Deciphering molecular and epigenetic mechanisms by which *Tex19* paralogs control male meiosis and transposable elements silencing

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Infertility is a major reproductive health problem affecting 10% to 15% of couples, with approximately equal contributions. A considerable number of cases (25 to 30%) remain idiopathic among infertile couples. This situation can be explained by a poor understanding of the basic mechanisms driving human male and female gametogenesis. *Tex19* is restricted to mammals, is present as a unique gene in human, and has been duplicated in mouse and rat giving rise to the paralogs *Tex19.1* and *Tex19.2.* Both genes are expressed in pachytene spermatocytes in adult testes. In addition, *Tex19.1* is expressed throughout the pluripotent cycle *in vivo*, from the preimplantation embryo to the gonads at the embryonic and adult stages, where its expression is limited to spermatogonial stem cells. To decipher Tex19 functions, we generated three types of knockout (KO): i) KO of Tex19.1 ii) KO of Tex19.2 iii) double KO (DKO) of both genes.

Tex19.1-/- males display a variable spermatogenesis defect, associated with an up-regulation of one endogenous retrotransposon, MMERVK10C. DKO show a fully penetrant phenotype similar to the most Tex19.1-/- severe phenotype. The main DKO phenotype consists in males exhibiting small testes. Despite the presence of spermatogonia and spermatocytes, spermatogenesis is blocked at the pachytene stage, associated with a chromosome asynapsis and an up-regulation of one endogenous retrovirus, MMERVK10C. Increased transposition of endogenous retroviruses in the germline of Tex19.1-/- mutant mice, and the concomitant increase in DNA damage, may be sufficient to disrupt the normal processes of recombination and chromosome synapsis during meiosis and cause defects in spermatogenesis. We performed RNA deep-sequencing experiments on 10 dpp DKO and WT testes, 114 genes are significantly up-regulated and 320 genes significantly down-regulated in the DKO versus WT. In order to identify Tex19.1 partners, we have performed GST-pull-down and immunoprecipitation IP experiments coupled to mass spectrometry experiments and identified 23 potential testis specific partners, among which PiwiL1, PiwiL2, Mael, Tdrd6, Tdrd8, Mvh, Edc4, Ranbp9 and Ddx20. The similarity with phenotypes observed in KO of genes of the piRNA pathway and the fact that Tex19 interacts with some actors of this pathway pushed us to check if Tex19s bind small RNA, which could correspond to piRNA. By immunoprecipitation using WT and KO testes, we show that both Tex19.1 and Tex19.2 bind small RNA of about 30 nucleotides. We performed Illumina deep sequencing of these small RNA. A total of 1.6 million reads from Tex19.1 perfectly mapped to the genome and peaked around 29 to 30 nt, similar to those in small RNA libraries prepared from PiwiL2. Detailed analysis of the repeat-associated piRNA revealed that the long terminal repeat (LTR, ~27.26%) retrotransposon dominate Tex19.1 libraries, whereas the long interspersed elements (LINEs, ~20.51%) and short interspersed elements (SINE, ~12.1%) form the remaining two major classes suggesting that Tex19.1 is implicated in transposable element silencing pathway and genome integrity protection during spermatogensis.

Synthesis of artificial sideromycins : towards new therapeutic weapons against Gram negative bacteria

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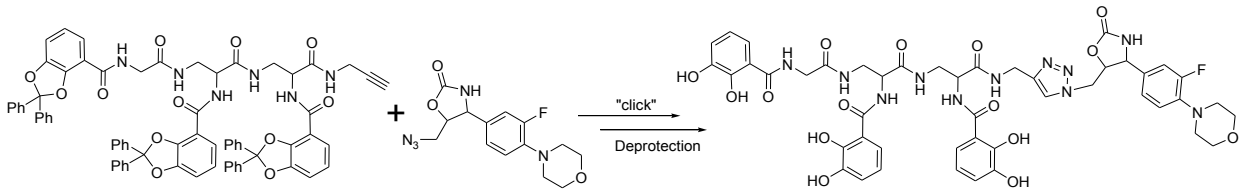
Although iron is one of the main components of the earth crust it's bioavailability is limited by the low solubility of iron(III) at physiological pH in aerobic conditions. Indeed, the iron(III) concentration is estimated at 10^{-9} M in the environment and only around 10^{-18} M in human biological fluids when many microorganisms require an iron(III) concentration in the micromolar range for an optimal proliferation.

