, UniFR

10:00 – 10:30 Chemistry and AMI approaches, Auditorium 3rd floor, AMI
Chair: Prof. Nico Bruns, Adolphe Merkle Institute, UniFR

10:00 – 10:30 Prof. Katharina Fromm, Dept of Chemistry, UniFR
«Silver based anti-microbial coating»

10:30 – 11:00 Prof. Michael Mayer, Adolphe Merkle Institute, UniFR
«Nanopore-based Analysis of Amyloid Proteins in the context of Alzheimer’s Disease»

11:00 – 11:30 Prof. Christoph Weder, Adolphe Merkle Institute, UniFR
«Polymer nanocomposites for biomedical uses»

11:30 – 12:45 Lunch (sandwiches) entrance hall, AMI

12:45 – 14:30 Guided Poster presentation: 3 min per poster, entrance hall, AMI

14:30 – 15:30 Biology and methodologies, Auditorium 3rd floor, AMI
Chair: Prof. Beat Schwaller, Anatomy, DepMed, UniFR

14:30 – 15:00 Prof. Jörn Dengjel, Dept of Biology, UniFR
«Mass-spectrometry based proteomics in biomedical science»

15:00 – 15:30 Dr. Laurent Falquet, Bioinformatics platform, UniFR
«The Bioinformatics core facility (BUGFri): a portfolio of data analysis expertise»

15:30 – 16:00 Coffee break, entrance hall, AMI

16:00 – 17:00 Medicine and HFR approaches, Auditorium 3rd floor, AMI
Chair: Prof. Anna Lauber-Biason, Endocrinology, DepMed, UniFR

16:00 – 16:30 Prof. Curzio Rüegg, Pathology, DepMed, UniFR
«Dissecting the metastatic cascade»

16:30 – 17:00 Dr. Serban Puricel, Cardiology, DepMed, UniFR
«Bioresorbable stents»

17:00 – 17:15 End of day – Poster Prize, entrance hall, AMI
Dr. Marie-Noëlle Giraud, Cardiology, UniFR
Address
Adolphe Merkle Institute
Chemin des Verdiers 4
CH-1700 Fribourg
Switzerland

Getting there
- by road
  From A12 (north & south) leave at Fribourg-Sud
  From A1 (Zurich) to Berne then join A12 (Geneva) and leave at Fribourg-Sud

- by rail / bus
  Geneva / Zurich line: get off the train at Fribourg
  From Fribourg station, AMI is a five-minute bus ride (n°1, 3 or 7 in front of the station); get off at
  «Charmettes» (1 and 3) or «Jardin Botanique» (7)

Organisation Research day 2017
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State-dependency in inhibitory control proficiency: an electrical neuroimaging study

Michael De Pretto, Etienne Sallard, Lucas Spierer

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Abstract

Behavioral and brain responses to stimuli not only depend on their physical features but also on the individuals' neurocognitive states before stimuli onsets. While the influence of pre-stimulus fluctuations in brain activity on low-level perceptive processes is well established, the state dependency of high-order executive processes remains unclear. Using a classical inhibitory control Go/NoGo task, we examined whether and how fluctuations in the brain activity during the period preceding the stimuli triggering inhibition influenced inhibitory control performance. Seventeen participants completed the Go/NoGo task while 64-channel electroencephalogram was recorded. We compared the event-related potentials preceding the onset of the NoGo stimuli associated with inhibition failures false alarms (FA) vs. successful inhibition correct rejections (CR) with data-driven statistical analyses of global measures of the topography and strength of the scalp electric field. Distributed electrical source estimations were used to localize the origin of the event-related potentials modulations. We observed differences in the global field power of the event-related potentials (FA > CR) without concomitant topographic modulations over the 40 ms period immediately preceding NoGo stimuli. This result indicates that the same brain networks were engaged in the two conditions, but more strongly before FA than CR. Source estimations revealed that this effect followed from a higher activity before FA than CR within bilateral inferior frontal gyri and the right inferior parietal lobule. These findings suggest that uncontrolled quantitative variations in pre-stimulus activity within attentional and control brain networks influence inhibition performance. The present data thereby demonstrate the state dependency of cognitive processes of up to high-order executive levels.
Anti-oxidative role of cytoglobin in podocytes and its association with chronic kidney disease

