JCI 2014

Communications orales / Talks

SynAggreg: a new high-throughput *in vitro* aggregation assay to identify synergistic effects of aggregation modulator compounds and for drug screening

Hubert Aviolat[1], Sophie Gioria[2], Chantal Weber[1], Pascal Villa[2], Yvon Trottier[1] and Fabrice A. C. Klein[1]

[1]*Translational Medicine and Neurogenetics Department, Institute of Genetics and Molecular and Cellular Biology (IGBMC), Illkirch, France.*
[2] *Platform of Integrative Chemical Biology of Strasbourg (PCBIS), Illkirch, France.*

The intracellular aggregation of mutant huntingtin (mHtt) fragments is a hallmark of Huntington disease (HD). While the toxicity of the aggregates is still debated, small molecules that modulate *in vitro* the aggregation of mHtt have protective effects in simple *in vivo* cellular and small organism models of HD. Thus, besides their interest as fundamental research tools to study the aggregation cascade and its role in HD pathology, aggregation modulators are molecules of potential therapeutic interest. However, such compounds tested in HD mice models have often failed to efficiently slow down the evolution of the pathology. There is thus a need to develop strategies to improve the efficiency of aggregation modulators for therapeutic intervention.

Bioavailability, blood-brain barrier crossing and dose/toxicity are classical issues for drug development. Additionally, mHtt fragments adopt a wide range of conformations that are difficult to target individually with high specificity. We reasoned, and obtained a first *in vitro* proof of principle, that combining compounds that target different conformations can modulate synergistically the aggregation of polyglutamine. Our goal is now to select *in vitro* combinations of compounds that efficiently modulate at low doses the aggregation of mHtt fragments, before testing the efficiency of this combinatorial strategy *in vivo*.

Existing *in vitro* high-throughput methods used to monitor the aggregation kinetics of amyloids are not suited to study combinations of compounds: they either lack sensitivity and reproducibility (e.g. the filter assay method), or their read-out relies on the direct interaction of a reporter molecule with a specific amyloid conformation (e.g. Thioflavin-T or SEPRION) that may be affected by cocktails of tested compounds.

We present here SynAggreg, a new sensitive, accurate, reproducible and cost effective high-throughput *in vitro* technology, adaptable to different amyloid sequences including mHtt fragments, that allows to study the effect of combinations of compounds, as well as to screen for new aggregation modulator compounds. This new method is based on the use of fluorescently labeled amyloid, whose aggregation can be specifically triggered and followed over time in a fully automated manner.

Characterization of mouse models for the 16p11.2 microdeletion and microduplication syndromes - likeness in the mirrors

Thomas ARBOGAST[1], Abdel OUAGAZZAL[1], Marie-France CHAMPY[2], Marie-Christine BIRLING[2], Alexandre REYMOND[3], Yann HERAULT[1,2]

[1] Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS/INSERM/UNISTRA, 67404 Illkirch, France

[2] Institut Clinique de la Souris, CNRS/INSERM/UNISTRA, 67404 Illkirch, France

[3] Center for Integrative Genomics, UNIL, CH-1015 Lausanne, Switzerland

---

Since 2005, genome-wide association studies have identified an important number of copy number variations (CNVs) responsible for human diseases. Here we were interested in the 16p11.2 microdeletion and microduplication syndromes associated with intellectual disabilities. Most common rearrangement corresponds to a 600 kb fragment including 29 genes. Whereas deletion and duplication has been associated with autism[1], a reciprocal effect of 16p11.2 gene dosage on BMI [2] and head size[3] has been noted, as deletions are associated with obesity and macrocephaly, whereas duplications are associated with been underweight and microcephaly. The reciprocal impact on BMI and head size for 16p11.2 copy-number variants indicates that some phenotypes could have mirror etiologies depending of changes in transcript levels for genes present in the CNV region.

We used Cre/Lox technology in targeted meiotic recombination strategy to generate recombination of 16p11.2 mouse homologue regions. We fulfilled behavioral and metabolic characterization of deleted and duplicated mice. Several mirror phenotypes were detected in our models affecting activity, memory, motor coordination and metabolism of mice. Interestingly some phenotypes were similar than those observed in patients but other were mirroring the human effects. This suggests that 16p11.2 rearrangements can influence expression of other genes than those located in the 16p11.2 region in a different way from human to mouse.