Under iron starvation conditions, almost all microorganisms synthesize and excrete siderophores.[1] These secondary metabolites chelate iron(III) in the extracellular medium and resulting ferric-siderophore-iron(III) is then translocated through the membranes into the cytoplasm, by a multiprotein system. Catechol is the most common bidentate moiety found in siderophores described to date. In the last decades, catechol siderophores, and their synthetic analogs, found many applications in crop science, bioremediation, or human health.

In this context, the use of catechol siderophores as vectors in antibiotic Trojan Horse strategies is probably one of the most impressive issue.[2] For pathogenic bacteria, the low permeability of membranes is the first defense against antibiotics. Siderophore-dependant iron uptake pathways are gates in this barrier and therefore means of introducing antibiotic compounds into the bacteria. For this purpose several groups around the world described the synthesis of conjugates between siderophores and antibiotic. In these approaches many siderophore vectors contains at least one catechol group to promote iron chelation.

We developed a new methodology in order to synthesize efficiently natural catechol siderophores. This methodology was then fruitfully applied to the synthesis of siderophore analogs bearing a terminal alkyne group. This function will be used to connect azide-antibiotics thanks to a "click chemistry" reaction (Scheme below is a representative example).



The resulting siderophore-antibiotic conjugates will be further evaluated for both their ability to cross bacterial envelop using the siderophore-dependent iron uptake systems and to inhibit bacterial proliferation.

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State-of-the-art hyphenated techniques HPLC/DAD-MS/SPE/NMR interface for new approaches in phytochemical exploration of orchids

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Orchidaceae is one of the largest botanical families and some species have been used as ingredients of traditional preparations with beneficial properties. Despite the high popularity of these plants, there are really few studies concerning their chemical composition. In the aim to acquire more basic knowledge about metabolites from different tropical orchids, several species were selected. The present study deals with a rapid on-line identification of metabolites present in one species never explored before.

Currently, main phytochemical approaches are based on several chromatographic and spectrometric techniques, like LC-DAD, LC-MS or NMR 1D and 2D. However, NMR and MS data are decoupled and a NMR analysis requires a prior total purification of some milligrams of compound of interest and consequently hundreds of grams of dried plant. The availability of raw material is often an obstacle to further analysis. Furthermore, this traditional phytochemical approach can take several months.

With hyphenated LC/DAD-MS/SPE/NMR, analyses are performed on-line, directly linking the complementary nature of NMR and MS data for phytochemical analysis. This state-of-the-art technique enables a rapid identification of all natural compounds present in hundreds of micrograms of a simplified fraction, without any other step of purification.

The analysis of two minor fractions of our studied orchid permitted the identification of six phenanthrene derivatives. Their structures were determined with MS and NMR (1D and 2D) data obtained only from some micrograms of compound of interest thanks to the capNMR probe.

This recently acquired equipment appears to be a great interest in pharmacognosy field to quickly describe known metabolites and target further analysis on the unknown ones. Finally, this performing tool permits a rapid on-line identification of chemical markers, and using few quantities of raw materials to better understand the chemical diversity of plant species.

Contribution of mu opioid receptor expressed in GABAergic forebrain neurons to addiction: a conditional knockout approach

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Morphine is the most used analgesic in clinic, despite adverse effects. Its effects are mediated by the mu opioid receptor, a G-protein coupled receptor. This receptor is widely expressed throughout the nervous system, mostly in GABAergic neurons, within reward circuits (habenula, striatum, ventral pallidum, ventral tegmental area) and the nociceptive pathways (spinal cord, thalamus). Using mice deficient for the mu receptor (constitutive knockout for mu receptor gene *Oprm1*) we have previously shown that mu receptor activation is responsible for both morphine analgesic and addictive effects [1].

In order to explore the specific contribution of mu receptor expressed by GABAergic neurons, we developed a conditional knockout mouse line (cKO), using the Cre/loxP system, where mu receptor is specifically deleted in forebrain GABAergic neurons. We bred mice with a floxed mu receptor gene with a transgenic Dlx5/6-Cre line [2]. Resulting cKO mice were compared with their control floxed littermates. We first determined brain distribution of mu receptors using qRT-PCR and radiolabelled ligand-binding experiments. There was a strong reduction of receptor expression in ventral pallidum and the whole striatum of cKO mice, both at mRNA and protein levels. In amygdala and hippocampus, receptor deletion was partial, and no change was detectable in the ventral tegmental area, habenula, thalamus and spinal cord. We then studied behavioral modifications of the cKO animals. We examined the effects of two prototypic opiates, morphine and heroin, in several behaviors: analgesia (hot plate, tail flick and tail immersion tests), physical dependence (naloxone-precipitated withdrawal), locomotor activity (opiate-induced hyperlocomotion) and reward (conditioned place preference). No difference in both morphine-induced analgesia and withdrawal syndromes were detected in cKO mice in our conditions, in accordance with the anatomical characterization of cKO mice, showing no difference of receptor expression compared with control mice in regions involved in nociception and physical dependence. Our data of heroin-induced hyperlocomotion showed a striking decrease of locomotor activity, consistent with the reduced level of mu receptor in cKO striatum. Remarkably, opiates conditioned place preference was preserved in spite of the marked decrease of mu receptor in the ventral striatum, a major region involved in reward.

