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Abstract book

Oral communications

Involvement of NPFF1 and NPFF2 receptors in hyperalgesia and analgesic tolerance associated with chronic morphine, and inflammatory pain in mice

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Résumé

There are two main types of pain: acute pain, which is intense but short-lived, and chronic pain, which is long-lasting and disabling. Worldwide, 30% of adults suffer from chronic pain. 50% of them are unable to relieve their pain causing major social and economic repercussions. During the 1990s, the use of opioids for pain management began to increase, particularly in the United States. Today, opioid abuse remains the leading cause of preventable death in the United States. Opiates analgesia in the clinics is mainly due to the activation of mu-opioid receptor. Their chronic use results in the development of pain hypersensitivity, analgesic tolerance and dependence, the most commonly observed side effects of opioids. In the laboratory, we are particularly interested in studying the involvement of GPCRs of the RFamide family in the modulation of nociception and adaptations associated with chronic opioid administration and inflammatory agents. To this purpose, we first assessed the effect of peripheral morphine administration on nociceptive threshold and/or morphine-induced analgesia, hyperalgesia and analgesic tolerance in wild-type, NPFFR1 and NPFFR2 KO mice. In addition, we evaluated the effect of two inflammatory agents, Freund's Complete Adjuvant (CFA) and carrageenan, on the development of inflammation and hyperalgesia in these same animals.

Our data show that (i) hyperalgesia induced by repeated morphine administration is reduced in NPFFR1 KO animals in the thermal modality, equivalent to the WT group in the mechanical modality, and absent in NPFFR2 KO animals in both thermal and mechanical modalities. (ii) analgesic tolerance is aggravated in NPFFR1 KO animals in the thermal modality, equivalent to the WT group in the mechanical modality and reduced in NPFFR2 KO animals. (iii) hyperalgesia induced by CFA or carrageenan is present in WT and NPFFR1 KO animals and absent in NPFFR2 KO mice in both heat and mechanical modalities. (iv) inflammatory edema appears to be greater in NPFFR1 KO animals than in WT and NPFFR2 KO mice.

In conclusion, our data show that NPFFR2 plays a critical role in the development of hyperalgesia and analgesic tolerance associated with chronic morphine as well as hyperalgesia induced by inflammatory agents. Conversely, NPFFR1 appears to play a limited role in these phenomena.

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Mots-Clés: RF, amide, Receptor, Inflammation, Pain, Hyperalgesia

The mRNA decay factor Pat1 mediates a global control of poly(A) tail length that is suppressed by a ribosome mutation

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Résumé

Eukaryotic mRNAs are key actors of gene expression; thus, their levels need to be finely tuned. Post-transcriptionally, levels of mRNAs are governed by their turnover in the cytoplasm. Critical steps of mRNA decay encompass deadenylation, decapping, and exonucleolytic mRNA body digestion, that constitutes the 5' -> 3' pathway.

The conserved decapping regulator Pat1 appears to impinge on all those processes. Despite evidence that Pat1 is a conserved and central actor of the 5' -> 3' mRNA decay pathway, some studies have reported that only a fraction of the transcriptome is impacted by its deletion. Moreover, Pat1 was described both as a translational repressor of oligoadenylated mRNAs and as a global translation initiation enhancer. Altogether, published data fail to provide definitive clues about the role of Pat1 in cellular mRNA control.

To elucidate Pat1's function, we performed multi-omic and genetic analyses of a pat1 mutant in the yeast Saccharomyces cerevisiae. Transcriptome and proteome analyses revealed that the expression of only a subset of genes is affected by Pat1's inactivation. In contrast, a FLEP-seq analysis, allowing the sizing of the poly(A)-tail of each transcript in the cells by Nanopore sequencing, revealed that the absence of Pat1 impacts the length of the poly(A)-tail at the transcriptome level. Unexpectedly, we have observed that the growth defects caused by Pat1's inactivation are partially suppressed by a mutation in a ribosomal protein. This suppressor mutation also partly rescued the abnormal poly(A)-tail length of pat1 cells. Moreover, we observed that polysome profiles are altered in the absence of Pat1 and further modulated by the suppressor.