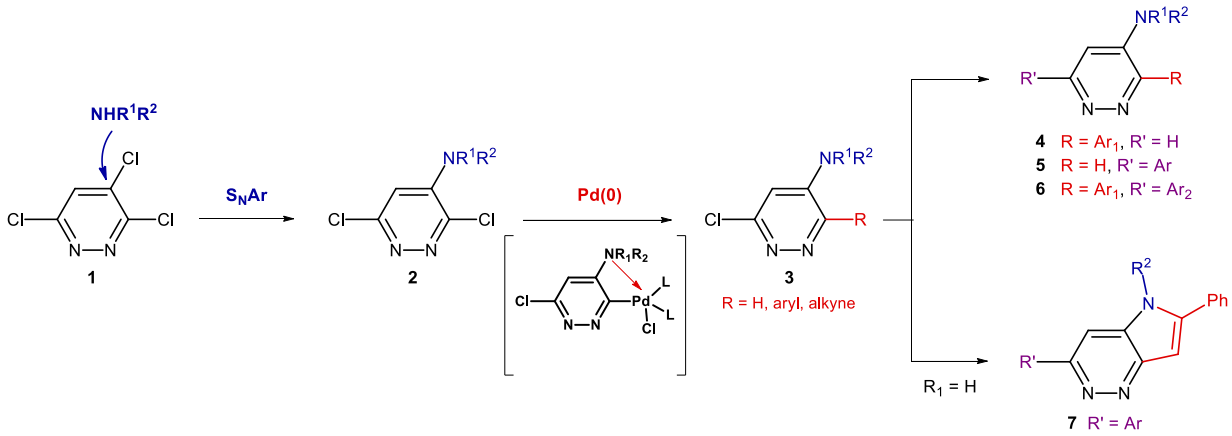
1. Weiss, L.A.*, et al.* Association between microdeletion and microduplication at 16p11.2 and autism. *New England Journal of Medicine* **358**, 667-675 (2008).
2. Jacquemont, S.*, et al.* Mirror extreme BMI phenotypes associated with gene dosage at the chromosome 16p11.2 locus. *Nature* **478**, 97-U111 (2011).
3. Shinawi, M.*, et al.* Recurrent reciprocal 16p11.2 rearrangements associated with global developmental delay, behavioural problems, dysmorphism, epilepsy, and abnormal head size. *Journal of Medical Genetics* **47**, 332-341 (2010).

Access to new polyarylated 4-aminopyridazines by regioselective palladium cross-coupling reactions

Emilie Blaise,[1,2] Arthur E. Kümmerle,[3] Hassan Hammoud,[1] Joao Xavier Araujo,[4] Frédéric Bihel,[1] Jean-Jacques Bourguignon,[1] Martine Schmitt[1]

[1]*Laboratoire d'Innovation Thérapeutique - UMR 7200, Faculté de Pharmacie, Université de Strasbourg, 74 route du Rhin, BP60024, 67400 Illkirch, France*
[2]*Prestwick Chemical, Boulevard Gonthier D'Andernach, 67400 Illkirch, France*
[3]*Departamento de Quìmica, Instituto de Ciências Exatas, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ 23890-000, Brazil*
[4]*Instituto de Quìmica e Biotecnologia, Universidade Federal de Alagoas, Maceiò-AL, Brazil*

Pyridazines are a family of structures with high interest in drug discovery.[1] Since 1970, the number of publications dealing with biologically active pyridazine derivatives has been in constant increase. Some pyridazines are already FDA-approved, like the antidepressant minaprine or the anti-Parkinson's drug moxiraprine[2]. However, while considerable attention has been devoted to the development of various 3-aminopyridazines[3], 4-aminopyridazines were poorly described.[4,5] Therefore, our aim was to investigate a novel synthetic approach leading to the various functionalized 4-aminopyridazines **4-6** and the pyrrolopyridazines **7**.



Starting from easily available and highly electrophilic 3,4,6-trichloropyridazine **1**, a first amination reaction occurred regioselectively at position 4, leading to 3,6-dichloropyridazine derivatives **2**. Interestingly, when submitting intermediates **2** to palladium-catalyzed reactions (Suzuki-Miyaura or Sonogashira cross-couplings, dechlorination), the modification always occured at the most hindered position 3. This regioselectivity towards position 3 might be the result of a specific coordination of palladium with the amino group at position 4. The second iminochloride could be further hydrogenated to afford pyridazine derivatives **4**, or substituted through a second Suzuki-Miyaura reaction, providing pyridazine derivatives **5-6** and pyrrolopyridazines **7**.

References:

[1] Wermuth, C. G. *et al.*, *Med. Chem. Commun.*, **2011**, *2*, 935-941.
[2] Wermuth, C. G. *et al.*, *J. Med. Chem.*, **1989**, *32*, 528-537.
[3] Bourguignon J. J. *et al.*, *Curr. Org. Chem.*, **2006**, *10*, 277-295.
[4] Boger D. L. *et al.*, *Chem. Rev.*, **1986**, *86*, 781-794.
[5] Rivero, R. A. *et al., J. Org. Chem.*, **2008**, *73*, 7204

Magnetic Resonance Elastography (MRE) for real-time guidance of minimally-invasive percutaneous procedures

Nadège CORBIN, Elodie BRETON, Quentin BOEHLER, Laurent BARBE, Pierre RENAUD, Michel de MATHELIN and Jonathan VAPPOU