To better characterize the role of mu receptor expressed on GABAergic neurons in reward, we will further examine the rewarding effects of opiates and drugs of abuse (THC, alcohol, cocaine) using operant behavioural paradigms.

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Helical oligourea as scaffold to multimerize TRAIL-R2 specific peptides: potential application in cancer therapy

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Activation of apoptotic pathway in tumor cells by targeting death receptors that belong to the Tumor Necrosis Factor Receptor (TNF-R) family represents an attractive therapeutic strategy. Within this family, the Tumor Necrosis-Related Apoptosis Inducing Ligand (TRAIL) has the unique characteristic of inducing apoptosis in a wide variety of cancer cells, while sparing the normal cells.[1] TRAIL, like other TNF family members, binds as a homotrimer to its receptors. Four membrane-bound TRAIL specific receptors have been described: two death receptors DR4 and DR5 that mediate the apoptogenic signal and two decoy receptors DcR1 and DcR2 that act as inhibitory receptors. Currently, recombinant TRAIL or agonistic TRAIL-receptor antibodies[2] are tested in clinical trials. We propose a peptide-based strategy to activate the TRAIL pathway for cancer therapy.

Recently, we reported on small apoptogenic peptides that bind DR5 and induce tumor cell apoptosis *in vitro* and *in vivo* in xenograft models.[3] As it is well established that DR5 oligomerization is necessary for activation of TRAIL signaling[4] in the present work, we used helical oligourea scaffold to multimerize the DR5 specific peptide and therefore to increase its apoptogenic effect. The helical oligourea scaffold are foldamers that remind of small proteins in terms of size and complexity, and allow the binding of numerous peptides on each helices and therefore increase the valence of the ligand[5]. Up to now we obtained helical structures bearing monomer, dimer and trimer of the DR5 specific peptide.

Experiments in Surface Plasmon Resonance (SPR) technology revealed that the various helical structures bind selectively to DR5 receptor with an increase of the binding affinity with the oligomerization of the DR5 specific peptide. Using a BJAB cell line model (Burkitt lymphoma) consisting of cells that express (BJAB DR5+) or lack (BJAB DR5-) DR5 receptor, we showed that helical structures bearing dimer or trimer of DR5 specific peptide induced apoptosis in the BJAB DR5+ cells and, as expected, had no effect in the BJAB DR5- cells. Helical structures bearing monomer had no effect in BJAB DR5+ cell lines. In the near future, the helical structures bearing peptide dimer and trimer, will be evaluated for their anti-tumoral effect *in vivo* in xenograft models of human HCT116 colon carcinoma cells.

With these new helical structures, we will develop entirely synthetic structures mimicking the apoptotic effect of oligomeric protein with potential application in cancer therapy.

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Deciphering how CentroNuclear Myopathy genes control Nuclear Positioning

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Centronuclear myopathies (CNM) are rare muscle disorders characterized by skeletal muscle weakness and the histological hallmark of the disease is the abnormal position of nuclei at the center of myofibers, while they are normally at the periphery. The mechanism leading to this abnormal position of the nucleus is poorly understood.

We decided to investigate the genetic and molecular pathway that leads to the nuclear positioning defects observed in CNM searching for protein partners of amphiphysin 2 (AMPH2), a membrane curvature sensing and membrane remodeling protein mutated in the autosomal CNM form [1,2].

By yeast two hybrid we found that human AMPH2 binds to Nesprins, a family of protein highly conserved through the evolution that has a pivotal role in the nucleocytoplasmic/skeleton coupling and in nuclear positioning/anchorage [3,4]. We confirmed this interaction by both GST pull down and Co-IP.