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Abstract

Cytoglobin (CYGB) is a member of the mammalian globin family, in addition to hemoglobin, myoglobin, neuroglobin and androglobin. Despite extensive research efforts, its physiological role remains unknown, but potential functions include reactive oxygen species (ROS) detoxification and signaling. Gene array expression analysis of biopsies from chronic kidney disease (CKD) and genome-wide association studies independently indicate that CYGB may be potentially implicated in CKD, particularly in diabetic nephropathy (DN). Accumulating evidence suggests that ROS play a crucial role at the onset of DN leading to podocyte detachment and/or apoptosis. To assess the putative anti-oxidative function of CYGB in podocytes, we established stable CYGB knockdown in the human podocyte cell line AB8/13. CYGB deficient podocytes displayed increased cell death and accumulation of ROS as assessed by H2-DCF-DA assays and the redox sensitive probe roGFP2-Orp1. Transcriptome analysis of control and CYGB knockdown cells identified dysregulation of multiple genes involved in apoptosis and redox balance. Interestingly, Seahorse technology demonstrated that CYGB knockdown cells consume less oxygen and are less metabolically active compared to control cells. To assess the role of Cygb in vivo, murine Cygb expression during nephrogenesis was assessed, showing a pronounced upregulation in the late phases of kidney organogenesis. Additionally, preliminary results on global Cygb knockout mice revealed that Cygb may be involved in maintaining kidney function. In conclusion, data of our study demonstrate that CYGB protects podocytes from oxidative stress and cell death, and may be involved in CKD, particularly in DN.
Malaria derived extracellular vesicles influence human neutrophils function

Kehinde Adebayo Babatunde¹, Michael Walch¹, Isabelle Fellay¹, Solange Kharoubi Hess¹, Luis Filgueira¹, Ionita Ghiran², Pierre Yves Mantel¹

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Abstract

Introduction
A dysfunctional innate immune response is believed to provide immune evasion of the malaria parasites, but also to cause increased susceptibility to bacterial infections. Neutrophils are the most abundant cells found in the blood circulation in direct contact with parasite infected red blood cells (iRBCs). However neutrophils population with reduced oxidative burst activities are present during malaria infection. These observations suggest that neutrophil responses are fundamentally defective in malaria patients. Extracellular vesicles (EVs) are iRBCs derived vesicles and contain both parasite and host materials, including microRNAs. In this present work we investigated, how EVs modulate neutrophil response.

Results
Interestingly, we have reported that malaria EVs contain miR451a, a miRNA that is known to regulate neutrophil activity. We have also monitored the uptake of EVs by neutrophils by fluorescence microscopy and real time polymerase chain reaction techniques. To address the role of miR451, we investigated the influence of malaria-induced EVs on human neutrophil functions in vitro and the effect of miR451a on the transcriptional response in differentiated HL60 cells to bacterial infections. We demonstrated that EVs inhibit neutrophil function by inhibiting neutrophils ability to produce ROS and suppression of cytokine secretion. The neutrophils were also affected in their bactericidal activity.

Conclusion
Our data indicate that malaria EVs deliver miR451 into neutrophils to interfere with their capacity to kill bacteria. We describe a new mechanism of cellular communication between parasites and the host immune system. While EVs might increase tolerance to the parasites, they dramatically affect the resistance to a co-infection by bacteria. The elucidation of the immune regulatory role of EVs might lead to the development of new diagnostic tools and therapies.
Effects of prefrontal transcranial direct current stimulation on language production in post-stroke aphasia

Pestalozzi M.I.¹, Di Pietro M.², Martins C.², Chouiter L.³, Spierer L¹, Schnider A², Annoni J.M.¹, Jost L.B.¹

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Abstract

A successful interplay between prefrontal and domain-specific language areas has been shown to be crucial for language processing. Non-invasive brain stimulation is increasingly being used as a promising therapeutic tool for neurological diseases. The aim of the present study is to investigate the effects of transcranial direct current stimulation (tDCS) of the prefrontal cortex (PFC) on language production in chronic post-stroke aphasic patients.

We used a randomized, sham-controlled and double-blind within-subject design. tDCS was applied for 20 minutes with a current density of 0.04 mA/cm², with the anodal electrode placed over the left dorsolateral prefrontal cortex and the cathodal electrode placed over the right supraorbital area. As outcome measures, a picture naming task, a repetition task, a verbal fluency task and a nonverbal executive functions task were performed both during (online) and immediately after stimulation (offline).

Preliminary results of the first five participants with anomic aphasia and mild language-related disability revealed significant offline-effects of anodal tDCS as compared to sham tDCS in repetition and verbal fluency. Significantly more words were produced in the fluency task and voice onset times in the repetition task were significantly shorter. Importantly, also on the individual subject level, the effects point towards the same direction for all participants. However, results indicate no online effects as well as no effects in the picture naming and in the nonverbal executive functions tasks.

These preliminary results suggest that increasing prefrontal excitability during language tasks might have beneficial after-effects on language production in chronic patients with aphasia.
Effects of continuous theta burst stimulation over the DLPFC on language production in late bilinguals

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Abstract

Clinical and neuroimaging studies indicate that language processing relies both on dedicated language networks and on a domain-general cognitive control network. The involvement of a domain-general executive network in language is also supported by the results of (r)TMS studies, showing that stimulation over the left dorsolateral prefrontal cortex (DLPFC) influences verbal working memory, sentence comprehension and language switching. The present study investigated the effects of an inhibitory continuous theta burst stimulation (cTBS) protocol over the left DLPFC on language production in healthy late bilingual subjects.