ICube, University of Strasbourg-CNRS, Strasbourg, France

Magnetic Resonance Imaging (MRI) provides a good contrast in soft tissues and enables fast image acquisition in any orientation and position. Therefore, MRI-guidance is particularly adapted to visualize and control minimally-invasive percutaneous ("through the skin", with a needle) procedures in interventional radiology. However, some tumors remain difficult to precisely delineate during the procedure. Magnetic Resonance Elastography (MRE) is a non-invasive method that allows to measure mechanical properties of soft tissues[1] Given that mechanical properties of tumors differ from those of healthy tissues[2], this work aims at developing real-time MRE in order to provide elasticity maps of tissues as a new contrast for accurate tumor localization. MRE requires: 1/ a mechanical actuator that generates a shear wave inside the tissue; 2/ a specific MR pulse sequence including motion-sensitizing gradients; and 3/ an inverse problem solver that computes the elastogram from acquired images. In this feasibility study, an interventional MRE system is developed and tested in heterogeneous gel phantoms. Adapting MRE to interventional MRI sets new specifications given the real-time requirement and constrained space. A compact and ergonomic MRI-compatible piezoelectric actuator is designed to make the interventional needle vibrate inside the tissue directly within the region of interest. The stimulation can vary from 50 Hz to 150 Hz with typical motion amplitude of 100 µm. A fast interactive motion encoding sequence is developed based on a real-time interactive spoiled gradient echo sequence used in interventional MRI[3]. An online inverse problem solver based on the local frequency estimation algorithm[4] is implemented for real-time elastogram reconstruction and requires 2 phase images to reconstruct an elastogram. Experiments are carried out on a 1.5T open bore interventional MRI system (MAGNETOM Aera, Siemens, Germany). The excitation device generates a shear wave inside the phantom without resulting in significant artifacts on phase images. The total acquisition time, meets the real-time interventional MRI requirements, with an elasticity map calculated every 2 seconds. The real-time elasticity maps clearly reflect the distinct regions of the heterogeneous phantoms in terms of mechanical properties. This study demonstrates the feasibility of interventional MRE in phantoms with adapted deep mechanical actuation, interactive MRE sequence and online elasticity map reconstruction. The additional information of elasticity in real-time is expected to benefit to both the exact tumor location during needle insertion as well as to the monitoring of thermal tumor therapy.

1. Muthupillai, R. *et al.* Magnetic resonance elastography by direct visualization of propagating acoustic strain waves. *Science* 269**,** 1854–1857 (1995). **2.** Venkatesh, S. K. *et al.* MR elastography of liver tumors: Preliminary results. *Am. J. Roentgenol.* 190**,** 1534–1540 (2008). **3.** Pan, L. *et al.* An integrated system for catheter tracking and visualization in MR-guided cardiovascular interventions. in Proc Intl Soc Mag Reson Med 19, 195 (2011). **4.** Knutsson, H., *et al.* Local multiscale frequency and bandwidth estimation. in *Image Process. 1994 Proc. ICIP-94 IEEE Int. Conf.* **1,** 36–40 vol.1 (1994).

Reducing dynamin 2 rescues a severe congenital myopathy in mice

Belinda Cowling[1], Thierry Chevremont[1], Ivana Prokic[1], Christine Kretz[1], Arnaud Ferry[2], Catherine Coirault[2], Vincent Laugel[3], Norma B. Romero[4,5], Jocelyn Laporte[1].

[1] Dpt Translational Medicine and Neurogenetics, IGBMC, Illkirch, France
[2] UMRS974, Université Pierre et Marie Curie-Paris, Paris, France
[3]Department of Pediatrics, Strasbourg-Hautepierre University Hospital, Avenue Moliere, F-67098 Strasbourg, France
[4]Université Paris 6 UR76, Inserm UMR 974, CNRS UMR 7215, Institut de Myologie, Unité de Morphologie Neuromusculaire,
[5]Centre de référence de pathologie neuromusculaire Paris-Est, Groupe Hospitalier La Pitié-Salpêtrière, Paris, France

Centronuclear myopathies (CNM) are congenital disorders associated with muscle weakness and abnormally located nuclei in skeletal muscle. An autosomal dominant form of CNM results from mutations in the gene encoding dynamin 2 (DNM2), and loss-of-function mutations in the gene encoding myotubularin (MTM1) result in X-linked centronuclear myopathy (XLCNM), which promotes severe neonatal hypotonia and early death. Currently, no effective treatments exist for XLCNM.

The main goal of this study was to validate a novel rescue approach for XLCNM. Recent data suggested some CNM-causing DNM2 mutations increase the dynamin oligomer stability and GTPase activity. Also, we and others showed that overexpression of wildtype DNM2 in skeletal muscle cause a CNM-like phenotype. We hypothesize myotubularin and dynamin 2 function in a common pathway, where either MTM1 loss-of-function or DNM2 gain-of-function lead to the CNM phenotype. To test this hypothesis, we reduced the expression of DNM2 in $Mtm1^{-/y}$ mice that reproduce a CNM phenotype with a progressive myopathy leading to death by about 12 weeks. $Mtm1^{-/y}Dnm2^{+/-}$ mice survived up to 2 years (normal lifespan). Classical CNM histological features including fiber atrophy and nuclei mispositioning were prevented or strongly delayed and reduced, and muscle strength was increased. Downregulation of Dnm2 selectively in skeletal muscle during embryogenesis or in young mice after onset of the disease showed that the rescue is cell autonomous and that downregulation of Dnm2 can stop and potentially revert the progression of the phenotype.

In conclusion, we identified MTM1 and DNM2 are in a common pathway regulating muscle organization and force. We introduce the original concept of '**cross-therapy'** where one form of the disease (XLCNM, MTM1) can be rescued by decreasing expression of another gene mutated in CNM (DNM2 in ADCNM). While DNM2 is a key mechanoenzyme for important cellular processes, its reduction is strongly beneficial for centronuclear myopathy and represents a novel potential therapeutic approach.