To investigate the biological significance of this interaction, we turned to *C. elegans* and we studied the position of nuclei in the hypodermal seam cells. Seam cell nuclei in adult hermaphrodites were visualized by a seam cell marker::gfp (scm::gfp) fusion. We found that a null mutant of the *C. elegans* ortholog of AMPH2 (*amph-1*) displays abnormal position of the hypodermal seam cell nuclei that resembles the defects observed in mutants for ANC-1 (homologous to mammalian Nesprin 1 and 2). We characterized these nuclear defects by using the CLEM technique developed in our laboratories [5]. We also created an *anc-1;amph-1* double mutant that shows percentage of mis-positioned seam nuclei comparable to each single mutant. These data suggest that there is no additive/synergistic effect and that the two proteins might be in the same pathway. To confirm this hypothesis we are testing the rescue of the phenotype using *amph-1* and *anc-1* constructs. Finally by RNAi screening we found that the knocking down of two microtubule binding proteins lead to seam nuclear mis-positioning like in *anc-1* and *amph-1* mutant.

We propose that defect in the AMPH2/Nesprin interaction affects the correct nuclear positioning in CNM and we are investigating the implication of the cytoskeleton in this process.

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Protein/ligand interactions: From a simple representation to 3-D alignment using a graph matching algorithm

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The increasing number of proteins in complex with small molecules has led to the necessity to understand biological recognition at a molecular level. Since a protein/ligand complex is composed of thousands of atoms and bonds, we have chosen to simplify the situation by only focusing on key spots: interactions between the protein and the ligand.

We present a novel suite of interaction detection (InterGen), alignment and comparison (Grim) tools. Starting from a protein ligand complex, we detect, thanks to geometrical rules such as distances and angles, 8 different interactions (Hydrophobic, Aromatic Edge-to-Face and Face-to-Face, Ionic Protein cationic, Ionic Ligand cationic, H-Bond Protein donor, H-Bond Ligand donor and Metal/Acceptor) [1]. Each time an interaction is detected, we create 3 pseudo-atoms, located at the protein atom, at the ligand atom, and at the center of the interaction. A name is set to each pseudo-atom to distinguish the interaction type and the 'positioning' (center, ligand, protein). We therefore convert a complex into a set of points, encoding key interactions between the protein and the ligand.

This new representation is easily applicable to find local and/or global similarity between two complexes. For this purpose, we developed an alignment tool (Grim) based on a graph matching algorithm using a clique detection method [2,3]. It first creates all possible pairs between a reference and a comparison pseudo-atom (P_1=(R_1, C_1)), only if both represents the same interaction type and 'positioning', giving a list of all possible matches between two complex. Pairs are then linked when distances between both reference (R_1,R_2) and comparison (C_1,C_2) are similar encoding the spatial orientation between pseudo-atoms. We finally search for the maximal clique, i.e. the maximal set of pairs that respect all distances between each other.

This concept has proved to be efficient in multiple scenarios. Grim is able to correctly align complexes despites low ligand or binding sites similarity. Moreover, when structure-based or ligand-based alignment methods fails, it can still align proteins with only a few matched points. Grim also gives the possibility to find local interaction conservations and can be used to design chemical libraries. Finally, we can use Grim to post-process docking poses by taking into account the binding mode of all known ligands for a particular target or target class.[4]

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Pallado-catalyzed reactions applied to solid phase synthesis of dipeptidic GPR54 agonists

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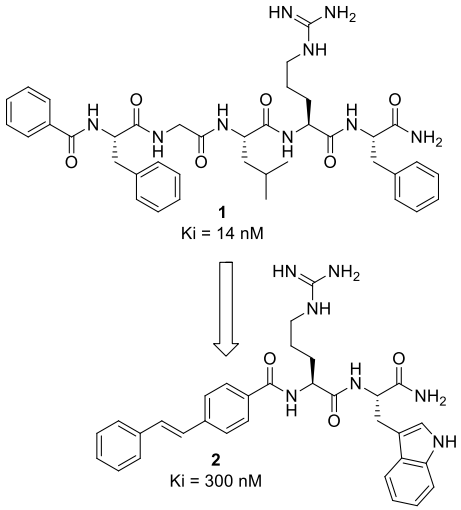
GPR54, also known as Kiss1R, belongs to the G-protein coupled receptor superfamily. Mainly expressed into the CNS, GPR54 recognizes an endogenous 54 amino-acid peptide named Kisspeptin. Originally discovered for its activity as metastasis-suppressor, Kisspeptin was later identified as a critical modulator of the neuroendocrine regulation of reproduction, especially involved in the production of the Luteinizing hormone (LH).