We used a randomized, sham-controlled, and single-blind within-subject design coupled with EEG recordings. The cTBS protocol consisted of a continuous train of 801 pulses delivered in 267 bursts; each burst contained 3 pulses at 30Hz, repeated with an interburst interval of 100 ms. Immediately after the stimulation, participants performed a picture naming task in their first (L1) and second language (L2), a translation task and a non-verbal task measuring executive functions (‘Eriksen’-Flanker-task).

Preliminary behavioral results obtained in 16 participants show no significant effects of cTBS, neither in the picture naming task nor in the Flanker task. In the picture naming task, we replicated the well-established effects of more accurate and faster responses for L1 as compared to L2. In the flanker task, reaction times were faster for the congruent as compared to the incongruent condition, which is in line with the results of previous studies.

Although according to the preliminary analyses cTBS did not induce significant behavioral effects, the ongoing electrophysiological neuroimaging analyses of the ERPs recorded during the tasks may provide new information concerning the interactions between the language networks and the more domain-general cognitive control network.
Parvalbumin modulation: treatment for autism spectrum disorders?

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Abstract

Autism spectrum disorders (ASD) consists of a group of neurodevelopmental disorders characterized by three core symptoms: impaired social interactions, communication deficits and stereotyped behavior. In post-mortem brains from autistic individuals and in different ASD mouse models, the numbers of immunoreactive interneurons positive for the expression of the calcium-binding protein parvalbumin (Pvalb neurons) were found to be decreased. We have demonstrated that in Shank mutant models this reduction was the result of parvalbumin (PV) protein down-regulation and not a loss of this interneuron subpopulation. The same held true for Pvalb mutant mice with reduced (PV+/-) or absent (PV-/-) PV expression; both genotypes showed an autistic-like phenotype. Since we hypothesized that PV-down-regulation might be sufficient to elicit the ASD-like traits, an upregulation of PV protein levels in PV-/- and PV+/- mice, possibly restoring them to wildtype levels, might diminish or possibly even abrogate the ASD-like phenotype. For these aims different strategies, including the production of transgenic mice with inducible up- and/or down-regulation of PV are generated. In one strain, the expression of PV can be reversibly blocked by an inducible shPvalb strategy. This allows for the testing whether the down-regulation of PV at a later (adult) stage will lead to an ASD-like phenotype in this transgenic line. Other strategies are focusing on re-expression an/or upregulation of PV expression in PV-reduced (PV+/-) mice at selected time points. All mice with genetic and/or pharmacologic interventions will be tested at the behavioral level including the sociability of mice in the 3-chamber test.
Cardiac Bone Marrow-Derived Cell-based Therapy associated with scaffold for Heart regeneration

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Abstract

Purpose
The development of patched associating of bone marrow derived cells (BMDC) and a substrate/scaffolds provided evidence of beneficial outcomes and optimal delivery approaches on myocardial infarction (MI). Preclinical trials are usually performed with BMDC isolated from healthy donor. However, for clinical application, BMDC isolated from infarcted patients are favoured as an autologous cell source. We hypothesized that the therapeutic capacity of the implanted biological patch may vary with BMDC origin. To test this hypothesis, we compared the regenerative potential of a biological patch composed of BMDC isolated from healthy or infarcted donor as a treatment of MI in a rat model.

Methods
MI was induced by Left Anterior Descending Artery ligation. BMDC were isolated from healthy and infarcted Lewis male rats. MI was induced in 52 female Lewis rats; two weeks post MI, 34 rats with an ejection fraction (EF) between 35-60% were selected and randomised in different treatment groups, sham operation (Sh, n=9), epicardial application of the biological patches obtained with 2 million cells isolated from healthy donors (HD, n=12), or from infarcted donors (ID, n=7) or substrate only (S, n=6). Four weeks post treatment, cardiac function and regional contractility (strain imaging) were measured by high-resolution echocardiography, the infarct expansion was quantify using systematic sampling of total heart and image analyses of trichromestained histological sections.

Results
Four weeks post MI, heart function decreased in control groups (ΔEFS=−1.21±3.2% and ΔEFS=−7.4±6.4%). Cells and substrate significantly induced stabilizations of heart function compared to substrate alone (ΔEFHD=1.1±5.6%,p=0.02 and ΔEFID=0.99±4.9%,p=0.4). Nevertheless, it is important to underline that heterogeneity in response to the treatments was observed, 42% responded positively to the treatment (ΔEF≥3%). In addition, the regional LV myocardial contractility showed that the LV non-contractile region decreased significantly for HD group from 10.8±3.3mm to 8.3±4.0mm (p=0.02). Furthermore, the index expansions (EI) decreased with the implantation of the biological patches, and were respectively EISh=0.21±0.11 vs. EIHD=0.08±0.04(p=0.05) vs. EIID=0.15±0.07(p=0.47). Interestingly, the LV volume significantly increased 4 weeks post ID patch implantation. These results suggested that although ΔEF for both biological patches are comparable, LV dilatation observed with infarcted cells may be detrimental in long term.