Altogether, our analyses are consistent with the model where the activation of decapping, that becomes rate-limiting, is the primary defect in the absence of Pat1. Our study supports the conclusion that Pat1 is a general mRNA decay factor even if its absence results in transcript-specific changes in mRNA levels. Altered mRNA decay in Pat1 deficient cells, and consequent translational impairments, can both be partly rescued by altered ribosomes.

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Mots-Clés: mRNA decay, decapping, ribosome, translation, yeast

New TURN-ON probes for the detection of bacteria in body fluids

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Résumé

Due to the spread of antibiotic-resistant pathogens, bacterial infectious diseases became one of the first causes of mortality and morbidity in the world1,2. Early administration of an appropriate antibiotic considerably reduces the risk of mortality3. However, the current methods of clinical detection and identification of bacteria in body fluids (urine, blood...) are insensitive and time-consuming due to the bacterial culture step. In our team, we aim at developing rapid and direct next-generation methods for clinical diagnostics of bacterial infections using fluorescent probes.
We have conceived a family of environmentally sensitive fluorescent probes for bacteria by conjugating a fluorogenic and solvatochromic dye Nile Red to antimicrobial peptides (AMP) and antibiotics targeting different components of the bacterial cell envelope. The probes were characterized by excellent fluorescence turn-on between aqueous and apolar media, and a positive solvatochromism similar to those of the parent dye. Applied to the analysis of bacteria by radiometric flow cytometry, these probes should allow the analysis of the difference in the microenvironment of the components of the cell envelope.4

With the goal to increase the fluorogenic character of the Nile Red-derived probes and to reduce their non-specific fluorescence in the presence of serum, we developed a family of bacteria-targeting probes based on aggregation-caused quenching (ACQ)5. The probes are composed of a peptide backbone to which are attached covalent dimers of Nile Red. In an aqueous medium, the dimeric probes exist in the form of non-fluorescent π-stacked H-aggregates, whereas in an apolar medium (bacterial cell membrane), the fluorescence is restored, leading to a strong fluorescence turn-on. Moreover, the strong interaction between the two fluorophores prevented dimer dissociation in the presence of serum, thereby opening up prospects for the application of Nile Red-based dimers in body fluids.

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*Intervenant

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Mots-Clés: fluorescent probes, fluorogenic detection, Nile Red, bacterial detection

Chemical mapping and bio-guided fractionation of macromycetes for the discovery of new and bioactive natural products

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Résumé

Throughout history, natural products have been a cornerstone of drug discovery(1), with the origins of many modern medications traceable to compounds isolated from plants, marine organisms, bacteria, or fungi. Antibiotics are a striking example, as most are derived from natural products-primarily of bacterial or fungal origin. During the so-called "golden era of antibiotics" (1950–1970), industrial high-throughput screening of natural extract libraries was widespread. However, this approach eventually yielded diminishing returns in terms of novel scaffolds, leading to a shift in focus toward synthetic chemistry(2). Simultaneously, the widespread and often inappropriate use of antibiotics-such as prophylactic use in the agri-food industry or inadequate treatment in human medicine-has contributed to the alarming rise of multidrug-resistant bacteria(3). Today, more than ever, there is an urgent need for new scaffolds with original modes of action to combat bacterial infections.
Macromycetes, commonly referred to as mushrooms, have been relatively underexplored as potential sources of antibiotics compared to filamentous fungi like *Penicillium* and *Fusarium*, despite preliminary evidence of promising antimicrobial activity(4). These organisms share ecological niches with plants, bacteria, animals, and micromycetes, yet have distinct resource requirements. As a result, they produce a range of unique compounds likely involved in signaling or defense, including antimicrobial agents. Research has largely focused on traditional medicinal mushrooms from Chinese pharmacopoeia(5)-such as *Ganoderma lucidum*, *Cordyceps militaris*, *Lentinula edodes*, and *Hericium erinaceus*-while few European species have been historically recognized for medicinal properties(6), with most attention limited to edible or toxic varieties.

