2018 – Talks

Role of ShcA in Chondrocyte Hypertrophic Differentiation and the Physiopathology of Osteoarthritis

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Osteoarthritis (OA) is the most prevalent joint disease. The hallmark of this pathology is the gradual loss of articular cartilage (AC), composed of a single cell type, the chondrocyte. Chondrocytes synthesize an abundant extracellular matrix (ECM) that mainly consists of type II collagen (Col II) and aggrecan. During OA development, stable chondrocytes undergo hypertrophic maturation leading to the synthesis of type X Collagen (Col X) along with enzymes that degrade the ECM, resulting in the decreased production of Col II, the mineralization of the matrix and subsequent ossification of the cartilage.

To directly test the role of ShcA (Src Homology and Collagen A) in chondrocyte differentiation *in vivo*, our lab generated conditional mice lacking ShcA in chondrocytes using the Cre-lox system (Twist 2 ShcA KO). Mice that lack ShcA, exhibit a dwarfism phenotype, associated with a diminished bone-to-cartilage ratio, and a disorganized growth plate with a decreased hypertrophic maturation of chondrocytes indicating a crucial need for ShcA in the terminal chondrogenic hypertrophy process. In addition, tibio-femoral sections analysis showed that these mice exhibit larger proteoglycan and Col II and less col X expression and are protected from severe age-related OA development. These results were further validated *in-vitro* by using dedifferentiated chondrocytes that underwent either chondrogenic or hypertrophic differentiation in collagen structures later implanted in nude mice (collaboration with A. Barbero). We observed that ShcA-/- cells showed increased expression of stable cartilage markers and a decrease in the expression of hypertrophy markers accompanied by a reduced metalloproteinase activity. Furthermore, ShcA-/- cells showed a diminished activation of the MAPK pathway and subsequent decrease in the activation of RunX2, a master transcription factor for chondrocyte hypertrophy, along with a significantly reduced ColX mRNA stability. We have also uncovered a novel mechanism by which ShcA controls the activity of the Hippo pathway effector YAP1 in hypertrophic conditions.

An advanced understanding of the exact role of ShcA has the potential to identify the gene as a therapeutic target. Also, chondro-induced ShcA-deleted mesenchymal stem cells could be useful to engineer a stable cartilage tissue for proper cartilage matrix regeneration *in-vivo* and to bring the possibility of using this novel approach to a pre-clinical level.

Wnt5a Decreases mTORC1/SREBP2 Activity and Promotes Endosomal Cholesterol Trafficking to the ER in Mice and in Human Vascular Smooth Muscle Cell

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We are interested in VSMCs plasticity and in how cholesterol accumulates within these cells. Accumulation of cholesterol within the artery wall is a fundamental feature of atherosclerosis. Macrophages are considered the primary source of foam cells in atherosclerosis. However, in advanced human coronary atherosclerosis 40-50% of cells identified as monocyte-derived macrophages, are in fact vascular smooth muscle cells (VSMCs) derived, and the development of therapies to limit their formation may help to protect against atherosclerosis.

We previously uncovered that in VSMCs the low-density lipoprotein receptor-related protein (LRP1), a multifunctional cell surface receptor that belongs to the LDL receptor (LDLR) gene family protects from atherosclerosis. LRP1 controls the transcriptional activation of a Wnt family member, Wnt5a that limit cellular cholesterol accumulation in fibroblasts and pre adipocytes. Wnt5a promotes cholesterol export, and interestingly we also established that it blocks nuclear translocation of the transcription factor SREBP-2 leading to a decreases in the expression of its target genes HMG-CoA reductase and HMG-CoA synthase and of cholesterol biosynthesis. However, whereas Wnt5 limits cholesterol accumulation and is expressed in atherosclerotic lesions, it is unclear whether it protects against atherosclerosis

