UN SOUFFLE DE COLLABORATION

Symposium québécois des chercheurs et cliniciens FK

Cystic Fibrosis Canada
Fibrose kystique Canada

Jeudi 9 et vendredi 10 mai 2019
Hôtel Mortagne

I would like to extend a warm welcome to the 2019 CFC-CFTRc symposium « An Era of Collaboration». This symposium will bring us up to date on CF research being carried out here in Quebec and hopefully will foster collaboration between different labs with common interests and complementary expertise. It also provides us with an opportunity to invite outstanding international speakers including Dr. Lap Chee Tsui, lead researcher responsible for cloning the CF gene, and Dr. Ray Frizzell, who has made many seminal contributions to our understanding of epithelial chloride transport. We are looking forward to their talks and to other exciting presentations throughout the day on diverse topics. There will also be several interesting flash presentations from graduate students and postdocs and we encourage them to discuss their work with other participants including speakers to benefit from their insights and feedback.

This symposium is an annual event for the CF Translational Research centre (CFTRc), a virtual CF centre that was formally established in the fall of 2015. It is based at McGill but includes many other institutions. Its main goal is to accelerate the development of a cure for CF by providing access to state-of-the-art equipment, CF cells and other materials, by promoting collaboration between laboratories, and by organizing symposia, workshops, seminars, and travel awards for trainees to present their work at conferences and obtain feedback.

This is my opportunity to acknowledge the tremendous efforts of Dr. Annick Guyot in putting together this symposium. I also thank Cystic Fibrosis Canada for their helpful collaboration and our sponsors for making the symposium possible.

I wish you all an interesting and productive meeting.

Dr. John Hanrahan
Director of the CFTRc

Cystic Fibrosis Translational Research
CENTRE
de Recherche Translationnelle sur la Fibrose Kystique

9h30 – Guest speaker – Conférencier invité

Retrospective on CF research and future directions

Lap-Chee Tsui

GBM, O.C., O. Ont.,
Founding President of the Hong Kong Academy of Sciences,
President of Victor and William Fung Foundation

Briefly, I would go over the key steps that led to the identification of the Cystic Fibrosis Transmembrane Conductance Regulator gene and what we learned from the genetics of the CF disease. The gene identification has allowed better definition of the basic defect in CF, deeper understanding of its pathophysiology and research into new strategies to treat the patients in the past 30 years. Further advances in CF disease management continue to require close collaborations among scientists and medical service providers, and, even patients themselves.

📖 Prof. the Honourable Tsui Lap-Chee, world-renowned molecular biologist, is currently Founding President of the Hong Kong Academy of Sciences, President of Victor and William Fung Foundation, Director of Qiushi Academy for Advanced Studies and Master of Residential College in International Campus of Zhejiang University, and University of Toronto's Emeritus University Professor. He was the 14th Vice Chancellor of The University of Hong Kong. Prior to his appointment at HKU, Prof. Tsui was Geneticist-in-Chief and Head of the Genetics and Genomic Biology Program of the Research Institute at The Hospital for Sick Children in Toronto. He is world renowned for his research work in human genetics and genomics. He has also made significant contributions to the study of the human genome, especially the characterization of chromosome 7, and identification of additional disease genes. He has over 300 peer-reviewed scientific publications and 65 invited book chapters. He is the recipient of many national and international prizes, including the 2018 Warren Alpert Foundation Prize. His other awards include 16 honorary doctoral degrees from prestigious universities around the world.

10h15 – Flash Presentation – Présentation éclair

Synergistic rescue of ΔF508 and CFTR2 mutation functional expression defects by structure-guided corrector combinations

Guido Veit[1], Haijin Xu[1], Elise Dreano[2], Radu G Avramescu[1], Miklos Bagdany[1], Lenore K Beitel[1], Ariel Roldan[1], Mark A Hancock[1], Cecilia Lay[3], Wei Li[3], Katelin Morin[3], Sandra Gao[3], Puiying A Mak[3], Edward Ainscow[3], Anthony P Orth[3], Peter McNamara[3], Aleksander Edelman[2], Saul Frenkiel[3], Elias Matouk[3], Isabelle Sermet-Gaudelus[2], William G Barnes[3], Gergely L Lukacs[1]