Alsace is a region characterized by a diverse landscape, dominated by a calcareous alluvial plain nestled between two mountain ranges (the Vosges in France and the Black Forest in Germany). Approximately 4000 mushroom species have been recorded in this area, the

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majority of which remain chemically unstudied. To assess the potential of macromycetes as antibiotic sources, a mycotheque of 150 wild mushroom species was assembled. Fungal extracts were subjected to bioautography(7) against members of the pathogenic ESKAPE family-specifically *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*-as well as *Serratia marcescens* and the fungal pathogen *Candida albicans*. These microorganisms are known for their ability to develop multidrug resistance and evade conventional antibiotics.

Preliminary screenings identified several extracts and compounds with antimicrobial activity. Additionally, UHPLC-MS/MS analysis coupled with data processing via the MZMine-GNPS pipeline allowed us to visualize the chemical diversity of the extracts as a molecular network, facilitating the identification of rare versus ubiquitous compounds. The integration of chemical and biological data guided the selection of promising extracts for fractionation and further characterization of active constituents. Two such extracts will be discussed in this talk to illustrate the effectiveness of the described approach.

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Compensatory effect of Dnm2 gain-of-function and loss-of-function mutations in centronuclear myopathy and Charcot-Marie-Tooth neuropathy

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Résumé

Dominant mutations in DNM2, encoding a GTPase implicated in membrane trafficking, result in two distinct neuromuscular diseases. Centronuclear myopathy (CNM) is characterized by muscle weakness and structural myofiber anomalies, and Charcot-Marie-Tooth neuropathy (CMT) is associated with sensory loss and neuron defects. CNM and CMT presumably involve an inverse pathomechanism with DNM2 gain-of-function in CNM and loss-of-function in CMT. However, the precise effect of the mutations is poorly understood, and no cure is approved for any of the diseases.

In order to investigate the potential compensatory effect of CNM and CMT mutations, we crossed Dnm2S619L/+ CNM with Dnm2K562E/+ CMT mice, and the Dnm2S619L/K562E offspring underwent behavioral, functional, morphological and biochemical investigations at 8 weeks. Dnm2S619L/K562E mice were larger than Dnm2S619L/+ and Dnm2K562E/+ littermates and manifested an increased general muscle force. Moreover, Dnm2S619L/K562E mice did not display the coordination defects observed in Dnm2K562E/+mice on the treadmill, and the in situ muscle force was significantly higher compared with Dnm2S619L/+ littermates. Compared with Dnm2S619L/+ and Dnm2K562E/+ mice, histological analyses of Dnm2S619L/K562E muscle sections showed an increase of myofiber diameter, and a normalization of myofiber architecture with a normal localization of nuclei and mitochondria and restored collagen thickness. Sciatic nerve analysis of both individual disease models revealed a decreased g-ratio, attributed to axonal hypotrophy, while Dnm2S619L/K562E nerve fibers presented a normalized g-ratio due to axonal hypertrophy and myelin thickening. Taken together, these results show an improvement of muscle function, structure, and peripheral nerves defects in Dnm2S619L/K562E mice, compared with Dnm2S619L/+ and Dnm2K562E/+ mice.

Overall, this study is the first to report a compensatory effect of two different mutations in

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the same gene, causing two different disorders. This study confirms the loss-of-function mechanism in DNM2-CMT, and suggests the increase of DNM2 activity as potential therapeutic strategy.