To test this, we generated mice deleted for Wnt5a in VSMCs (smWnt5a-). Mutant mice accumulated foam cells and develop 100% more atherosclerosis lesions than controls when fed with a cholesterol rich diet. Using CRISPR/Cas9 guided nuclease we generated human VSMCs knockout for Wnt5a (VSMCs Wnt5a-/-) and controls. We quantified free cholesterol inside cells, Confocal analysis shows that cholesterol-enriched cytosolic vesicles that accumulate in VSMCs Wnt5a-/- are Lamp1 positive endosomal compartments. Here we present evidence for the first time that Wnt5a regulates cholesterol trafficking in VSMCs. It inactivates the nutrient-dependent mTORC1 signaling pathway, this promotes cholesterol trafficking to the ER, suppresses SREBP-2 activity, and limits cholesterol accumulation. Conversely, low Wnt5a which correspond to high mTORC1 activity leads to less membrane cholesterol that reaches the ER and inhibition of autophagy. This activates SREBP-2, and increases cholesterol biosynthesis.

An advanced understanding of studies regarding wnt5a, its signaling & trafking can play role in treating different diseases like cancer, and cardiovascular system etc.

Neuropsychiatric Lupus: the Neuroinflammatory Model

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Neuropsychiatric systemic lupus erythematosus (NPSLE) is a severe complication that can occur in patients with SLE. The disease negatively impacts the overall SLE outcome (tenfold increase in mortality rates1) and the quality of life of up to 75% of lupus patients2. Clinically and biologically NPSLE remains poorly understood and there is no accurate marker allowing its diagnosis and/or prognosis. It can affect both the central and peripheral nervous systems. In our ongoing studies, we are trying to understand the mechanisms of central manifestations, such as psychosis, depression and memory loss.

Currently, we are investigating the disease progression in a spontaneous lupus murine model, the MRL/lpr strain3. As the disease is yet to be thoroughly investigated, our experimental scope is large: it ranges from behavioural to molecular pathway studies. To date, we have performed various behavioural tests3 showing that the diseased mice are suffering from cognitive deficits. These observations led us to look into the anatomical structures of their brains using magnetic resonance imaging, which unveiled a degenerative state of the structure. We also investigated the possible peripheral immune cell infiltration of the brain by flow cytometry, and we observed elevated numbers of various lymphocytic cells in the central nervous system of our mice. In conclusion, our current evidence is pointing to a neuroinflammatory development of the disease.

In the future, we would like to look in greater detail on how does the disease progress in the brain of these mice and relate our discoveries to human disease. We would like to explore the complement pathway, as proposed by Bialas and collegues4. Another possible target that interests us is the NLRP3 inflammasome5. Current approaches involve confocal microscopy on mice brains and cell culture experiments on human cell lines, the latter could help us explore molecular pathways of neuroinflammation in a genetically human background.

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The transcription factor E2F6 represses germline genes via specific DNA methylation recruitment to gene promoters.

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The methylation on cytosines is an epigenetic modification that represses gene transcription when located on promoter-proximal CpG islands. During mouse embryonic development, a global methylome reprogramming event occurs. The genome is first demethylated after fertilization and is then *de novo* methylated after the embryo implantation. During this step only 1% of promoter CpG islands gain methylation, mainly on germline genes promoter, a class of genes regulating gametic functions that need to be repressed in somatic cells, However the mechanism by which the genome is specifically *de novo* methylated during mammals developement is unknown.

Recent studies showed that the transcription factor E2F6 is invovled in some germline genes repression making it a good candidate to study DNA methylation recruitment. To adress its specific role, comparative methylome and transcriptome using high-throughput sequencing technologies were achieved beween mouse WT and E2f6 -/- embryos and embryo-derived primary cells.

Preliminary data revealed that (i) E2F6 is involved in the recruitment of DNA methylation at a small number of CpG-rich promoters of germline genes, (ii) germline genes repression is defined during a specifc temporal window,

My data provide evidence that a sequence specific DNA binding transcription factor adresses DNA methylation to repress germline genes during early embryogenesis. Further studies are necessary to shed light on the molecular mechansims of specific DNA methylation recruitment throught the binding of E2F6.