Conclusion
Our study demonstrated that independent of the cell origin, patches stabilized heart function. However, only healthy donor cells showed a regenerative capacity. Noticeably, LV dilatation following infarcted cell implantation questioned the long-term safety. Heterogeneity of outcomes revealed the presence of respondent and non-respondent subjects to the trea
Androglobin: a newly identified globin required for male fertility in mice

Anna Keppner, Sara Santambrogio, David Hoogewijs

Abstract

Globins are small globular metallo-proteins, involved in different cellular functions via their reversible binding capacity to gaseous ligands (O₂, CO and NO) and their storage, transport and detoxification. Besides the well-known hemoglobin (Hb) and myoglobin (Mb), various new globin types have been discovered in vertebrates, including cytoglobin, neuroglobin, and more recently also androglobin (Adgb). Adgb is a chimeric protein, consisting of a calpain-like domain and a globin-like domain, and is mainly expressed in testis tissue. In this study, we aimed to analyse the in vivo function of Adgb, using transgenic and knockout mice. A gene-trap construct was inserted into the mouse Adgb gene, comprising a lacZ reporter sequence, and three loxP sites, thereby generating the Adgb<sub>tm1a</sub> line. By crossing these mice with CMV-promoter driven Cre-recombinase-expressing mice, Adgb<sub>tm1b</sub> mice were obtained, in which exons 13 and 14 of the Adgb gene were removed, with remaining lacZ gene expression. Finally, by crossing Adgb<sub>tm1a</sub> mice with both Flp-recombinase and subsequent Cre-recombinase-expressing mice, full knockout Adgb<sub>tm1d</sub> mice were obtained, lacking the complete gene-trap insert and exons 13 and 14. All three mouse lines display male infertility at the homozygous state, indicating spermatogenesis-related defects. Our preliminary data suggest that Adgb plays a role in elongating spermatids as revealed by FACS analysis, and X-gal staining on microdissected seminiferous tubules shows predominant expression in the midpiece of elongating spermatids and mature spermatozoa. Interestingly, in humans, ADGB expression is strongly reduced in infertile men as compared to fertile individuals. Since the cell proliferation rate during spermatogenesis is extremely high, and considering the high oxygen gradient present in testis, the discovery of a globin domain-containing protein is of high interest and opens a new route towards the understanding of oxygen-dependent mechanisms involved in sperm formation.
Detection of rare circulating tumor cells with a nanoparticle-based amplification system

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Abstract

Breast cancer remains a leading cause of cancer-related women mortality in developed countries. Over one third of all breast cancer patients will die the disease due to metastasis. The presence of circulating tumor cells (CTC) is associated with systemic disease, resistance to therapy and relapse. Thus CTC can be considered as easily accessible surrogate markers or systemic disease progression. Detection of CTC remains challenging, in part due to their very low frequency among circulating blood cells, and nowadays require time consuming manipulation or complex and expensive equipment. Nature has developed many mechanisms to specifically amplify weak signals. One notable system is blood coagulation, a process by which the blood forms clots following endothelial injury or other major homeostatic perturbations.

In this project we take inspiration by nature to develop an in vitro diagnostic (IVD) test based on sensor-responsive nanoparticles (NPs) capable of dynamic self-assembly through amplification cascades allowing sensitive detection of cancer cells.

We propose a concept where a first nanoparticle (NP1) is engineered to specifically target breast cancer cells and upon binding, to initiate the enzymatic polymerization of fibrinogen. A second NP (NP2) carrying detection moieties is added and will then be recruited to the polymerized fibrin to render the decorated CTC detectable.

We have developed NP1 by coupling an antibody directed against HER2 (Trastuzumab) and thrombin to 40nm gold nanoparticles coated with Protein G (InnovaCoat®). We generated NP1 and analyzed their ability to detect HER2^{high} (SKBR3), HER2^{low} (MDA-MB-231) and HER2^{neg} (MDA-MB-468) cells and initiate coagulation. Obtained results showed that NP1 can specifically detect HER2 expressing cells (flow cytometry, microscopy) with high discrimination and initiate coagulation. Currently, we are characterizing the sensitivity and specificity of NP1 to detect CTC in blood samples and devising NP2.
Respiratory health hazard assessment of a biomedical candidate, graphene oxide, using a 3d lung tissue model