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The most common cystic fibrosis mutation, ΔF508 in nucleotide binding domain 1 (NBD1), impairs CFTR-coupled domain folding, plasma membrane (PM) expression, function, and stability. Robust ΔF508-CFTR correction is achieved by stabilization of NBD1, the interfaces between NBD1 and membrane-spanning domains (MSDs), as well as NBD2, the former two representing primary conformational defects, established by using combination of genetic and pharmacological means. Thus, a rationally designed, structure-guided corrector strategy may require the combination of type I correctors supporting the NBD1-MSD1 and NBD1-MSD2 interface formation, type II correctors targeting NBD2, and type III correctors stabilizing the NBD1 domain.

VX-809 (lumacaftor), the first approved corrector, exhibits a type I mechanism and in combination with the gating potentiator VX-770 (ivacaftor) provides only modest clinical benefit to patients carrying two copies of the ΔF508 mutation.

Here we report the identification of compounds for all three corrector types in a screen of ~600,000 small molecules by monitoring the PM expression of the HRP-tagged ΔF508 in CFBE41o- (CFBE) epithelia. Compounds that increased ΔF508 PM densities both in the presence and absence of VX-809 contained type III correctors, compounds that required VX-809 included type II correctors, and compounds exhibiting redundancy with VX-809 encompassed type I correctors. The mechanisms of action (MOAs) of correctors were determined by domain-interrogation and domain-specific binding assays, competition with reference compounds, and ΔNBD2-CFTR PM density measurements. While type I-III correctors alone displayed only modest correction, combination of correctors from all three classes synergistically increased the Δ508 PM density and function in CFBE by up to ~9 fold in comparison to VX-809 alone, augmented the mutant ER maturation and the abundance of the complex-glycosylated form, promoted the peripheral stability, and largely normalized the single channel function of ΔF508. These results correlated well with CFTR gain-of-function in human bronchial epithelia and human nasal epithelia from $CFTR^{\Delta F508/\Delta F508}$ patients. Corrector combinations lead to ~50% of wild-type-level correction in human nasal and bronchial epithelia and in mouse nasal epithelia. Likewise, corrector combinations were effective against rare missense mutations in various CFTR domains, probably acting via structural allostery, suggesting a mechanistic framework for their broad application.

This study provides proof of principle for synergy screening to identify correctors with distinct MOAs, which, when used in structure-guided combinations, achieve therapeutically relevant correction levels of ΔF508 and other processing mutants.

10h20 – Flash Presentation – Présentation éclair

Synergistic inhibition of CFTR dependent chloride secretion by urban air pollution particulate matter and oxidative stress in airway epithelial cells

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The FDA recommends that clinically relevant drug-drug interactions be defined during drug development and assessed in prospective clinical studies prior to filing a new drug application. Consequently many interactions of approved CFTR modulators with other drugs are known. By contrast, the interactions of CFTR modulators with environmental factors have not been studied although they could impact efficacy. For example rescued F508del-CFTR is downregulated by lower concentrations of cigarette smoke extract compared to wild-type CFTR (1). Urban air pollution particulate matter (PM) induces ER stress and the unfolded protein response (2) and elevates oxidative stress that may contribute to the cellular response to PM (3), however the effect on rescued F508del-CFTR after corrector treatment is not known. To examine the effects of PM and oxidative stress individually and in combination on airway epithelial cells, comparing responses of non-CF and CF cells. Polarized CFBE cell monolayers and primary HBE cells mounted in Ussing chambers were used to measure chloride secretion. qPCR was performed on targets associated with oxidative stress. PM (standard reference material 1648) from the National Institute of Standards and Technology was used as PM. CFTR-dependent secretion was unaffected by overnight exposure to PM alone but was strongly inhibited when cells were treated with both PM and the oxidant stressors tert-butylhydroquinone or hydrogen peroxide. CFBE cells expressing F508del-CFTR that had been partially rescued by the corrector drug VX-809 were more susceptible to PM + oxidant stress than cells expressing WT-CFTR. mRNA transcript levels for the antioxidant enzymes glutathione synthetase, superoxide dismutase 2, and catalase were elevated by oxidant exposure as expected, however this response was also impaired in cells expressing F508del-CFTR and further reduced by the presence of PM + oxidant stress. Immunoblots of primary human bronchial epithelial cell lysates revealed a slight decrease in CFTR protein expression, although a reduction in basolateral transport also contributed to the acute inhibition of CFTR-dependent secretion by oxidants. We conclude that oxidant stress and PM cause synergistic inhibition of airway secretion and antioxidant defenses are compromised in CF cells. Finally, much of this inhibition of CFTR-dependent secretion may occur at the basolateral membrane.