DICER1 levels of expression as a threshold for cellular homeostasis and life-span through canonical and non-canonical functions.

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Loss-of-function (knock-out) mouse models have established a fundamental role of the RNAse III enzyme DICER1 in animal development and tissue morphogenesis and homeostasis. These functions are currently assumed to result mainly from the DICER1-dependant biogenesis of micro-RNAs (miRNAs) which carry important roles in the regulation of gene expression. However non-canonical DICER1 functions recently emerged, such as interacting with the DNA-Damage Response (DDR) pathway and the processing of non-coding RNAs, suggesting that DICER1 might also participate in the regulation of major cellular process through miRNAs-independent mechanisms.

A correlation between reduced DICER1 levels of expression and ageing has been reported and furthermore, DICER1 expression is associated with overall sensitivity to stress. To establish the contribution of non-canonical roles of DICER1 in the initiation and/or progression of age-related disorders, I performed a thorough age-related phenotyping of hypomorphic *Dicer1* mutant (*Dicer d/d*) mice which exhibit decreased DICER1 expression.

My work has been mainly focused on adipose tissue and metabolism, as a fat-specific *Dicer1* knock-out mouse model has previously been associated with accelerated ageing and dramatic metabolism and adipocyte identity shifts under metabolic challenges (i.e. High-Fat Diet - HFD - and Dietary Restriction – DR - experiments). However those results are assumed to arise from changes in the miRNAs profile, yet non-canonical roles of DICER1 have not been mentioned so far.

We observed that, in *Dicer d/d* mice, compared to littermate controls, adipose tissue show an ageing-dependent (i.e in 70 weeks-old animals but not in 35 weeks-old mice) inflammatory signature associated with accumulation of non-coding B1 RNAs that might be linked with cellular senescence. Strikingly, fed with a lipid-rich diet, *Dicer1*-deficient mice appear resistant to the HFD-associated disorders: they gain moderate weigh, have less white adipose tissue and are more tolerant to glucose than the controls. These observations might be interpreted as the consequence of accumulating inflammation, mitochondrial dysfunction and cell-cycle disruption in different tissue (possibly gut and adipose tissue) associated with the accumulation of non-coding RNAs.

These preliminary results suggest that reduced DICER1 levels of expression following stress insults may act as a threshold regulating cellular homeostasis and, in chronic condition such as ageing, leading to major breakdown in tissue function.

Time-dependent luminescence loss of individual upconversion nanoparticles upon dilution in aqueous solutions

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Single-particle luminescence microscopy is a highly powerful method to extract key information on biological systems that is not accessible by ensemble-level methods. However, this technique is limited by the low signal-to-noise ratio of individual luminophores, background signal, blinking and low photostability. These drawbacks can be overcome by using upconversion nanoparticles (UCNPs), which make it possible to avoid biological autofluorescence by their anti-Stokes emission, and which provide an intrinsically stable and non-blinking luminescence [1,2].

However, ensemble measurements of diluted aqueous dispersions of UCNPs have shown an instability of luminescence over time due to particle dissolution-related effects, which can be especially detrimental for single-particle experiments [3]. Here, we investigated the luminescence response of individual UCNPs in aqueous conditions by quantitative wide-field microscopy. We observed that the particles exhibit a rapid luminescence loss, accompanied by large changes in spectral response, leading to a considerable heterogeneity in their luminescence and band intensity ratio. Moreover, the dissolution-caused intensity loss is not correlated with initial particle intensity or band ratio, which makes it virtually unpredictable.

These effects and the subsequent development of their heterogeneity can be largely slowed down by adding millimolar concentrations of sodium fluoride in the buffer. As a consequence, the presented data indicate that microscopy experiments employing UCNPs in aqueous environment should be performed in conditions that carefully prevent these effects.

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StatoMerocyanines: Ultrabright Fluorophores for Multicolor Imaging and Tracking of Lipid Droplets in Cells and Tissues

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Lipid droplets (LDs) are organelles that serve as the storage of intracellular neutral lipids. LDs regulate many physiological processes. Moreover, recent findings showed their involvement in metabolic disorders and diseases such as obesity, diabetes and cancers [1].