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Abstract

Graphene and related materials (GRM) exhibit promising properties for a wide range of new high-technology and biomedical applications. In particular, graphene oxide (GO), a subclass of GRM with a high content of oxygen-containing functional groups, has been recognized as a promising medical agent for drug delivery, imaging, tissue engineering, as well as in a new generation biosensors¹,². Mass production of GRM, including GO, has considerably increased in the past several years hence it is imperative to assess their possible interaction with humans during the life-cycle and their potential hazards to human health under realistic exposure scenarios³. Concerns have been raised especially regarding their interaction with the respiratory system in occupational exposure settings as inhalation is considered the primary route of exposure for airborne particles⁴. It has been shown that GO can be easily respirable and interact with lung cells thus resulting in induction of oxidative stress and pulmonary inflammation⁵. In the present study, a 3D human lung model composed of epithelial cells, and primary human immune cells, dendritic cells and macrophages⁶ was combined with a commercial aerosolization system (VitroCell®Cloud) which enabled a simulation of realistic exposures of inhaled GO to the lung epithelial barrier tissue. Deposited masses (ranging from ~ 300 to 1000 ng/cm², assessed by the quartz crystal microbalance)⁷ of the aerosolized material corresponded to an acute exposure scenario. The effect of GO was compared to that induced by the selected 2D (graphene nanoplateletes) and non-2D (carbon black) benchmark carbon-based materials. None of the investigated parameters, i.e. cell viability and morphology, pro-inflammatory response and oxidative stress, was elevated 24 hours post exposure which suggests an acute exposure to GO did not initiate biological response of the 3D human lung model.

CBX2.2 mutation as novel cause for 46,XY Disorder of Sex Development

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¹University of Fribourg, Department of Medicine, Division of Endocrinology, University of Fribourg, Switzerland, ²University of Sao Paulo, Medical School, Brazil

Abstract

Introduction
Sexual differentiation during embryonic development is one of the defining moments of human life. The chromatin regulator CBX2.1 has previously been identified as essential for human male development. However, less is known about the second isoform CBX2.2. We set to elucidate the role of CBX2.2, taking advantage of two distinct mutations in two unrelated 46,XY patients with dysgenetic gonads.

Methods
We performed Whole Exome Sequencing with genomic DNA samples from both patient, to rule out mutations in other 46,XY DSD related genes. For the identification of CBX2.2 binding targets, we performed DNA adenine methyltransferase and next generation sequencing in human testicular cells and analyzed the data with Pathway Studio and Gene Ontology Enrichment. The expression pattern of potential candidate genes has been validated using qRT-PCR under overexpression of either WT or mutant CBX2.2.

Results
We identified over 1900 direct binding targets of CBX2.2. Six were selected based on their known role in sex development: EMX2, MAK, HOXA13, WDR77, TWIST1 and BNC2. We validated the influence of WT and both CBX2.2 mutants on these targets using qRT-PCR. In particular, WT CBX2.2 increased the expression of EMX2, whereas the mutated CBX2.2 proteins were inactive.

Conclusion
It is intriguing to hypothesize that, at least in part, mutations in CBX2.2 impair EMX2 expression in the formation of the early gonad and cause gonadal dysgenesis in 46,XY individuals similarly to EMX2 haploinsufficiency. This study shows the importance of CBX2.2 and identifies several of its partners, broadening our understanding of sex development and disorder of sex development.
Arginase-II promotes tumor necrosis factor-α release from pancreatic acinar cells causing β-cell apoptosis in aging

Yuyan Xiong, PhD, Gautham Yepuri, Jean-Pierre Montani, MD; Zhihong Yang, MD; Xiu-Fen Ming, MD, PhD

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Abstract

Aims
Aging is associated with insulin resistance and pancreatic dysfunction. Our previous studies demonstrated that arginase-II (Arg-II) deficiency protects mice against atherosclerosis, vascular aging and obesity-associated type 2 diabetes. It has been reported that Arg-II is expressed in pancreas of rodents and humans However, functions of Arg-II in regulation of pancreatic β-cells and in age-associated glucose intolerance are not known.

Design & Methods
The WT and Arg-II−/− offspring from hetero/hetero cross were interbred to obtain WT and Arg-II−/− mice, respectively. Pancreatic cell apoptosis was evaluated by Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining.

Results
Here we show that targeted disruption of Arg-II improves glucose tolerance as a result of increased insulin secretion without significant change in insulin sensitivity as compared to age-matched old wild type (WT) mice, which is associated with larger pancreatic islet size and higher β-cells mass in the old Arg-II−/− mice. Arg-II is mainly expressed in acinar cells and upregulated with aging in female WT mice with concomitant enhanced TNF-α release from the pancreatic acinar-cells leading to apoptosis of the pancreatic β-cells. Moreover, conditioned medium of isolated acinar cells from old WT mice enhances apoptosis of cultured β-cells in vitro, which is reduced by neutralizing antibody against TNF-α.