10h25 – Flash Presentation – Présentation éclair

The dual phosphodiesterase 3/4 inhibitor RPL554 stimulates rare class III and IV CFTR mutants

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Over 2,000 mutations have been reported in the *cftr* gene, many of which cause disease but are rare and have no effective treatment. Thus there is an unmet need for new, preferably mutation-agnostic, therapies for cystic fibrosis (CF). Phosphodiesterase inhibitors (PDEis) are one potential class of therapeutics as they have been shown to elevate intracellular cAMP levels and stimulate CFTR-dependent secretion in human airway epithelia, however the number of people of CF that could be helped by PDEi's remains to be determined. Recently, we demonstrated that an inhibitor of human PDE3 and PDE4, RPL554 (Verona Pharma), was able to stimulate the class IV CFTR mutant R117H CFTR endogenously expressed in well-differentiated primary human bronchial epithelial cells. Therefore, we sought to assess whether RPL554 could also stimulate other class IV CFTR mutants and explored its effects on class III CFTR mutants for the first time. Fisher Rat Thyroid cells transduced with lentiviruses to stably express R334W or T338I CFTR (class IV) or S549R or G551D CFTR (class III) were used to study regulation by RPL554. We found that RPL554 elevates intracellular [cAMP] leading to a potentiation of forskolin-stimulated R334W, T338I, G551D and S549R CFTR when used either alone or in combination with the CFTR modulators VX809 and VX770. Furthermore, we obtained biochemical evidence that VX809 can increase the cell surface expression of T338I, G551D and S549R CFTR, which correlated with enhanced cAMP-stimulated activity. Together, our findings strengthen the therapeutic potential as RPL554 as an anti-CF therapy for CF patients with class III/IV mutations and expand its scope as a drug that can benefit numerous CF patients.

11h00 – Oral Presentation – Communication Orale

Airway smooth muscle, airway hyperresponsiveness and cystic fibrosis

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Cystic fibrosis is associated with an "asthma" phenotype in a high proportion of affected persons. Although primarily an epithelial disease CFTR deficiency in other tissues may be implicated in significant ways in the clinical expression of disease. We have focused on airway smooth muscle as a possible contributor to the pathogenesis of the asthma phenotype. Airway smooth muscle expresses CFTR and involvement in the contractile properties of the muscle has been demonstrated, primarily through the study of cultured smooth muscle cells. We have examined the airways for evidence of remodeling of the muscle and have confirmed greater muscle mass in airways from CF affected subjects, in agreement with previous studies. The intrinsic biomechanical properties of the muscle ex vivo were not different muscle harvested from previously healthy subjects, showing similar maximal force development on exposure to methacholine and normal dynamic properties when stimulated either electrically or with methacholine. However, the impairment of relaxation to β-agonist stimulation was shown after pre-treatment with interleukin-13, a T 2 cytokine associated with CF, compared to control muscle. In addition, we have found enhanced proliferation of CF smooth muscle in vitro compared to non-CF control cells. The mechanism is unclear but is associated with a smaller of proportion of cells in the G0/G1 phase of the cell cycle, suggesting an acceleration of entry to the S phase. An enhanced expression of myosin light chain kinase was also demonstrated but the significance of this finding is not clear.

In conclusion, CF airway smooth muscle shows distinct characteristics that may favour its remodeling in vivo and the inflammatory environment, associated with T 2 cytokine expression may lead to resistance to relaxation to β-agonist stimulation.