Liraglutide protects β cells from proinflammatory Tissue Factor bearing Microparticles, interest for pancreatic islets transplantation

Céline Gleizes [1], Andrei Constantinescu [1], Malak Abbas [1], Blandine Yver [1], Laurence Kessler, [1,2], Florence Toti [1,3]

[1] EA 7293, Stress vasculaire et tissulaire en transplantation, Fédération de Médecine Translationnelle, Université de Strasbourg
[2] Service d'endocrinologie diabète et nutrition, Hôpitaux Universitaires de Strasbourg.
[3] UMR CNRS 7213 : laboratoire de biophotonique, Faculté de pharmacie de Strasbourg

Keywords: IBMIR, Liraglutide, β cells, Tissue Factor, islets transplantation

IBMIR (Instant Blood Mediated Inflammatory Reaction) follows islets transplantation and is characterized by drastic cytokine secretion and the expression of tissue factor (TF). Microparticles (MPs) are plasma membrane fragments shed from stressed cells that act as cellular effectors. They bear active form of TF. Recently Liraglutide, a GLP-1 analog, has been used in the treatment in type 2 diabetes for its cytoprotective effects on β cells.

We evaluate the protective effect of Liraglutide on β cell dysfunction mediated by MPs, using an *in vitro* cell cross-talk model using an oxidative and cytokine stress.

Rat β cells, Rin-m5f, were stimulated by H_2O_2 or IL-1β + TNF-α. MPs generated were isolated and applied to naive Rin-m5f for 24h. Effects of 1µM Liraglutide were assessed on insulin secretion, apoptosis and, MP release and TF activity.

Direct protection by Liraglutide is revealed by a significant decrease in oxidative stress-induced apoptosis (10% vs. 18%) and by restored insulin secretion. Indirect protection of β cell occurs through a significant reduction in MP shedding (oxidative: -25% ; cytokinic -18%) and through a major decrease in MP-induced apoptosis and NF-κB activation. Moreover, TF-activity expressed at target cell surface and borne by MPs was limited by the addition of Liraglutide (MPox: -40%; MPcyt: -45%). Liraglutide counteracted significantly the decrease in insulin secretion induced by MPs (MPox: +70% ; MPcyt: +22%). Pre-incubation of MPs with antibodies to TF restored insulin secretion in naïve target cells.

In conclusion, MPs released in response to oxidative and cytokine stress disseminate TF activity and proinflammatory signals. Liraglutide counteracts noxious MP effects on beta-cell survival and function. Our data bring new hints on the Liraglutide cytoprotective mechanisms in islet transplantation and particularly during IBMIR.

This work has received the financial support of Novo Nordisk.

Red Fluorescent Turn-On Ligands for G Protein-Coupled Receptors in Living Cells

Iuliia A. Karpenko[1], Andrey S. Klymchenko[2], Rémy Kreder[2], Bernard Mouillac[3], Yves Mély[2], Marcel Hibert[1] and Dominique Bonnet[1]

[1] Laboratoire d'Innovation Thérapeutique UMR 7200 et [2] Laboratoire de Biophotonique et Pharmacologie UMR 7213, CNRS/Université de Strasbourg, Faculté de Pharmacie, 74 route du Rhin, 67401 Illkirch, [3] CNRS UMR 5203, INSERM U661, and Université Montpellier I et II, Institut de Génomique Fonctionnelle, Département de Pharmacologie Moléculaire, 141 rue de la Cardonille, 34094 Montpellier

Fluorescent turn-on systems[1] are probably the most powerful tools at the moment in biological sensing enabling direct quantification of an analyte without removal of unreacted probe and providing an encouraging possibility of *in-vivo* monitoring of molecular interactions.

Herein, we report the design, the synthesis and the evaluation of first red fluorescent turn-on ligands capable to probe G protein-coupled receptors at the cell surface in homogenous conditions[2].

Environment-sensitive Nile Red dye was introduced onto a peptidic ligand of the oxytocin receptor (OTR)[3] through a PEG spacer, which was found to have a profound effect on the functional and the turn-on properties of the ligand.

The developed turn-on ligands were successfully applied to visualize the OTR by confocal microscopy in homogenous conditions, to monitor the ligand-induced receptor internalization and to rapidly and roughly quantify the number of receptors at the cell surface.

We anticipate that the method will find application for any other type of membrane receptors for the rapid detection, imaging and quantification.

[1] J. B. Grimm, L. M. Heckman, L.D. Lavis, Prog. *Mol. Biol. Transl. Sci.* **2013**, *113*, 1–34.
[2] I. A. Karpenko, R. Kreder, C. Valencia, P. Villa, C. Mendre, B. Mouillac, Y. Mély, M. Hibert, D. Bonnet, A. S. Klymchenko, *ChemBioChem*, **2014**, DOI: 10.1002/cbic.201300738.
[3] G. Gimpl, F. Fahrenholz, *Physiol. Rev*. **2001**, *81*, 629–683.

Solvatochromic probes with FRET quencher detect selectively ordered and disordered phases in plasma membranes of living cells.

Rémy Kreder, Zeinab Darwich, Oleksandr A. Kucherak, Yves Mély, Andrey S. Klymchenko

Laboratoire de Biophotonique et Pharmacologie, UMR 7213 CNRS, Université de Strasbourg, Faculté de Pharmacie, 74, Route du Rhin, 67401 ILLKIRCH, France.