Conclusions
In this study, we demonstrate an age-associated Arg-II upregulation in pancreatic acinar cells, which promotes TNF-α release through p38mapk, leading to β-cell apoptosis, insufficient compensatory insulin secretion, and glucose intolerance in mice in a gender-specific manner.
Role of arginase-II in regulation of water balance

Ji Huang1,4, Jean-Pierre Montani1,4, François Verrey2,4, Eric Feraille3,4, Xiu-Fen Ming1,4, Zhihong Yang1,4

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Abstract

Aim
Arginase-II (Arg-II) is highly expressed in kidney with its most abundant expression in proximal tubule epithelial cells and also in collecting duct cells. However, the function of Arg-II in kidney remains largely unknown. In the present study, we aim to investigate the role of Arg-II in regulation of vasopressin-regulated water channel protein aquaporin 2 (AQP2) in collecting ducts and the impact on water balance.

Methods and results
In cultured mouse collecting duct cell line mCCDc11, desamino-d-arginine vasopressin (dDAVP), a synthetic vasopressin receptor V2-agonist, stimulated expression and membrane translocation of AQP2 as expected and upregulated Arg-II levels as assessed by immunoblotting and/or immunofluorescence staining. Silencing Arg-II further enhanced AQP2 expression and membrane translocation in response to dDAVP. Conversely, overexpression of wild type (WT) or an inactive Arg-II mutant suppresses the dDAVP's effects. In agreement with these findings in vitro, total and membrane-associated AQP2 levels were significantly higher in Arg-II-deficient (Arg-II−/−) than WT mice, demonstrating a negative regulation of AQP2 by Arg-II. Furthermore, the total and membrane-associated AQP2 levels in WT mice were increased by water deprivation paralleled with an increased Arg-II level in collecting duct cells and decreased urine volume and enhanced urine and plasma osmolality. Arg-II−/− mice, however, showed more pronounced water preservation effect under the water deprivation condition.

Conclusion
Arg-II in collecting duct cells controls water balance through negative regulation of AQP2 expression and membrane translocation independently of its L-arginine: ureahydrolase activity.
Androglobin is a recently discovered oxygen-binding globin with a crucial role in spermatogenesis

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Abstract

We recently identified a novel globin lineage, consisting of a large chimeric protein with an N-terminal protease domain and a central globin domain, named androglobin (Adgb) because of its specific expression in testis tissue. Intriguingly, this new member of the globin family is evolutionary ancient and extremely conserved, being present in mammals, vertebrates, more basal animal clades and even unicellular organisms. Hexacoordination of the Adgb heme iron and lack of transcriptional induction in hypoxia in mammalian cell culture and in mice as well as decreased expression in human tumor biopsies indicate a function independent of classical O₂ supply. Adgb expression is associated with postmeiotic stages of spermatogenesis and analysis of a newly generated Adgb knock-out mouse model suggests a crucial role in reproduction, consistent with decreased Adgb expression levels in semen and testis biopsies from infertile males. Phenotyping of Adgb-deficient mice demonstrates absence of mature spermatozoa and developing elongating spermatids in the lumen of seminiferous tubules, indicating an Adgb-dependent arrest of spermatogenesis prior to spermatid differentiation at the round haploid spermatid stage. To gain additional insights into the physiological function of Adgb we explored the Adgb-dependent interactome by performing immunoprecipitation (IP) followed by mass spectrometry analysis, and identified multiple spermatogenesis-related proteins with major roles in chromatoid body formation and RNA processing and storage. Validating co-IP and FRET experiments confirm the observed interactions further suggesting that Adgb is indispensable for male reproduction possibly via chromatoid body associated processes.
A new mechanism controlling cancer stem cells in breast cancer

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Abstract

Over the last years it has been shown that many tumor types are organized as a hierarchy in which cancer stem cells (CSC) are at the top. Recent data by a few research groups, including ours, demonstrate that CSC are not only responsible for tumor maintenance and resistance to therapy, but they are also the cells that lead metastatic colonization. Since over 90% of cancer-related deaths are due to metastatic disease, understanding the mechanisms that CSC use in order to colonize secondary organs is essential to the field of cancer biology. We have recently identified a molecule that is crucial to control mammary gland stem cell fate. It is expressed mainly by metastatic stem cells and fibroblasts. Interestingly, if we use genetic engineering to delete this gene in mice, mammary gland tumors then show reduced numbers of cancer stem cells and impaired metastatic potential. Moreover, limiting dilution assays demonstrate that mammary gland tumors lacking this molecule have a dramatic reduction in tumor initiating capacity and metastatic colonization ability. Mechanistically, our results indicate that this is due to a decrease in stemness in this population due to impaired communication of CSC with other tumor promoting cell types and the microenvironment. Human data mining shows that the factor we identified predicts poor prognosis in breast cancer and is significantly increased in claudin-low breast cancer cells, which are known to contain high numbers of CSC. Overall, these data reveal a new biological mechanism of CSC maintenance in breast cancer. Since this molecule is barely expressed in healthy tissues in the adult, we think that it could potentially be exploited to treat breast cancer from a CSC perspective.
Prenatal Valproate Exposure Differentially Affects Parvalbumin-Expressing Neurons and Related Circuits in the Cortex and Striatum of Mice