Mots-Clés: Myopathy, neuropathy, Charcot, Marie, Tooth, disease, dynamin, compensation

Unveiling the anti-Mycobacterium secrets of medieval remedies: advanced analytical approaches for a modern understanding

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Résumé

The recent proliferation and prevalence of antimicrobial multi-resistant infections has prompted the development of other strategies and alternatives to urgently combat this global threat. Rifampicin-resistant *Mycobacterium tuberculosis* was included in the Critical Priority class of pathogens defined by the World Health Organization, due to the global impact on public health, the severity of the disease on affected patients and the increasing incidence of multi-drug-resistant strains (1-2).
In this context, past mastering of remedies formulation appears as a wealth of resources for present research. In particular, Arab Medieval Pharmacopeias (AMP) were explored by our interdisciplinary team gathering researchers from biology, chemistry, humanities and informatics sciences (3) with the aim to discover new bioactive compounds from ancient preparations to combat actual health threats.

Modern approaches were conducted together for the selection a remedy from AMP, the targeting of potential antimicrobial compounds and their isolation. Three manuscripts from the 9th to the 12th Century were used to create an interactive database in order to bring out relevant anti-tuberculosis remedies (4). A formulation assembling plants resins, roots and metals was highlighted. After sourcing, extraction and in vitro evaluation against *Mycobacterium tuberculosis* H37Rv, five resins extracts showed anti-*Mycobacterium* activity (MIC values from 100 to 400 μg/mL). The study was then focus on Bdellium, a gum-resin from *Commiphora wightii* (Burseraceae) based on its selective action against *M. tuberculosis* strain. The targeted isolation of putatively bioactive compounds was guided by the combination of HPLC-HRMS/MS data dereplication and molecular networking, both with pharmacophoric deconvolution which is differential analyses of 2D NMR data linked to bioactivity. Pharmacophoric deconvolution indicates that diterpenes structural pattern could be responsible

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of the anti-*Mycobacterium tuberculosis* activity. Their targeted isolation and antimicrobial evaluation are ongoing to confirm our analytical and historical approaches.

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Mots-Clés: Resin, anti Mycobacterium, Molecular Networking, Pharmacophoric deconvolution

Light-Induced Mechanical Gain-of-Function in PIEZO Channels via a Chemical Optogenetic Approach

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Résumé

All cells are exposed to mechanical stimuli and respond to them with varying sensitivity. Several proteins can transduce mechanical signals into biological responses, among which the PIEZO1 and PIEZO2 play a prominent role. These proteins form mechano-activated ion channels and are essential in diverse physiological processes, including touch sensation, blood pressure regulation and red blood cell volume control. Dysregulation of PIEZO channels has been associated with various human pathologies. For example, a gain-of-function mutation (R2482H) in PIEZO1 is responsible for xerocytosis, a form of hereditary anemia. As such, PIEZO channels represent promising therapeutic targets.
Currently, most methods to study PIEZO activity rely on mechanical stimulation. Although physiologically relevant, this approach lacks specificity, as it can also activate other mechanosensitive pathways. To overcome this limitation, the laboratory developed a novel chemical optogenetic tool, called mOP1, which allows for rapid and specific activation of PIEZO1 using light. This approach is based on the chemical photoswitch maleimide ethylene azobenzene trimethyl ammonium (MAT), which contains an azobenzene moiety that toggles between *trans* and *cis* configurations under green (525 nm) and UV (365 nm) light, respectively. MAT is covalently attached to a cysteine residue introduced by site-directed mutagenesis (Y2464C) in the PIEZO1 pore domain. In the dark, the MAT adopts the *trans* configuration and the channel remains closed. Upon 365 nm irradiation, MAT switches to *cis* form, inducing a conformational change that rapidly opens the channel, without mechanical stimulation. The goal of this study is to further investigate the biophysical properties of the light-gated ion channels.