However, in spite of an exponential interest for GPR54, there are only few reports dealing with the development of new GPR54 ligands. Tomita et al. described few years ago the pentapeptide FGIRW-NH_2 (**1**) as a nanomolar agonist, highlighting the importance of the Phe-residue at position 1. Starting from this information, we extended the deletion of the N-terminal part of Kisspeptin, identifying the dipeptidic sequence Bz-Arg-Phe-NH_2 with a Ki_{GPR54} of 8µM.

We next optimized the N-terminus part of this dipeptide in order to recover the aromatic interaction of the phenylalanine identified by Tomita. To explore the chemical space around the benzoyl moiety, we developed an innovative synthetic strategy associating palladium-catalyzed cross-coupling reactions (Sonogashira, Suzuki-Miyaura) and solid phase peptide synthesis (SPPS). More particularly, using two sequential sonogashira reactions, we carried out the convergent synthesis of several acetylenic compounds up to 90% yield over 6 steps. SAR analysis led us to identify the pseudopeptide **2** as a full agonist exhibiting a Ki_{GPR54} of 0.32 µM and an EC50 of 0.39 µM. Due to its short size, compound **2** is the first GPR54 agonist able to be tested in vivo by peripheral administration (i.v. or i.p.).



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FLIM imaging of lipid membrane domains using advanced fluorescent probes

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Biomembranes are ordered and dynamic nanoscale structures critical for cell functions. The biological functions of the membranes strongly depend on their physicochemical properties, such as electrostatics, phase state, viscosity, polarity and hydration. These properties are essential for the membrane structure and the proper folding and function of membrane proteins. Lipid domains in cell plasma membranes have been reported to play critical roles in a number of cell functions ranging from regulation of membrane protein activity to membrane trafficking, sorting and signal transduction. Lipid domains are enriched in saturated lipids (mainly sphingolipids) and sterols (mainly cholesterol) that form a liquid ordered (Lo) phase. Lo phases coexist with a liquid disordered (Ld) phase, mainly composed of unsaturated phospholipids. To monitor the properties of membranes and lipid domains, fluorescence techniques and notably, two-photon microscopy appear highly suited due to their exquisite sensitivity and their capability to operate in complex biological systems, such as living cells and tissues. In this context, we use a multiparametric environment-sensitive fluorescent probe of the 3-hydroxychromone family, characterized by an ESIPT reaction, which generates two tautomeric emissive species with well-separated emission bands. Response of this probe to changes in its environment could be monitored through changes in the ratios of the two bands. Using FLIM, we showed that F2N12S gives clearly different fluorescence lifetime signatures in these two phases. Using these differences in lifetimes, we could determine quantitatively how the probe distributes in the two phases. The probe was successfully used to image the plasma membranes of intact cells, as well as changes in the lipid phase on cholesterol depletion and apoptosis.

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High-resolution dose-response screening using droplet-based microfluidics

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Screening of compound libraries is a critical step in the hit discovery and hit optimization phases of drug discovery projects and today, robotic HTS programs using microtitre plates can process up to 100,000 compounds per day (~1 s^{-1}). However, despite the fact that compounds can display complex concentration-dependent relationships, varying in potency, efficacy, and steepness of response, usually just a single measurement at a single concentration (~10 μM) is obtained for each compound in the chemical library during a primary drug screen.

This results in high numbers of false positives and false negatives, as well as the inability to identify subtle, complex pharmacology, such as partial agonism or antagonism. Typically, promising compounds are identified in a primary screen and then more fully characterized in a dose-response analysis with 7-10 data points per compound. We have recently described a robust microfluidic approach that increases the number of data points to ~10,000 per compound [1]. The system exploits Taylor-Aris dispersion to create concentration gradients, which are then segmented into picoliter microreactors by droplet-based microfluidics. The large number of data points results in IC50 values that are highly precise (±2.40% at 95% confidence) and highly reproducible (CV = 2.45%, n = 16). In addition, the high resolution of the data reveals complex dose-response relationships unambiguously. We used this system to screen a chemical library of 704 compounds against protein tyrosine phosphatase 1B (PTP1B), a diabetes, obesity, and cancer target.

We identified a number of novel inhibitors, the most potent being sodium cefsulodine, which has an IC50 of 27 ±0.83 μM. During the first year of this project, we developed this system to provide a robust platform for screening: a first prototype has been developed capable of screening at a rate of 800 compounds per day in dose-response mode, and will be followed soon by a second prototype capable of screening 8000 compounds per day. We developed first generation assays based on enzyme inhibition, iPS cells, tumour stem cells and tumour spheroids.The vastly reduced assay volume offered by this technology should also permit screening on cells that currently cannot be produced in sufficient quantity for conventional HTS. In particular, primary cells and induced pluripotent stem cells could become amenable to HTS, dramatically increasing the relevance of the screening data and forestalling clinical dead-ends.