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Abstract

Autism spectrum disorders (ASD) comprise a number of heterogeneous neurodevelopmental diseases characterized by core behavioral symptoms in the domains of social interaction, language/communication and repetitive or stereotyped patterns of behavior. In utero exposure to valproic acid (VPA) has evolved as a highly recognized rodent ASD model due to the robust behavioral phenotype observed in the offspring and the proven construct-, face- and predictive validity of the model. The number of parvalbumin-immunoreactive (PVC) GABAergic interneurons has been consistently reported to be decreased in human ASD subjects and in ASD animal models. The presumed loss of this neuron subpopulation hereafter termed Pvalb neurons and/or PV deficits were proposed to result in an excitation/inhibition imbalance often observed in ASD. Importantly, loss of Pvalb neurons and decreased/absent PV protein levels have two fundamentally different consequences. Thus, Pvalb neurons were investigated in in utero VPA-exposed male (“VPA”) mice in the striatum, medial prefrontal cortex (mPFC) and somatosensory cortex (SSC), three ASD-associated brain regions. Unbiased stereology of PVC neurons and Vicia Villosa Agglutinin-positive (VVAC) perineuronal nets, which specifically enwrap Pvalb neurons, was carried out. Analyses of PV protein expression and mRNA levels for Pvalb, Gad67, Kcnc1, Kcnc2, Kcns3, Hcn1, Hcn2, and Hcn4 were performed. We found a 15% reduction in the number of PVC cells and decreased Pvalb mRNA and PV protein levels in the striatum of VPA mice compared to controls, while the number of VVAC cells was unchanged, indicating that Pvalb neurons were affected at the level of the transcriptome. In selected cortical regions (mPFC, SSC) of VPA mice, no quantitative loss/decrease of PVC cells was observed.

However, expression of Kcnc1, coding for the voltage-gated potassium channel Kv3.1 specifically expressed in Pvalb neurons, was decreased by 40% in forebrain lysates of VPA mice. Moreover, hyperpolarization-activated cyclic nucleotide-gated channel (HCN) 1 expression was increased by 40% in the same samples from VPA mice. We conclude that VPA leads to alterations that are brain region-and gene-specific including Pvalb, Kcnc1, and Hcn1 possibly linked to homeostatic mechanisms. Striatal PV down-regulation appears as a common feature in a subset of genetic (Shank3B−/−) and environmental ASD models.
Proton Pump Inhibitors Impair Vascular Function by Accelerating Endothelial Senescence


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Abstract

Objective
Proton pump inhibitors (PPIs) like Esomeprazole (Nexium) are extensively used drugs for the treatment of gastroesophageal reflux disease (GERD). Recently, the use of PPI has been associated with increased cardiovascular (CV) risk. In this study, we hypothesized that PPIs pose CV risk by accelerating vascular aging and endothelial dysfunction. Therefore, we aimed to investigate the mechanisms that may lead to the development of CVD.

Methods and results
Long-term incubation of human microvascular endothelial cells (ECs) with clinically relevant concentration of PPIs (esomeprazole) accelerated premature endothelial cell senescence by shortening telomere length. A significant increase in superoxide levels and decrease in nitric oxide production, decrease in DDAH, eNOS and iNOS gene expression were also observed upon PPI treatment. These factors are well known to increase oxidative stress and exacerbate endothelial dysfunction. Functionally, PPIs impaired the angiogenic and proliferative capacity of ECs as confirmed by Matrigel tube formation and cell proliferation assays. Investigation of the molecular pathways involved in PPI-accelerated endothelial senescence revealed that plasminogen activator inhibitor-1 (PAI-1), a gene commonly associated with EC senescence and telomere length, was significantly up-regulated by PPI treatment. PPI treatment increased pH of lysosomes inactivating lysosomal enzymes leading to accumulation of misfolded proteins and impaired proteostasis. Furthermore, PPIs downregulated all genes involved in Shelterin complex (a set of genes involved in regulation and maintenance of telomere function and also responsible for the regulation and signaling of DNA damage response pathways) providing a mechanistic evidence for the role of PPIs in accelerating endothelial senescence.