11h25 – Flash Presentation – Présentation éclair

Fenretinide mimics CFTR-induced correction of DHA/AA imbalance and blocks LPS-induced MUC5AC overexpression without affecting MUC5B

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Cystic fibrosis (CF) is the most common genetic disease in Caucasians. CF manifests through the accumulation of mucus in the lungs, which serves as the fertile soil for the growth of microorganisms, leading to recurrent infections and ultimately lung failure. Mucus in CF patients consists of DNA from dead neutrophils and mucins produced by goblet cells. MUC5AC mucin is responsible for the pathological plugging of the airways whereas MUC5B has a protective role against bacterial infection. Therefore, decreasing the level of MUC5AC without affecting MUC5B would be a desirable mucoregulatory treatment.

We demonstrated that pre-treating mice with fenretinide in a chronic model of *P. aeruginosa* lung infection efficiently prevents the accumulation of mucus. Fenretinide prevented lipopolysaccharide-induced increase of MUC5AC gene expression, without affecting the level of MUC5B in the lung goblet cell line. Furthermore, fenretinide treatment efficiently reversed pro-inflammatory imbalance of fatty acids by increasing the levels of docosahexanoic and decreasing the level of arachidonic acid in lung epithelial cell line and primary leukocytes derived from CF patients.

11h30 – Flash Presentation – Présentation éclair

Mechanism of action of the synergistic combination tomatidine-aminoglycoside against *Staphylococcus aureus* virulent and persistent phenotypes

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Background: *Staphylococcus aureus* (SA) can adopt two phenotypes, prototypical (WT) and its small-colony variant (SCV). These two phenotypes are often recovered from the lungs of cystic fibrosis (CF) patients and are frequently co-isolated with *Pseudomonas aeruginosa* (PA). Aminoglycosides such as tobramycin or gentamicin (AMG) were shown efficient to control and reduce PA prevalence to 40% in patients with CF. Although PA is one of the most deleterious pathogens in CF, prophylaxis targeting PA over the past few years did not profoundly modify the overall number of exacerbations per year or the number of days of hospitalization per exacerbation per year based on the CF registry data. On the other hand, *S. aureus* recovery rate from patients increased to more than 53% of the patients. Furthermore, reports indicate that co-isolation of both SA (MRSA or SCVs) and PA worsen patients' health. Tackling all three pathogens seems imperative. Tomatidine (TO), a phytomolecule extracted from tomatoes, exerts a strong bactericidal activity on the *S. aureus* SCV phenotype (minimal inhibitory concentration [MIC] of 0.06 µg/mL) and is part of the novel steroidal alkaloid antibiotic class. Moreover, when TO is combined to an aminoglycoside, the combination shows a strong synergistic activity against WT SA (AMG MIC of 0.06 µg/mL). We recently determined that the molecular target of TO was the ATP synthase subunit c (*atpE*) and that TO reduced ATP production in *S. aureus*. We report here how TO, with AMG, exerts its bactericidal activity against both the WT and SCV phenotypes of SA.

Methods: Since TO affects the bacterial ATP synthase, we measured the membrane potential. Bacteria in broth were incubated with various concentrations of antibiotics (TO or TO-AMG). Bacteria were then washed in PBS, the fluorophore $DiOC_2$ was added and incubated for 30 min before flow cytometry. To assess the production of reactive oxygen species (ROS), bacteria were suspended in broth and incubated 2h at 35°C before the addition of 10 µM H_2DCFDA for 1h. Bacteria were then washed and transferred to a 96-well plate containing broth and antibiotics (ciprofloxacin as a control). Fluorescence was measured (λ exc 494_{nm}, λ emi 521_{nm}) over a 13-h period. AMG uptake was measured using a Texas-Red tagged AMG with the addition of TO. All results are reported as a percentage of that measured for WT without antibiotic.