This platform is already used for internal Medalis projects such as Microproteomix.

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Design and synthesis of non peptidic agonists to explore central roles of vasopressin and oxytocin receptors

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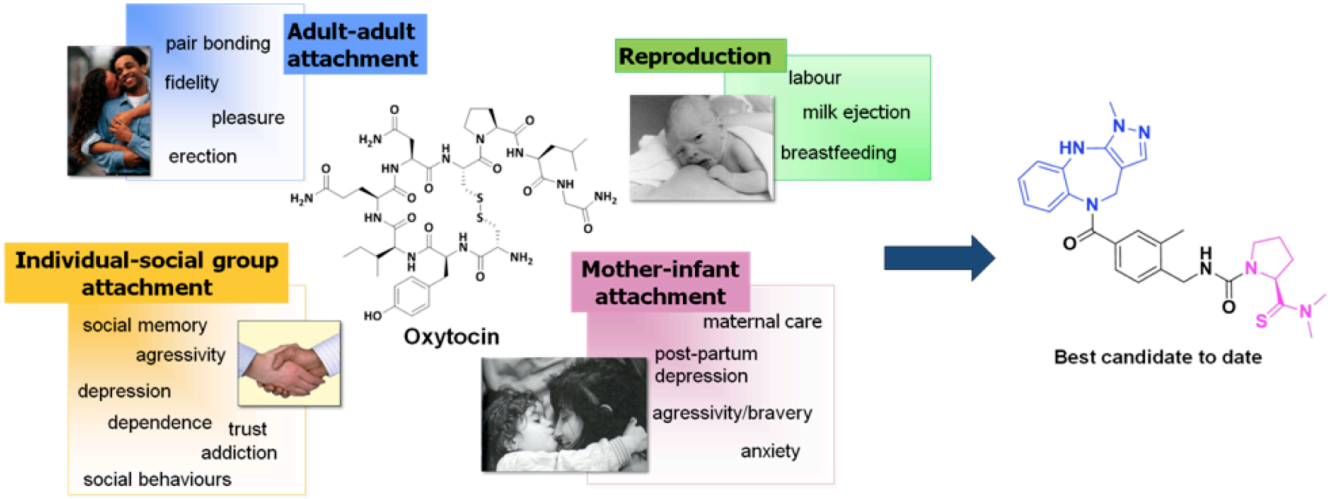
G protein coupled receptors are the largest family of transmembrane proteins and are the target for many drugs on the market (30% of drugs target GPCRs). The oxytocin/vasopressin system represents a good model to explore GCPRs, from the architecture to the function. Oxytocin and vasopressin neurohormones, through their receptors V_{1a} and OT, play a crucial role in a broad range of attachment behaviors (social behavior, anxiety, mother-child attachment, loyalty in couples). Recent studies showed that oxytocin can decrease fear associated to social phobia and symptoms related to autism[1]. In vivo studies are hampered due to the poor bioavailability (poor intestinal and blood-brain barrier penetration, short half-life, rapid clearance) of peptidic hormones. In this context, it's important to design and to synthesize new non peptidic agonists of the oxytocin receptor that will be able to cross the blood-brain barrier and act on the central nervous system.

Our first strategy consists in developing an accelerated approach to discover original ligands. A rapid and efficient assay using time-resolved fluorescence resonance energy transfer (TR-FRET) has been developed to screen V_{1a} and OT receptors. The second approach is based on the rational drug design starting from previous studied performed in our lab. These studies[2] give a better understanding of the essential determinant for the ligand-oxytocin receptor interaction.

This work is the starting point of the rational design of a centrally active, potent nonpeptide agonist of the oxytocin receptor to modulate and study the activity of this receptor *in vivo*.



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pPEI polymer is an effective reagent for intracellular delivery of proteins and antibodies in live cells

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Recent advances in biotechnology have led to therapeutic monoclonal antibodies able to mediate a benefit to patients by targeting extracellular receptors. Expending the therapeutic scope of antibodies will depend on our ability to develop specialized delivery systems that can successfully shuttle them into the cytoplasm for targeting intracellular pathology-mediating proteins.

Several methods have been described for introducing membrane-impermeable proteins into cells. Direct mechanical/physical membrane destabilizing techniques like electroporation, microinjection or pore-forming reagents showed effective. Alternatively, it is possible to keep the integrity of the plasma membrane by exploiting endocytic pathways through the formation of delivery systems. Cationic lipids, that are also efficient in delivering siRNAs, were shown to deliver large amounts of antibodies into the cytoplasm of mammalian cell lines.