The method used in this study is the patch-clamp technique, which is the gold standard for measuring currents in cells. Specifically, I employed the cell-attached configuration, which allows the recording of activity from a small number of channels in a native-like membrane environment. The cells used were HEK293 cells lacking endogenous PIEZO1 activity (HEK P1KO). These cells were transiently transfected with either a plasmid encoding wild-type mouse PIEZO1 (mP1) or a plasmid encoding a cysteine mutant. Prior to recording, cells were incubated with the MAT and extensively washed before patch-clamp recordings. During patch-clamp experiments, negative pressures (suction) were applied through the pipette

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to mechanically stimulate PIEZO1 channels and responses were compared before and after cells were briefly illuminated at either 365 nm or 525 nm to induce photoisomerization of the bound MAT.

Our results show a two-fold increase in current amplitude and slower inactivation kinetics of mechano-induced currents in cells expressing the cysteine mutant when MAT is in the *cis* configuration compared to the *trans* state. These effects are fully reversible and mimic the gain-of-function phenotype of the R2482H mutation linked to xerocytosis. Importantly, these light-dependent effects were not observed in cells expressing wild-type mP1, confirming the specificity of the MAT-mediated modulation.
These findings demonstrate that MAT can induce a reversible gain of function in a mutant PIEZO1, offering a novel and precise way to probe channel activity. This approach enables modeling of PIEZO1 function and dysfunction with high temporal control, paving the way for future *in vivo* studies.

Mots-Clés: PIEZO channels, Chemical optogenetic tool, Patch Clamp, Gain of function

Development of PROTACs targeting kinases for the treatment of cancers

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Résumé

Cancer is a major public health issue worldwide. In France, it is considered as the leading cause of mortality in men and the second in women.1,2 This high incidence is primarily attributed to metastases, which account for 90% of cases and which pose significant treatment challenges.3,4 Recent studies have demonstrated the involvement of CDK5 in tumours, with functions ranging from metastasis to angiogenesis. CDK5 plays an essential role in cell motility, invasiveness and metastatic spread. 5,6,7

Most protein kinase inhibitors approved by the FDA and used in the clinic target the ATP pocket; however, their selectivity remains relatively low due to their mechanism of action, which relies on ATP-pocket binding. Several strategies have been proposed to target other pockets, essential protein/protein interactions and conformational transitions. A promising strategy which has emerged for therapeutic targeting leverages the Ubiquitin-Proteasome System (UPS) to induce degradation of a protein of interest (POI), thanks to Proteolysis-Targeting Chimeras (PROTACs), hetero-bifunctional molecules composed of a ligand of the protein of interest (POI) connected via a linker to a ligand for the E3 enzyme of the UPS (Figure 1).

This project focuses on the design and synthesis of PROTACs that target the ATP pocket of CDK5. We have synthesized several of the PROTACs derived from a selective CDK5 inhibitor to induce selective degradation of CDK5.8 We have characterized the affinity of our PROTACS to inhibit the catalytic activity of CDK5 in vitro and have investigated their potential to promote degradation of CDK5 in lung cancer cell lines.

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Mots-Clés: PROTAC, metastasis, CDK5, degradation, allosteric modulation, selective inhibition, kinase

*Intervenant

ORAI1 downregulation ameliorates the multi-systemic signs of Tubular aggregate myopathy (TAM) and Stormorken Syndrome (STRMK)