In this work we introduce a family of new fluorogenic merocyanine named StatoMerocyanines (SMCy). SMCy are based on an indolenine moiety and a dioxaborine barbiturate derivative. In oil, their fluorescence range from yellow to the near-infrared (NIR) while the fluorescence is quenched in aqueous media. Additionally, SMCy display remarkable brightness and photostability. All the members of this new family selectively stain the LDs in live cells in background-free manner. Unlike Nile Red, a commercial lipid droplet marker [2], SMCy dyes possess narrow and sharp absorption and emission bands allowing multicolor imaging. SMCy proved to be compatible with fixation and led to high-quality 3D images of lipid droplets in cells and tissues. Their high brightness allowed efficient tissue imaging of adipocytes and circulating LDs. Moreover, their remarkably high two-photon absorption cross-section as well as their capacity to efficiently fluoresce in the NIR region led to two-photon multicolor liver tissue imaging.

Taking advantage of the wide range of color palette, we were able to track and image lipid droplet exchange between cells demonstrating intercellular communication [3].

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Protein-sized Dye-loaded Polymer Nanoparticles for Free Particle Diffusion in Cytosol

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Despite broad applications of fluorescent NPs in bioimaging, their use inside cells is complicated by a poor understanding of how size of NPs influences their behavior in the cytosol. To address this issue nanoparticles based on the same material with varied size down to that of single protein molecules are required. In this work, we achieved this using dye-loaded polymer NPs, which is an emerging class of fluorescent NPs characterized by high brightness, low toxicity and design flexibility.

We investigated the influence of fraction and type of charged groups (negatively charged: carboxylate and sulfonate, positively charged: trimethylammonium) introduced into the polymer poly(methyl methacrylate) (PMMA) on the size of particles prepared by nanoprecipitation. In this way sizes could be tuned from 50 nm to 7 nm, while the NPs preserved their capacity to encapsulate large amounts of cationic fluorophores with bulky hydrophobic counterions generating NPs 20-fold brighter than quantum dots. Then these NPs were microinjected into living cells and the influence of the NP size on their diffusion in the cytosol was studied using single-molecule imaging. It was found that the smallest particles were able to reach all parts of the cytosol with a critical size of 17 nm for free diffusion and spreading in the cytosol.

The proposed concept paves the way to new extremely bright NPs with size down to that of proteins suitable for intracellular bioimaging with high localization precision.

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Development and characterization of stable suspension of layersomes as original drug delivery systems

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Among a variety of drug delivery systems, liposomes are very promising candidates considering their biocompatibility, biodegradability and their drug loading capacity for both hydrophobic and hydrophilic molecules. However, their major drawback is their instability in the gastro-intestinal environment and their enzymatic degradation by lipases (1). Lots of efforts have been made to overcome this major hurdle. One approach to increase their stability is the layer-by-layer coating with oppositely charged polymers to obtain structures called 'layersomes' (2).

Based on previous work, the poly-L-lysine (PLL) and poly-L-glutamic acid (PGA) combination was chosen by our group for the layersome formulation. Indeed, PLL and PGA coating was shown to increase the robustness of liposomes (3). Small unilamellar liposomes (Lp) composed of phosphatidylcholine, phosphatidylglycerol and cholesterol were used as starting material. Alternate deposition of positively and negatively charged polymers was achieved by slowly dropping the liposomes into a PLL solution and then by slowly dropping the obtained Lp-PLL suspension into a PGA solution. Excess of polymer was removed after each coating step by filtration.

We first demonstrated that the alternate deposition of positively and negatively charged PLL and PGA, respectively leads to an increase of the average diameter and a variation of the zeta potential values compared to primary liposomes, which is consistent with a layer-by-layer coating. The formulation procedure was optimized to obtain homogenous, stable and quite small (< 200 nm) layersome structures. We succeeded in developing stable and monodisperse layersome formulations with two polyelectrolyte layers of about 100 nm average diameter (half-value width: 15 nm). We are currently tuning the number of layers deposited on the liposomes to increase their resistance in simulated media.