remy.kreder@unistra.fr

Detecting and imaging lipid microdomains (rafts) on cell membranes remain a challenge despite intensive research in the field [1]. Two types of probes are usually used for this purpose: one specifically labels a given phase (liquid ordered, Lo or liquid disordered, Ld), while the other, being phase-sensitive, stains the two phases in different colors. Here, we combined both approaches, which enabled specific probing of one given phase with a phase-sensitive probe. As phase-sensitive probe we selected a recently developed NR12S, which was designed for studies of plasma membrane in live cells [2]. To probe specifically Lo phase with NR12S, we developed a fluorescence quencher that binds exclusively the Ld phase. The quencher (bQ10S) is based on BHQ-2 unit (efficient absorption in 450-650 nm range), which is designed as membrane probe, similarly to NR12S, but bearing a bulky alkyl chain. Experiments in model membranes showed that the new membrane quencher exhibits a strong preference to the Ld phase and does not flip-flop between the leaflets. Added at micromolar concentration to vesicles presenting both phases, it quenched efficiently the fluorescence of NR12S in Ld phase, so that the blue-shifted fluorescence characteristic for ordered phase was observed both in spectra of large unilamellar vesicles and in ratiometric images of giant vesicles. To achieve specificity to Ld phase, we developed an analogue of NR12S, bearing a bulky hydrophobic chain (bNR10S). Spectroscopy and microscopy studies confirmed its strong preference for Ld phase in large and giant unilamellar vesicles. Finally, studies of NR12S, bNR10S and bQ10S in live cells revealed the coexistence of separated phase domains for three cell lines (HeLa, CHO and 293T). Moreover, the relative contribution of two phases depended strongly on the cell line. The developed tools provided one of the first direct evidences for the phase separation in cell plasma membranes.

1. Lingwood, D., Simons, K. Lipid rafts as a membrane-organizing principle Science 2010, 327, 46

2. Kucherak, O.; Oncul, S.; Darwich, Z.; Yushchenko, D. A.; Arntz, Y.; Didier, P.; Mély, Y.; Klymchenko, A. S. Switchable Nile Red-based probe for cholesterol and lipid order at the outer leaflet of biomembranes. J. Am. Chem. Soc. 2010, 132, 4907–4916.

The cystathionine beta-synthase is necessary and sufficient to induce learning and memory deficits in mouse model of Down syndrome.

Maréchal Damien (1), Lopes-Pereira Patricia (2), Birling Marie-Christine (3), Hérault Yann (1,3).

(1) IGBMC, 1 rue Laurent Fries, 67404 ILLKIRCH, France;

(2) TAAM, UPS44, 3B rue de la Férollerie 45071 Orléans Cedex 2, France;

(3) ICS, 1 rue Laurent Fries, 67404 ILLKIRCH, France;

Down syndrome (DS) or Trisomy 2, is the most frequent live-born autosomal aneuploidy in human. This complex genetic disorder leads to abnormal levels of HSA21 genes expression, disturbing a variety of physiological, morphological and biochemical systems either directly from the increased of specific HSA21 gene products or indirectly through the interaction of these genes products with the whole genome or proteome. Mouse models are used to find out the HSA21 regions that are implicated in the appearances of the different symptoms. I focused my project on the telomeric part of the HSA21, and analysed the Ts1Yah mouse model trisomic for the HSA21 homologous *Abcg1-U2af1* region present on mouse chromosome 17. Behavioral and electrophysiological analyses of this model highlighted the role of the *Abcg1-U2af1* region in the maintenance of cognitive specific functions. Indeed, trisomy of this region leads to immediate and short term memory deficit. We proposed *Cbs* as a candidate for the phenotypes observed in Ts1Yah mice. To test this hypothesis, we proposed 2 approaches: analysed transgenic mice overexpressing *Cbs* and turned back to two copies of this gene in the Ts1Yah model. Overexpressing the human CBS protein in restricted brain areas lead to short-term memory deficits similar to those observed in the Ts1Yah model while decreasing the amount of Cbs in Ts1Yah rescued this deficit. This discovery shows that *Cbs* is necessary and sufficient to alter cognition in the Ts1Yah model and paves to new therapeutic perspectives for Down syndrome people.

Acknowledgement: All the team members and the ICS for the technical support.

Evaluation of a multi-modal passive and active tracking approach for real-time automatic scan plane alignment in interventional MRI

Markus NEUMANN[1], Elodie BRETON[1], Loïc CUVILLON[1], Michel DE MATHELIN[1]

[1]ICube, Université de Strasbourg, CNRS, Strasbourg, France

Purpose: In recent years, magnetic resonance imaging (MRI) has been used increasingly for guiding minimally invasive percutaneous interventions (tumor ablations, biopsies), where accurate and fast automatic scan-plane alignment is highly beneficial. The previously presented active [1] and passive [2] tracking approaches for automatic scan plane alignment suffer from drawbacks such as the use of heavy additional instrumentation or dedicated tracking images, respectively. In this work, a hybrid tracking workflow is developed, combining MR image tracking of a passive marker and RGB-D (red green blue - depth) sensor images for real-time automatic scan-plane alignment. **Methods and Results:** All experiments are performed in a 1.5T MRI system using an interactive, real-time, multi-slice SSFP sequence [3]. A program performs the implemented workflow (both MR and RGB-D image reception & processing, scan-plane control) on an external PC connected to the MRI console PC and to the RGB-D sensor. The RGB-D sensor is positioned in the back of the MRI scanner capturing the scene inside the MRI tunnel. The tracking target consists in a contrast-agent (Gd-DTPA) filled cylinder with 2 pink balls attached at its distal ends in order to be simultaneously detectable in MR and RGB-D sensor images. The proposed workflow controls 2 alternately acquired orthogonal real-time MR scan planes aligned to the marker axis. For this purpose, the 3D marker pose is detected in both modalities and then fused using an Information Filter (IF). Based on the predicted marker pose from the IF, a marker-aligned scan-plane is calculated and the corresponding command is sent to the MRI console PC. One strength of the workflow is that when detection fails in one modality, the IF uses the available measurements from the other modality. An online registration procedure is performed in the beginning of the workflow in order to transform detected 3D marker poses from the RGB-D sensor to the MRI frame: 20 marker positions are detected in both modalities for determination of the rigid transformation between MRI and RGB-D sensor frames. As the transformation is unknown in the beginning of the workflow, tracking is first performed based on MR images only [4]. As soon as a valid transformation has been found, measurements from both modalities are fused for tracking (hybrid mode). A typical root mean square error of 7 mm has been obtained for registration between MRI and RGB-D sensor frames. **Conclusion:** The presented hybrid workflow combines through an IF the tracking performances of an MR image based passive approach and an active approach based on high frequency measurements from an RGB-D sensor. It could be easily integrated in the clinical routine as only little additional instrumentation is needed and no explicit calibration step has to be performed.