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Résumé

Tubular aggregate myopathy (TAM) and Stormorken syndrome (STRMK) are clinically overlapping diseases affecting skeletal muscle, bones, spleen and platelets. They are caused by gain-of-function mutations in the Ca2+ sensor STIM1 or the Ca2+ channel ORAI1, both regulating Ca2+ balance through the ubiquitous store-operated Ca2+ entry (SOCE) mechanism. Functional investigations have shown that the TAM/STRMK mutations induce overactive SOCE, resulting in excessive influx of extracellular Ca2+. We previously generated a mouse model (*Stim1R304W/+*), recapitulating the main clinical signs of TAM/STRMK patients, and representing a unique tool to assess therapeutic strategies.
Currently, no therapies have been approved for TAM/STRMK. However, SOCE is amenable to manipulation. We aimed to rebalance Ca2+ homeostasis in *Stim1R304W/+* mice through shRNA-mediated downregulation of *Orai1*. Intravenous injections of AAV9 carrying *Orai1*-specific shRNAs significantly reduced *Orai1* expression by 80%. Compared with non-injected controls, treated *Stim1R304W/+* mice manifested increased body size and improved muscle force production and relaxation kinetics 3 months post injection. Moreover, histological analyses of muscle samples evidenced a normalization of fiber size and shape, and we also noted a partial restoration of spleen size, architecture and the number and distribution of megakaryocytes, the platelet precursor cells.
In conclusion, shRNA-mediated downregulation of *Orai1* improved the multi-systemic signs of TAM/STRMK. The treatment efficiently corrected the muscle, bone and spleen phenotypes in *Stim1R304W/+* mice, but had no measurable effect on platelet numbers and bleeding diathesis. In view of the high conservation of the targeted *Orai1* sequences in mouse and human, this approach represents a viable strategy for prospective clinical trials.

Mots-Clés: Tubular aggregate myopathy, Stormorken syndrome, ORAI1, Calcium, shRNA, ASO

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The Alpha5 GABAA inverse agonist treatment alleviates cognitive impairments in a Down syndrome mouse model

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Résumé

Down syndrome (DS) is a major cause of intellectual disability (ID) with a genetic origin. One of the leading hypotheses concerning ID in DS is a shift in the excitatory/inhibitory balance towards inhibition in the central nervous system. Indeed, several molecular markers specific to inhibitory neurons are increased in rodent models. Thus, modulating GABAergic activity could be a potential therapeutic approach with promising results on rodent models using a complete blocker of GABA receptors (PTZ). However, PTZ is known to increase seizure risk. To reduce this risk, we repurposed the a5IA drug developed by Merck, which is a negative allosteric modulator of GABAa5 receptors located post-synaptically after Martinotti cells. Acute injection of the molecule rescued long-term, working and spatial memory without any sign of adverse effects in our previous study in Ts65Dn mice. In this project, we explored the cognitive and motor effects of chronic administration of a5IA on the Dp(16)1Yey mice (a more complete Down syndrome model).
We evaluated working memory, learning capacity, spatial memory and motor function using the Y-maze test, the pattern dissociation paradigm, the Barnes maze and the Rotarod, respectively. Dp(16)1Yey (Dp16) mice and control (WT) littermates were injected with either a5IA or vehicle two times a week throughout the pipeline starting at 8 weeks of age and lasting 7 weeks. We evaluated the effects of a5IA on both males and females, but we did not identify significant sex-related variations.

The treatment restored the working memory in the Y-maze of Dp16-treated mice. These mice showed functioning memory with significantly more spontaneous alternations than random exploration, which was also observed in WT vehicle and WT treated but not in Dp16 vehicle mice. The molecule modulated motor performance. Dp16-vehicle mice performed significantly worse on the rotarod than WT-vehicle mice, whereas Dp16-treated mice displayed intermediate performances. WT-treated mice also displayed a slight improvement compared to WT vehicle mice. Surprisingly, the deficits observed in Dp16 vehicle mice regarding learning in the pattern dissociation task and spatial memory were not rescued by a5IA.
Altogether, a5IA restored working memory but not memory anchoring and spatial learning, despite the localisation of the a5IA targets, the GABAa5 receptors, in the hippocampus. This partial restoration of cognitive abilities might be explained by the fact that the a5IA treatment does not affect all steps in a circuit. For instance, electrophysiological dysregulation

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in Martinotti cells and parvalbumin interneurons were detected, and both cell populations modulate pyramidal neurons. Hence targeting only GABA receptors specific to Martinotti cells could be insufficient to restore all cognitive functions. Future studies will explore the activity modification of these two cell populations to understand the ID linked to DS further.