In parallel to formulation, we performed the synthesis of PLL and PGA covalently coupled to rhodamine B and fluorescein, respectively, to demonstrate the alternate coating of liposomes. So far, both PLL-rhodamine B and PGA-fluorescein with different grafting percentages were synthetized and used to image and confirm the layer-by-layer coating of liposomes by fluorescence intensity measurements. A preliminary light FRET (Fluorescence Resonance Energy Transfer) was observed twice and needs to be confirmed. In parallel, a covalent locking of the peptide side chains was permitted via amide bonds to strengthen the polymeric coat. Stability experiments in simulated media are currently in progress.

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Spexin, a novel neuropeptide that modulates nociception via Galanin R2 receptor.

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Chronic pain is a major health issue in our society that clearly impacts quality of life and costs a lot to society. Today, opiates still represent the gold standard analgesics but their use is clearly associated with numerous adverse side effects including analgesic tolerance and opioid-induced hyperalgesia. There is therefore a need to develop novel analgesics that display fewer side effects.

The novel neuropeptide spexin (SPX) was discovered using bioinformatics. Mature SPX is an amidated peptide of 14 aminoacids that is highly conserved in vertebrate. Recent ligand-receptor interaction studies showed that SPX activates human GalaninR2 and R3 (GALR2/3) receptor subtypes, but not GalaninR1 (GALR1), suggesting that SPX is the endogenous agonist of GALR2/3. The SPX expression profile in brain and peripheral tissues of human and rodents suggests that this peptide may be involved in the modulation of several physiological functions including appetite control, anxiety, cardiovascular/renal function and nociception. Moreover, GALR2 is also expressed in pain pathways. We recently showed that central administration of Spexin in mice produces a dose dependent analgesic effect.

Furthermore, in collaboration with D. Bonnet 's team we identified novel and patentable fluoro-SPX derivatives with increased efficacy at GALR2 receptors and increased stability. In plasma We further showed that two of these derivatives display the same analgesic activity than endogenous SPX (10 nmol) but at a dose 100 times lower (0.1 nmol).

Finally, in a model of CFA-induced inflammatory pain we showed that peripheral administration of these derivatives produces anti-hyperalgesic activity at low doses and that this activity is not dependent of the opioid system. Altogether, our data strongly suggest that Spexin-GalR2 is a novel system involved in the modulation of pain and that spexin derivatives could represent novel therapeutic tools for pain treatment.

Organic nanoantenna for amplified fluorescence detection of nucleic acids

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Fluorescence detection of ultra-small concentrations of biomolecules in cells, their lysates and blood samples remains a challenge, because of a strong background noise from biological media and a poor brightness of organic dyes.

Previously, based on dye-loaded polymeric nanoparticles (NPs),1,2 we developed an organic nanoantenna, which provided giant signal amplification enabling detection of single molecules in ambient sunlight conditions.3 In the present work, we developed a robust approach to modify small (3000 of donor dyes inside a particle to ~20 acceptors hybridized with oligonucleotides at the nanoantenna surface, leading to an average signal amplification of 75. Owing to high brightness and strong amplification of nanoantenna particles, the developed nanoprobe can detect in solution low concentrations of the target oligonucleotide sequence, encoding cancer marker survivin, with limit of detection (LOD) of 5 pM using conventional fluorometer. After immobilization on the glass surface the nanoprobe can operate at the single particle level enabling detection of the target nucleic acids with LOD of 0.25 pM. Moreover, the nanoprobe remains functional in the biological media, including serum and complex mixture of non-target nucleic acids.

Such unique properties of functionalized organic nanoantennas open the route to new generations of ultrasensitive probes for different biomolecules.