1. Qing K. et al. “A multi-slice interactive real-time sequence integrated with the EndoScout tracking system for interventional MR guidance,” ISMRM 2010: p.1860;
2. de Oliveira A et al. “A New System for Passive Tracking of a Prostate Biopsy Device with Automatic 3D Needle Position Estimation.” ISMRM 2008: p.3003;
3. Pan L et al. “An Integrated System for Catheter Tracking and Visualization in MR-Guided Cardiovascular Interventions”, ISMRM 2011: p.195;
4. Neumann M et al. “Evaluation of an Image-based Tracking Workflow with Kalman Filtering for Automatic Image Plane Alignment in Interventional MRI”, EMBC 2013: p.2968-2971

Genetic analysis of epidermal cell mechanical properties during *C. elegans* embryonic elongation

Gabriella Pásti[1], Christelle Gally[1], Huimin Zhang[1,2] Michel Labouesse[1]

[1]IGBMC, Strasbourg
[2] Institute of Biology and Medical Sciences, SooChow University, CHINA

We focus on C. elegans embryonic elongation, a process, controlled by cell shape changes that transform a ball of cells into a tube-shaped animal. The contribution of mechanical forces to development is gaining wider acceptance. But the detailed molecular mechanism by which cells can sense and respond to these forces remains elusive.
C. elegans embryonic elongation consists of two phases. The first phase is driven by the contractions of an epidermal actomyosin system. In the second one, muscles contribute to elongation through a recently reported mechanotransduction pathway. PAK-1 (p21-activated kinase) is a key regulator of both phases: it is a good starting point to aim at a better view on the molecular landscape of elongation. To reach this aim, we have two approaches.

First, we carried out a systematic search for genetic interactions by an RNAi screen, targeting approximately 350 genes in *pak-1* and three other mutant backgrounds affecting elongation.

Second, we looked for potential PAK-1 interactors by a yeast two hybrid screen. I tested the *in vivo* relevance of the most interesting candidate, a putative cytoskeletal protein. I established a genetic interaction between the two genes by dynamic analysis of the elongation process: double mutants showed a novel elongation defect, not observed in single mutants. To reveal the underlying molecular mechanism, I analysed the major molecular players of elongation (cytoskeleton, adherens junctions, hemidesmosomes). We saw serious changes only at the level of hemidesmosomes: attachments structures and force-transmitters between the epidermis and the muscles. Moreover, In order to better characterize this transmission, I optimized a spinning disk time-lapse imaging method, that enables us to simultaneously monitor the muscle contractions and epidermal deformation in a dynamic way. Our results suggest that mechanical properties of the epidermal cells might be affected in the double mutant background. To further confirm these findings, our ongoing experiments involve laser ablation of the epidermal actin cables, that allows us to measure the viscoelastic properties of epidermal cells in vivo.

Consistent with the genetic interaction, *in vivo* expression studies revealed similar localization between the two proteins at the level of hemidesmosomes. Furthermore, whereas *pak-1* knock-down did not affect our putative cytoskeletal protein, the lack of this protein disturbed PAK-1::GFP localization.

Our results show that the predicted interaction could exist in vivo and has an important role in *C. elegans* embryonic elongation.

Human Papillomaviruses (HPV) Interactome: from the ubiquitin-proteasome system to the PDZ domain proteins

Juline Poirson[1], Marie-Laure Straub[1], Patricia Cassonnet[2], Caroline Demeret[2], Gilles Travé[1], Yves Jacob[2], Murielle Masson[1]

[1] Equipe oncoprotéines, UMR7242, IREBS, 67404 Illkirch, France

[2] Unité Génétique Moléculaire des Virus ARN (GMVR), URA3015, Institut Pasteur, 75017 Paris, France

Our study aim is to understand the mechanisms by which the oncogenic viruses hijack the cellular machinery. Oncogenic viruses interact in particular with the ubiquitin-proteasome system (UPS) effectors and with the PDZ domain proteins, involved in cellular polarity.

Our work is focus on the human papillomavirus viruses (HPV), which are the archetype of DNA oncogenic viruses. Nowadays more than 180 HPVs different genotypes are identified, only a few are closely associated with the development of cancer. It is mainly the high-risk mucosal HPVs (HPV16 and HPV18), which are involved in many cancers. These HPVs are implicated in 95% of cervical cancer, the third most prevalent cancer in women worldwide. Despite the recent elaboration of vaccination, HPV infection is still an important public health problem. Moreover, some high-risk cutaneous HPVs, involved in skin lesions, appear to be implicated in the development of non-melanoma skin cancer and in epidermodysplasia verruciformis. The HPV oncogenic properties are mainly due to the expression of the two viral proteins E6 and E7. These one could hijack the UPS effectors to induce the degradation of cellular proteins like the members of the p53 and pRb family and could interact with some PDZ domain proteins (MAGI-1, SCRIB), which contribute to carcinogenesis.