Mots-Clés: Down Syndrome, Cognition, Mouse model, GABA, Therapy

Bacteriocins: From Isolation to Innovation – Harnessing Natural Peptides Against Multidrug-Resistant Pathogens

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Résumé

The growing threat of multidrug-resistant (MDR) bacteria is making it increasingly difficult to treat infections with standard antibiotics. As these pathogens continue to evolve and resist multiple drug classes, there's an urgent need to explore new antimicrobial strategies. In this context, bacteriocins (small, naturally occurring peptides produced by bacteria) are emerging as promising alternatives due to their targeted activity and low potential for resistance development. In this study, we focused on five clinically relevant MDR pathogens: *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus epidermidis*, *Escherichia coli*, and *Klebsiella pneumoniae*. Strains from these species were successfully isolated from clinical sources and selected for their resistance to multiple antibiotic classes. To identify potential bacteriocins with antimicrobial activity, we isolated lactic acid bacteria (LAB) from diverse Moroccan fermented matrices. Purification of the active peptides was performed using cation-exchange chromatography (SP Sepharose), followed by semi-preparative and analytical C18 reversed-phase HPLC. Structural characterization was conducted using MALDI-TOF mass spectrometry, leading to the identification of several peptides-specifically pediocin PA-1, plantaricin, and a modified form of the class IIb bacteriocins L50A and L50B. However, in the antibacterial tests, we only used pediocin PA-1, modified forms of L50A/L50B, and a commercial Nisin A (used as a control). Clear inhibitory effects were observed particularly against MDR *P. aeruginosa, S. epidermidis,* and *E. faecalis*, with certain combinations showing enhanced activity, suggesting potential synergy. No antimicrobial effects were observed against *E. coli* and *K. pneumoniae* under the tested conditions. These findings support the idea that bacteriocins may be useful additions to the antimicrobial arsenal, especially against Gram-positive pathogens and certain Gram-negative strains such as *P. aeruginosa*. Their use in combination with conventional antibiotics could be a promising strategy to lower the minimum inhibitory concentration (MIC) and improve treatment efficacy against resistant infections.

Mots-Clés: Multidrug resistance, Bacteriocins, HPLC, MIC.

*Intervenant

Multi-omics study of intracellular transport defects impacting focal adhesion in myotubular myopathy in mice

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Résumé

X-linked myotubular myopathy (XLMTM) is rare and a severe form of centronuclear myopathy (CNM) caused by the loss-of-function mutations in *Myotubularin 1* (*MTM1).* Previous studies have reported significant impairments in focal adhesion dynamics and integrin intracellular localization in 8 weeks *Mtm1*−/y mice. However, the underlying causes of these defects and their progression across different disease stages remain poorly understood. To address this, we performed transcriptomic and proteomic analyses on the *Mtm1*−/y mouse model at pre-symptomatic (E18.5), early (2w) and late (7w) developmental stages. Our results reveal that the pathways related to intracellular transportation, integrin activation and recycling, vesicle trafficking, and extracellular matrix (ECM) organization were consistently altered. An early upregulation of caveolin-dependent endocytosis and ECM components along with impaired fast recycling of integrins, were observed. These findings were confirmed by measuring the mRNA and protein expression. This ultimately led to the intracellular accumulation of active β1-integrin at the late disease stage. In silico analyses further indicated that these defects occur at the early endosomal level due to the absence of MTM1 and not at the late endosomal stage. Additionally, in-vitro studies have validated the overexpression of slow recycling transporter and caveolins during the later stages of disease progression.
Overall, these findings suggest that major defects in various intracellular transport systems have impact on the dysregulation of focal adhesion and cytoskeleton dynamics. This highlights intracellular transporters as a strong promising therapeutic targets for restoring cellular homeostasis at early disease stages in *Mtm1KO* mouse models.

Mots-Clés: Myotubularin 1 (MTM1), Intracellular transport, Integrin signalling, Extracellular Matrix (ECM), Multi, omics

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