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Heterogeneity of the alpha5 integrin subunit expression in glioblastoma

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Glioblastoma (GBM) is the most aggressive primary brain tumor. Despite major research efforts to find new therapeutic target and encouraging pre-clinical results, all trials failed to improve patient survival. Treatment failure and recurrence are explained by intratumoral heterogeneity. Our previous results showed that the integrin α5β1, the fibronectin receptor, is implicated in GBM aggressiveness and represents a pertinent therapeutic target. Recently, we observed that its expression was heterogeneous between patient tumors but also between different areas in a given tumor. We hypothesized that this intratumoral heterogeneity may be linked to different glioma initiating cells (GIC).

We characterized α5β1 expression in 9 GICs cell lines before and after differentiation. Our results show that α5 integrin is not expressed in stem cell culture conditions. However, α5 expression is induced after differentiation in about half of the cell lines supporting the notion of inter-tumoral heterogeneity of GICs. Two cell lines were selected and were genetically modified by depletion (CrisprCas9) or transfection of the α5 integrin gene. Different clones were selected expressing or not the integrin. We noticed that when GICs are programmed or forced to express α5 integrin, differentiated cells became more aggressive. Notably, differentiated cells, expressing the integrin had a capacity of migration and proliferation increased and acquired a fibronectin-dependent motility and a proliferative phenotype We also observed with single cell-derived clone analysis that intra-tumoral GICs heterogeneity exists. The *in vivo* assays demonstrated that GICs, programmed to express the integrin, were prone to form larger tumors.

Our data support the hypothesis that some GICs are programmed to express the α5 integrin subunit to form a more aggressive tumor. Further studies will be needed to explore the implication of such heterogeneity in resistance to anti-integrin therapies but also to conventional chemo/radiotherapies. By understanding mechanisms, mediated by the integrin α5β1, to induce a most aggressive phenotype, we will propose new marker to classified patients and to find new therapeutic strategy.

Fluorocarbon-peptide conjugates (FPC): new concept to increase the metabolic stability of peptides for therapeutic applications.

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Over the past decade, peptides have shown an increasing interest for therapeutic applications as they are selective and efficacious signaling molecules. To date, 60 therapeutic peptides have been already approved by the FDA1. However, they are often not directly suitable for use as convenient therapeutics because they have intrinsic weaknesses, including poor chemical and physical stability, and a short *in vivo* half-life due to rapid enzymatic degradation2, 3.

To address the peptides instability issue for therapeutic applications, we propose an unprecedented strategy based on the grafting of fluorocarbon chains (F-chains) onto peptides. Thereby, the hypothesis was to induce the self-organization of fluoropeptides in aqueous solution, resulting in the protection of the native peptide from enzymatic degradation. To demonstrate the efficacy of our approach the apelin-17 peptide, a neuro-vasoactive peptide which presents a short plasma half-life, was selected as model4,5. Different F-chains were then grafted onto apelin-17 following a solid-phase approach. The highest plasma stability fluoroapelin (LIT01-196) was then evaluated in rat model demonstrating the positive impact of F-chain to greatly improve the *in vivo* efficacy of apelin-17 (Gerbier, R *et al.* FASEB J., 2017, 31, 687-700). In this communication, we will present also some preliminary results to gain insight into the mechanism leading to the increase of human plasma stability of LIT01-196.

Altogether, these promising results should open the route to a convenient, safe and general approach to greatly increase the metabolic stability of numerous peptides for their *in vivo* use as pharmacological tools and/or therapeutic agents.

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Role of Tip60 in UHRF1 regulation in human cancer cells

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Nuclear epigenetic integrator UHRF1 (Ubiquitin-like containing PHD and RING finger domains 1) plays an important role in maintenance of DNA methylation, histone modifications, cell cycle progression and DNA damage response [1]. UHRF1 is believed to have an oncogenic potential as its expression levels are high in many cancers and it also promotes cellular proliferation [2]. High UHRF1 levels can serve as a powerful diagnostic tool for cancer [3]. An important partner of UHRF1 is Tip60 (Tat-interacting protein of 60 kDa), which is present in the same macro-molecular complex as UHRF1 and DNMT1 [4]. Tip60 belongs to MYST family and has a role in epigenetic regulation through its acetyltransferase activity. Tip60 maintains cellular and genomic stability and suppression of tumorigenesis [5]. The aim of this study was to investigate that how Tip60 regulates the UHRF1?