In this work, we demonstrated that delivery can be even enhanced using a PEI-based polymer (pPEI) with pH-sensitive self-aggregating properties [1]. This polymer is able to entrap proteins in stable polyplexes, which are then uptaken by cells *via* endocytosis. Acidification of the polyplex-contained endosomes in turn led to self-dissociation of the polyplex and membrane rupture, leading to effective translocation. Our current study was notably performed using a monoclonal antibody directed against the HPV16 E6 oncoprotein, which is able to neutralize the E6-mediated degradation of p53 [2].

Our result showed pPEI to deliver the anti-E6 into HPV-16 infected CaSki cells as seen by a resurgence of nuclear p53 and may open novel therapeutic perspectives for antibodies.

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Identification of α5β1 integrin allosteric antagonists by *in silico* screening and cell-based assays.

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Introduction

Integrin α5β1 emerges as a pertinent target in solid cancers[1], especially in glioblastomas (GBM), the most aggressive brain tumour. Integrin α5β1 is implicated in physiological processes often deregulated in cancers such as proliferation and migration. This integrin, also called the fibronectin receptor, recognizes the RGD sequence of fibronectin. The few antagonists currently known are designed as RGD analogues. They have been selected for their capacity to inhibit the binding of α5β1 integrin to fibronectin[2]. Due to their resemblance to fibronectin, they can induce integrin activation. In order to avoid these unwanted side effects, we designed an alternative way to get a new generation of integrin antagonists. We used an *in silico* approach to select original integrin ligands which we further characterized for their inhibitory activity on **α5β1-dependent cell migration.**

Materials and Methods

In silico selection of compounds was realised by a pharmacophore-based screening of the BioInfo database (http://bioinfo-pharma.u-strasbg.fr/bioinfo) after computer-assisted modelling of the target, followed by a molecular docking. Evaluation of cell migration was carried out by a single cell migration tracking assay with the U87MG glioma cell line expressing different levels of the α5 integrin subunit. Selected compounds effects on cell adhesion to fibronectin were also investigated.

Results

A three-dimensional model of the α5β1 integrin headpiece domain was obtained by homology to the x-ray structure of the αVβ3 integrin. A druggable pocket localised mainly on the α5 subunit, remote to the fibronectin binding site, was identified. A 3 million commercially-available molecules (Bioinfo) were screened for their suitability to fit a pharmacophore generated from the novel allosteric pocket and to be properly docked out of which 70 ligands were selected. These compounds purchased and tested for their inhibitory potency of U87MG cell migration. We already showed that U87MG cell speed was correlated with the expression of α5 integrin at the cell surface. 5 molecules proved able to inhibit U87MG cell migration in an α5β1 integrin-dependent manner. Interestingly, none of these compounds were able to inhibit the integrin binding to fibronectin in contrast with the RGD-like antagonists.

Conclusions

With our original approaches, we identified 5 lead-compounds behaving as allosteric antagonists of α5β1 integrins. They may represent a second generation of integrin antagonists potentially more efficient that conventional antagonists. They will also help to understand more deeply the molecular characteristics of α5β1 integrins. Lastly, these antagonists will represent new therapeutic options for the treatment of glioblastoma.

Notes or short references

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ATF7 transcription factor is implicated in melanoma cell growth

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Cells are exposed to different sources of stress that will affect many cellular signalling pathways. Transcription regulation is controlled by transcription factors that bind DNA. ATF7 is a transcription factor belonging to the b-ZIP family implicated in many cellular mechanisms including cellular proliferation (Olsen et al., 2006). Cells exposed to stress (UVs irradiation, osmotic stress) present a fast increase of ATF7 phosphorylation, which leads to its transcriptional activation that in turn enhance the expression of target genes (Camuzeaux et al., 2008). As ATF7 -/- primary cells cannot be immortalized (Maekawa et al., 2010), we decided to investigate the role of ATF7 in the cell cycle.

Recent studies in the lab have shown that the ATF7 threonine residue 112 is specifically phosphorylated during mitosis in the absence of external stimulus. Our researches focus on the elucidation of the effect of this post-translational modification. We have designed different stable melanoma cell lines overexpressing ATF7. These cells are growing faster than the parental lines, and their cell cycle is shortened. One hypothesis is that ATF7 induces the expression of a soluble growth factor released in the medium.