The identification of new cellular proteins targeted by HPV relies on a high throughput screening of the interaction between E6 and E7 and the UPS effectors or the PDZ domains with a protein-protein interaction assay, the protein complementation assay (PCA), based on the *Gaussia princeps* luciferase (Cassonnet *et al.* (2011))

Two cDNA libraries, adapted to the PCA, dedicated to the effectors of the UPS and to the PDZ domains, were generated. The first screenings with HPV16 and HPV18 E6 have already identified new potential partners of E6. The results will provide novel insights on viral carcinogenesis.

Cassonet, P., Rolloy, C., Neveu, G., Vidalain, P.-O., Chantier, T., Pellet, J., Jones, L., Muller, M., Demeret, C., Gaud, G., Vuillier, F., Lotteau, V., Tangy, F., Favre, M. & Jacob, Y. (2011) Benchmarking a luciférase complémentation assay for detecting protein complex. *Nature Methodes* **8** (12), 990-992.

Development of bioorthogonal tools for biological applications

Marion Recher[a], Claire Bedez[b], Claire Batisse[b], Jean-Christophe Amé[c], Valérie Lamour[b], Valérie Schreiber[c], Wojciech Krezel[d], Alain Wagner[a]

[a] *UMR 7199 CNRS, UdS, LabEx MEDALIS, 74 Route du Rhin, 67401 Illkirch, France*
[b] *IGBMC, Inserm U 964, UMR 7104 CNRS, UdS, CS10142, 67404 Illkirch, France*
[c] *UMR7242, CNRS, UdS, LabEx MEDALIS, IREBS, Bld S. Brant CS10413, 67412 Illkirch, France*
[d] *IGBMC, Inserm U 596, UMR 7104 CNRS, UdS, CS10142, 67404 Illkirch, France*
mrecher@unistra.fr, alwag@unistra.fr

With the increasing difficulties to lead new drugs on the market, the idea of improving the ones already existing is of high interest. The major drawbacks of most FDA-approved drugs are secondary effects and administration/elimination of the drug. In order to improve that, we propose to use the recent developments of bioorthogonal chemistry. A reaction is considered as bioorthogonal if it can occur in the biological media without interfering with the native biological processes.

To determine proteins responsible for secondary effects, we are elaborating chemical proteomics approaches.[1,2] For that purpose, our interest was to develop new affinity probes, cleavable under non-denaturating conditions, to extract proteins targeted by a drug from a cell lysate and identify them by mass spectroscopy.

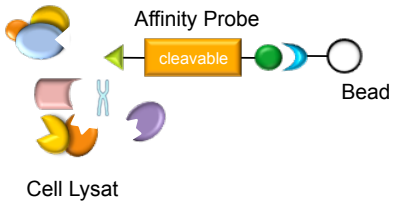


Figure 1. Principle of Chemical Proteomics

In order to provoke the elimination of a circulating drug, we proposed to use the recent development of 'click' reactions.[3] In this project, we try to neutralize a drug circulating in a living mouse by the injection of an antagonist probe. The two compounds, bearing an azide and a cyclooctyne moiety respectively, would undergo a strain-promoted cycloaddition, leading to an easily eliminated product.

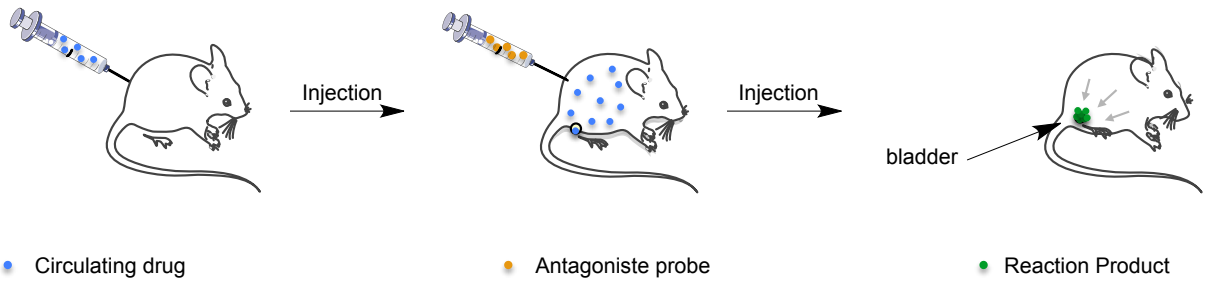


Figure 2. Principle of provoked elimination

Références

1. Leriche *et al.*, *Bioorg. Med. Chem.* **2012**, *20*(2), 571–582
2. Budin *et al.*, *ChemBioChem* **2010**, *11*(17), 2359-2361
3. Bertozzi *et al.*, *Angew. Chemie*. **2009**, *48*(38), 6974–6998

Targeting α5β1 integrin improves the pro-apoptotic arm of p53 activation in glioblastoma.

Guillaume RENNER, Hana JANOUSKOVA, Anne-Marie RAY, Fanny NOULET Laurence CHOULIER, Nelly ETIENNE-SELLOUM, Maxime LEHMANN, Isabelle LELONG-REBEL, Sophie MARTIN, Monique DONTENWILL.