Conclusion
Our data provides the first molecular and mechanistic evidence that PPIs pose a major risk for CVDs by accelerating endothelial senescence and endothelial dysfunction through, at least in part, activation of PAI-1 protein, lysosomal dysfunction and telomere shortening. Given the widespread and long-term use of PPIs in the absence of medical supervision, our data raises a major safety concern.
Parvalbumin-expressing ependymal cells in rostral lateral ventricle wall adhesions contribute to aging-related ventricle stenosis in mice

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Abstract

Aging-associated ependymal-cell pathologies can manifest as ventricular gliosis, ventricle enlargement or ventricle stenosis. Ventricle stenosis and fusion of the lateral ventricle (LV) walls is associated with a massive decline of the proliferative capacities of the stem cell niche in the affected subventricular zone (SVZ) in aging mice. We examined the brains of adult C57BL/6 mice and found that ependymal cells located in the adhesions of the medial and lateral walls of the rostral LVs overexpressed parvalbumin (PV) and displayed reactive phenotype, similarly to injury-reactive ependymal cells. However, PV+ ependymal cells in the LV-wall adhesions, unlike injury-reactive ones, did not express GFAP. S100B+/PV+ ependymal cells found in younger mice diminished in the LV-wall adhesions throughout aging. We found that periventricular PV-immunofluorescence showed positive correlation to the grade of LV stenosis in non-aged mice (< 10-month-old), and that the extent of LV-wall adhesions and LV stenosis was significantly lower in mid-aged (> 10-month-old) PV-KO mice. This suggests an involvement of PV+ ependymal cells in aging-associated ventricle stenosis. Additionally, we observed a time-shift in microglial activation in the LV-wall adhesions between age-grouped PV-KO and wild-type mice, suggesting a delay in microglial activation when PV is absent from ependymal cells. Our findings implicate that compromised ependymal cells of the adhering ependymal layers upregulate PV and display phenotype shift to “reactive” ependymal cells in aging-related ventricle stenosis; moreover, they also contribute to the progression of LV-wall fusion associated with a decline of the affected SVZ-stem cell niche in aged mice.
Diversity of microvascular endothelial cell markers in different regions of the human brain

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Abstract

Background
Microvascular endothelial cells in the brain have various important functions, including contribution to the blood-brain barrier and supply of nutrients and oxygen to the brain, as well coagulation control, inflammatory responses and angiogenesis. Endothelial cells have observable morphological and molecular differences in different tissues and organs. However little is known about differences of microvascular endothelial cells in the human brain. The aim of this study was to characterize qualitatively the expression of endothelial cell markers within different parts of the brain.

Methods
Frozen sections from different anatomical regions (precentral and postcentral gyrus, hippocampus, rhinal and visual cortex) of human formalin fixed brains (n=3) were stained immunohistochemically for endothelial cell markers, including CD31, claudin 5, occluding, von Willebrand Factor, ZO-1, as well as astrocyte (glial fibrillary acid protein) microglia (Iba1) markers.

Results
The expression patterns of the markers was heterogenous in the different regions of the brain studied. CD-31 had the most positive expression among the different biomarkers especially in the precentral cortex. ZO-1 and and vWF had fairly positive expressions in the pre central and post central cortices compared to other regions.

Conclusion
The expression pattern of the different endothelial markers is heterogenous in the microvasculature within the different anatomical regions of the cerebral cortex. This results indicate that the diversity in microvascular endothelial cells contribute to functional differences in the different brain regions.
Characterization of AKR1C2: New Insights into the backdoor pathway for dihydrotestosterone biosynthesis and implications for Disorders/Differences of Sex Development (DSD)

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Abstract

In the “classic” pathway of androgen synthesis testosterone synthetized in the testis is peripherally converted to the more potent dihydrotestosterone (DHT) by 5α-reductase. Additionally, an alternative “backdoor” pathway leading to DHT without testosterone as a precursor has been described. Recent studies on mutations in the enzymes of the backdoor pathway AKR1C2/C4 in patients with 46,XY DSD indicated that both pathways are required for normal human male sex development.

AKR1C2 has 3 splicing variants encoding 2 different enzyme isoforms: variants 1 and 2 encode isoform 1, the canonical enzyme, and variant 3 encodes the shorter isoform 2. The transcript variant 3 differs in its 5’UTR, lacks multiple 3’-coding exons, and includes a different terminal exon. RT-qPCR showed that fetal steroidogenic tissues expressed all 3 variants. The enzymatic activity and function of AKR1C2 isoform 2 is unknown. Our biochemical characterization suggested that, at comparable stoichiometry, the short AKR1C2 is more active in producing DHT than the long isoform and might therefore play an important role in human fetal sex development. In parallel, DNA sequencing analysis of the 31 kb AKR1C2 gene in a cohort of DSD patients revealed complex rearrangements, such as loss of heterozygosity in at least one case.
Thank you for your participation!