We are also investigating the implication of the small alternative spliced ATF7-4 isoform. ATF7-4 lacks the BRLZ domain that enables ATF7 to bind DNA, and inhibits ATF7 transcriptional activity by trapping ATF7 specific protein kinases in the cytoplasm (Diring et al,. 2011). After a stress ATF7-4 is ubiquinylated and degraded by the proteasome. The kinases are then able to phosphorylate ATF7 in the nucleus. We generated ATF7-4 mutants that cannot be degraded even after cellular stress conditions. We want to decipher now if the ATF7-4 mutant inhibits melanoma cells growth.

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HR-MAS NMR studies of Proteins. Examples of Sensitivity, Easy of use, Sample stability

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High Resolution Magic Angle Spinning NMR spectroscopy (HR-MAS) is a powerful technique. HR-MAS was developped and largely used for solid support synthesis as shown by Shapiro[1]. HR-MAS found also various applications in metabolomic as the study about drug delivery in rat tissues[2] or recently to differenciate benin from malignant thyroid tissue[3]. An other application area was early opened by Viel[4] to investigate ligand-receptor binding events. This latter works presents examples on ligand screening on mass limited grafted protein samples.

HR-MAS experiments on proteins are investigated here. In comparison to 5mm liquid-state NMR, using HR-MAS on protein presents many benefits such as reduction in sample volume (50µL instead of 550µL), stabilisation of the protein on beads and faster purification during protein production. Working in HR-MAS allows to increase the protein concentration, because it protects the sample from aggregating.

Here we shown a new way to study grafted proteins using methyl-SoFast–HMQC experiment[5] by HR-MAS NMR. This technique provides a usefull tool for proteins studies, screening and site resolved studies of kinetics molecular process[6]. This method is largely used in liquid-state NMR biomolecular area. As shown in the litterature[7], Methyl-SoFast HMQC can be used in various and specific conditions and also well works with a mixture of proteins. The major benefit of this experiment is to work on non labeled molecules.

We combined HR-MAS technique and SoFast experiments to take advantage of each one, to studying proteins grafted onto a solid support (nickel beads). SoFast-methyl-HMQC was investigated to record sensitivity curves on samples of ^{13}C labeled proteins on 400MHz and 800MHz spectrometers[8].

Examples of spectra of several proteins grafted on beads, recorded at both magnetic fields, are presented. Solid phase effect of the beads on magnetization transfert is explored.

Using HR-MAS for protein studies is feasible and presents a lot of benefits, most liquid-state NMR experiments are available (as NOESY also used in this study). Several examples are described at a 800MHz and 400MHz field.

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The highly purified EPA:DHA 6:1 product-evoked endothelium-dependent NO mediated relaxation in the coronary artery involves a copper-dependent event triggering the redoxsensitive PI3-kinase/Akt pathway to activate eNOS by phosphorylation at Ser1177

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Aims: Omega-3 fatty acid products containing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been shown to reduce the risk of cardiovascular disease, in part, by stimulating the endothelial formation of nitric oxide (NO), a potent vasoprotective factor. This study determined whether the EPA:DHA ratio and purity affect the ability to cause endothelium-dependent relaxations in arterial rings, and characterized the mechanism leading to endothelial NO synthase (eNOS) activation. **Methods and Results:** EPA:DHA 6:1 caused significantly greater endothelium-dependent relaxations in porcine coronary artery rings than EPA:DHA 1:1, EPA and DHA alone, and EPA:DHA 6:1 with a reduced EPA + DHA amount. Relaxations to EPA:DHA 6:1 were slightly but significantly reduced by an eNOS inhibitor, not affected by inhibition of endothelium-dependent hyperpolarization and abolished by both treatments. Relaxations to EPA:DHA 6:1 were insensitive to cyclooxygenase inhibition, and reduced by inhibitors of oxidative stress, an inhibitor of either Src kinase or PI3-kinase, and copper chelating agents. EPA:DHA 6:1 induced phosphorylation of Src, Akt and eNOS at Ser 1177; these effects were inhibited by MnTMPyP and PEG-catalase. EPA:DHA 6:1 induced the endothelial formation of ROS in coronary artery sections as assessed by the redox-sensitive probe dihydroethidine, this effect was inhibited by MnTMPyP, PEG-catalase, and intracellular copper chelating agents. **Conclusion:** Omega-3 fatty acids cause endothelium-dependent NO mediated relaxations in coronary artery rings, which are dependent on the EPA and DHA ratio and purity, and involve an intracellular copper-dependent event triggering the redox-sensitive PI3-kinase/Akt pathway to activate eNOS by phosphorylation at Ser 1177.