CNRS UMR 7213, Université de Strasbourg, Faculté de Pharmacie, Illkirch, France.

INTRODUCTION

Glioblastoma is the most aggressive and malignant form of brain tumour. Despite advances in standard therapy, including surgical resection followed by radiation and chemotherapy with temozolomide (TMZ), the prognosis for patients with glioblastoma remains unfavourable, with a median survival of 12-18 months. We recently demonstrated that α5β1 integrin is a promising anti-cancer target in high grade glioma. We characterized an inverse crosstalk between this integrin and the tumor suppressor p53. The aim of this study was to investigate the potential benefit of combining α5β1 integrin antagonists with a non genotoxic p53 activator, Nutlin-3a, in glioma cells.

MATERIAL AND METHOD

U87MG (p53wt) cells overexpressing or depleted in α5 integrin subunit were treated with K34c (a RGD-like integrin antagonist) and/or Nutlin-3a. U373 (p53 mutant) and LNZ308 (p53 KO) cells were similarly investigated. Cell survival was assessed by clonogenic assays. p53 activation was determined by western blot analysis using a specific phospho-p53 (ser15) antibody and by RT-qPCR analysis of p53 target genes. Apoptosis level was recorded by flow cytometry analysis of Annexin V/PI staining and cleaved caspases/PARP cleavage analysis by western blots. Signaling pathways were investigated by specific RNA interference and/or inhibitors.

RESULTS AND DISCUSSION

Combined treatment of K34c + Nutlin-3a showed a synergistic decrease in cell survival and a huge increase in apoptosis in p53wt cells only. Nutlin-3a activated the p53 pathway and allowed an increase in pro-apoptotic genes (Fas, bax, noxa) and a decrease in anti-apoptotic genes (birc5, bcl2, myc) but was unable to trigger apoptosis. Integrin antagonist alone affected the PI3K/AKT signalling pathway but was unable to trigger apoptosis. It also impacted on PEA-15, an anti-apoptotic protein, expression and phosphorylation. Combining K34c with siRNA targeting survivin or siRNA targeting PEA-15 with Nutlin-3a recapitulated apoptosis in p53wt expressing cells.

CONCLUSION

We confirm the existence of an α5β1 integrin-p53 crosstalk which may be relevant for high grade glioma treatment. Convergent anti-apoptotic pathways, including survivin and PEA-15 proteins, were unraveled in this study. Re-activation of p53 by non genotoxic drugs in combination with α5β1 integrin targeted therapy may be a new therapeutic option in the subset of high-grade glioma expressing a functional p53 protein and a high level of α5β1 integrin.

Study of the interaction between the HIV-1 nucleocapsid protein (NC) and the cellular protein Unr : implication on the viral IRES dependent translation

Nedal Taha, Sarwat Zgheib, Kamal Kant Sharma, Eléonore Real, Yves Mely

Laboratoire de Biophotonique et Pharmacologie, UMR 7213 CNRS, Université de Strasbourg, Faculté de Pharmacie

The nucleocapsid protein (NC) of the human immunodeficiency virus type 1 (HIV-1) exists in the infected cell as a domain of the Gag polyprotein (55kDa) or as a mature form (NCp7) after Gag cleavage by the viral protease during virus maturation. In these two forms, NC plays crucial roles in several steps of the viral cycle including reverse transcription, integration, nucleic acid (NA) encapsidation and virion assembly.The mature NCp7 is a small basic protein of 55 amino acids which presents two CCHC zinc-finger motifs. Its important functional role and its high conservation across several HIV-1 strains place the NCp7 as a target of choice for the development of antivirals. Though NCp7 functions rely largely on its NA chaperone activity, there is increasing evidence that several functions of NC may also be mediated by its interactions with host cell proteins. The study of these interactions is thus essential for the understanding of the multiple roles of NC in the viral cycle. According to the recently published databases listing the cellular proteins potentially involved in HIV-1 infection or interacting with viral proteins, we selected and tested several proteins for their interaction with NC. Using biochemical techniques such as co-immunoprecipitations (Co-IP) and imaging techniques such as Fluorescence Lifetime Imaging Microscopy (FLIM) to measure Förster Resonance Energy Transfer (FRET) between labelled partners, we validated the interaction between NC (either in its mature form or as a domain in Gag) and the cellular protein Unr (Upstream of N-Ras). Unr acts as an ITAF (IRES-transactivating Factor) of rhino and polio viruses stimulating the cap-independent translation of their viral RNA by the binding to and the refolding of their IRES (Internal Ribosome Entry Site). The HIV-1 genome harbors two IRESes, the first one is localized in the 5' untranslated region (5'-UTR) and the second one is in the Gag coding region. The HIV-1 mRNA is capped and polyadenylated and can be translated by both a cap-dependent mechanism under physiological conditions and a cap- independent mechanism when the cell is blocked in G2/M by the expression of the HIV-1 protein Vpr. We showed that Unr is able to act as an ITAF of the 5'-UTR HIV-1 IRES when it is overexpressed in HeLa cells and is also able to inhibit the infection by a non replicative lentivector used as a model of the early phase of the infection. The ITAF action of Unr is inhibited by the co-expression of the mature NCp7 but not by the expression of Gag, suggesting a competition between NCp7 and Unr for the binding to the IRES.