Results: TO reduced WT membrane potential in a dose-dependent manner and reached a low of 35% at the highest doses (≥8 µg/mL). On the other hand, SCV membrane potential, which was about 10% of that of WT, further dropped to about ≤2% at very low TO concentrations (≥0.0035 µg/mL). This was also accompanied by 2 times more ROS production than that seen in the no antibiotic control. Besides, there was no difference in the membrane potential of WT when comparing the effect of AMG to that of TO-AMG. However, the combination TO-AMG generated 2.5 times more ROS compared to that caused by AMG alone. TO also increased AMG uptake by more than 1.5 times.

Conclusions: TO is able to reduce the membrane potential of both SA WT and SCV phenotypes, but the membrane potential only dropped to a critical level in the SCV. Also, significant ROS are only produced in SCV, which are highly susceptible to TO. Similarly, only the TO-AMG combination generated significant ROS production in the WT and increased AMG uptake, which explains the strong synergy with aminoglycosides. The TO-AMG combination may represent a novel therapeutic paradigm for lung infections in CF patients, targeting both *S. aureus* phenotypes (WT and SCV) and *P. aeruginosa* all together.

11h35 – Flash Presentation – Présentation éclair

Despite antagonistic activities in vitro, *Pseudomonas aeruginosa* enhances *Staphylococcus aureus* colonization in a murine lung infection model

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Background: A defective muco-ciliary clearance of bacteria in cystic fibrosis (CF) patients results in recurrent pulmonary infections. *S. aureus* (SA) and *P. aeruginosa* (PA) are the most prevalent lung pathogens in CF and are frequently co-isolated. Their co-infection is associated with a worse clinical outcome, as noted by decreased lung functions and frequent pulmonary exacerbations. However, despite their co-occurrence, PA and SA prototypic strains exhibit antagonism *in vitro*: PA reduces SA growth and induces the small-colony variant (SCV) phenotype. Therefore, we attempted to better understand the apparent conflict between the *in vitro* observations and the high SA-PA co-occurrence in CF. We previously described clinical SA-PA co-isolates not displaying such an antagonism. The present study compares the colonization of various strains, including reference, clinical co-isolates or virulence-attenuated mutants, in a murine co-infection model.

Methods: Growth kinetics were followed for co-cultures of five SA-PA clinical pairs isolated from adult CF patients. Selective plates were used to determine viable counts for each species. In other experiments, SA was spread over an agar plate where a PA spot was applied in the centre to visualize SA SCV formation around PA. *In vivo* interactions were characterized using a mouse lung infection model. Intra-tracheal inoculations of SA, PA or SA-PA pairs were performed, and infections developed for 24h. Lung homogenates were plated on selective media allowing CFU counts of either SA or PA. Inflammation was assessed by myeloperoxidase (MPO) quantification and expression of two known receptors for cellular adhesion of SA, ICAM-1 and ITGA-5, were measured by RT-qPCR.

Results: Growth kinetics showed that some PA antagonized their SA co-isolate with a drop of ~3.6 log10 CFU/mL initiated after 8h of co-culture. However, other pairs did not interact negatively showing equivalent growth for SA in mono- and co-cultures. The agar co-culture model revealed the formation of SA SCVs in pairs where PA antagonized SA growth kinetics, while some non-antagonistic pairs did not result in the formation of SCVs. Paradoxically, in the infection model, SA colonization was significantly higher in SA-PA co-infections, even for pairs showing antagonism *in vitro*. In fact, SA colonization was most enhanced in co-infections with antagonistic PA. The SA colonization increased up to 2.00 $\log_{10}$ in co-infections compared to their respective SA mono-infections ($P < 0.05$). PA did not benefit from the co-infection showing equivalent colonization of lung tissues in presence or absence of SA ($P > 0.05$). Upon compiling all results from 200 co-infections (35 PA and 10 SA strains, including clinical, reference and mutant strains), SA colonization was found to be proportional to PA colonization. Different virulence-attenuated mutants for both species were evaluated in the co-infection model, but no virulence or regulatory genes could specifically be associated to this phenomenon. The level of inflammation was measured via MPO quantification but was not correlated to the promotion of SA colonization. However, RT-qPCR of ICAM-1 and ITGA-5 showed that PA significantly increased their expression, both in mono- or co-infections with SA.