Our results showed that UHRF1 directly interacts with the MYST domain of Tip60 and increasing the levels of Tip60 leads to down regulation of UHRF1 and DNMT1 in cancer cells [1]. We also observed that higher levels of Tip60 decreases the association of UHRF1 with USP7 (deubiquitinase enzyme) and promotes degradation of UHRF1.

This suggests a role of Tip60 in regulation of UHRF1 which can be explored later to target high levels of UHRF1 in tumors for anticancer therapy.

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Inhibitor type 5 (PDE5), in: mitochondrial respiration, mitochondrial permeability transition pore (MPTP) opening and oxidative stress in the gastrocnemius muscle (GC) of mice during acute Ischemia-Reperfusion (IR).

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Ischemia-Reperfusion (IR) of the lower limbs is a public health problem, potentially causing irreversible tissue or even whole-body damage, which is life-threatening.

Ischemia is characterized by a lack of oxygenation of a tissue, secondary to an interruption of blood flow in an artery. However, reperfusion, necessary to save the limb, is accompanied by an increase in muscle damage (mitochondria, cell energy production center), mainly attributed to the release of free radicals. The association of changes in intracellular signaling during muscle alterations is also well-known including that of cyclic nucleotides governed by their specific phosphodiesterases (PDEs). In that way, we wondered whether PDEs should be implicated in the regulation of skeletal muscle mitochondria [1].

It has been shown in the laboratory that N-type natriuretic peptide (BNP) used during limb IR protects skeletal muscle against the deleterious effects of IR [2], interestingly BNP is known to increase intracellular levels. cyclic guanosine 3 ', 5'-monophosphate (cGMP) [3]. Knowing also the structural relationships existing between the receptors of natriuretic peptides and those of guanylate cyclase, we wondered whether an increase in the level of cGMP in skeletal muscle induced by a specific inhibitor of PDE5 might protect the muscle.

Our study is focused on sildenafil, a specific phosphodiesterase type 5 (PDE5) inhibitor, currently given in Human (3). We investigate the potential protective effect of this drug on the mouse gastrocnemius muscle during IR. This investigation involves a joint analysis of mitochondrial muscle respiration, oxidative stress production, and mitochondrial permeability transition pore (MPTP) behavior at the level of this muscle.

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Development of innovative antitumoral platinum (II) compounds to induce immunogenic cell death

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Some cancer treatments like chemotherapeutic agents (anthracyclines, platinum derivatives,...) are able to activate the antitumor immune response by inducing a particular cell death: the immunogenic cell death (ICD). This process is characterized by the exposition of the endoplasmic reticulum chaperone calreticulin at the cell surface as well as the release of ATP and non-histone chromatin-binding protein high mobility group box 1 (HMGB1) which serve as immunostimulatory damage-associated molecular patterns (DAMPs) and increase the antitumor immune response. We focused on *N*-heterocyclic carbene platinum complexes associated with polyethyleneimine, a transfection agent, to create multivalent cationic platinum compounds (NHC-Pt(II)-PEI) that induce apoptosis *in vitro* and *in vivo* in xenograft immunodeficient mouse model [1].

To evaluate the potential implication of the immune response on the NHC-Pt(II)-PEI *in vivo* effect, immunocompetent mice bearing tumors were treated with platinum particles and the results revealed an antitumor effect of our conjugates, in the same range than the clinical used platinum drug oxaliplatin, but with less side effects. We evaluated if NHC-Pt(II)-PEI were able to induce ICD. First results showed expression of calreticulin upon NHC-Pt(II)-PEI treatment. We are then evaluating if their association with immune danger signals could enhance this effect.

Altogether our results reveal the possibility of creating Pt(II) derivatives that can be used as chemotherapeutic agents by killing tumor cells and as immunotherapeutic agents by triggering the antitumor immune response.

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