Conclusion: The observation that lung colonization by SA is improved in presence of PA may explain the frequent co-infections by these pathogens in CF and may contribute to their combined detrimental effect on patient health. The more effective SA colonization in presence of PA could involve an increase of its cell surface receptors by PA.

11h40 – Oral Presentation – Communication Orale

Susceptibility of CFTR-mutant mice to *C. rodentium* infection: a new model to study the role of CFTR in the gut?

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Although commonly associated with a respiratory phenotype and chronic lung infection, Cystic Fibrosis (CF) is a multi-organ disease with manifestations in multiple organ systems, including the gastrointestinal tract. CF is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR), which is located in the membrane of various epithelial tissues and immune cells and plays a critical role in ion and fluid homeostasis. Mouse models of CF generally display little lung pathology, but do possess an overt intestinal phenotype, characterized by obstruction, inflammation, and bacterial overgrowth. To probe the intestinal phenotype of CF mutant mice, we infected □F508 CFTR mice (Cftrtm1Eur) and *Cftr*$^{-/-}$ mice with *Citrobacter rodentium*, a Gram-negative intestinal mouse pathogen. We found that CFTR mutant mice were highly susceptible to infection with *C. rodentium,* displaying high levels of mortality and atypical localization and greater colonization of bacteria than WT littermate mice. Detailed immunophenotyping revealed differences in the intestinal immune status of CFTR mutant mice at steady state and following infection. Further experiments using conditional knockout and gut-corrected mouse models suggest that loss of CFTR expression in the intestinal epithelium is not sufficient to cause *C. rodentium* infection susceptibility. We propose that *C. rodentium* infection provides a new model system to study the role of CFTR in the gut which may be relevant to intestinal disease observed in CF patients.

12h05 – Oral Presentation – Communication Orale

Correction of the CFTR3849+10kb C>T mutation using a CRISPR-Cas9 NHEJ strategy delivered by receptor-targeted nanocomplexes

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Introduction: Cystic Fibrosis is an autosomal recessive disorder caused by mutations in the CFTR gene. The 10th most common mutation, 3849+10kb C>T, generates a cryptic splice site, resulting in the formation of a pseudoexon containing a PTC, producing a truncated version of the protein. CRISPR/Cas9 allows for precise targeting of mutations by a guide RNA targeting molecule followed by double strand DNA cleavage by Cas9 nuclease. However, delivery of the CRISPR components into cells and target organs remains a challenge.

Aims: Our approach is to deliver CRISPR with a non-viral nanoparticle, previously described for in vivo DNA and siRNA delivery. These nanoparticles comprise peptide and lipid components, which package nucleic acids and target their delivery to epithelial cells. Gene editing of airway epithelial cells is permanent, therefore repeated delivery with these non-immunogenic nanoparticles could be performed to reach a sufficient level of genetic correction.

We designed pairs of Cas9 guide RNAs to create targeted double-stranded breaks in CFTR either side of the mutation, resulting in high efficiency excision via non-homologous end-joining repair, when tested in a mini-gene assay in HEK293T cells. Experiments were then repeated in bronchial cells isolated from a patient homozygous for this mutation to confirm functional restoration of the CFTR protein channel.

Methods: Primary CFBE cells were lentivirally transduced with the *BMI-1* proto-oncogene to expand proliferative potential for the course of experiments. Pairs of gRNAs were complexed with Cas9 protein and formulated with our lipid-based nanoparticles for transfection. 48 h post-transfection, genomic DNA was isolated, and cells were re-seeded for repeat transfections. Cells were then expanded for ALI culture. After 5 weeks differentiation on ALI, CFTR mRNA was analysed via qRT-PCR, and protein expression analysed by Ussing Chamber.

Results: After one transfection, a DSB efficiency of 26% was achieved in primary CFBE cells, as measured by Inference of CRISPR Edits (ICE) software, and T7 endonuclease assay. Of this, 10% had the expected 187 bp excision, indicative of successful cutting by both guides. After four repeat nanoparticle transfections, a DSB efficiency of 82% was achieved, 61% of sequences having the expected excision. This was able to restore CFTR mRNA expression and, importantly, CFTR channel function as measured by Ussing Chamber.

Conclusion: This approach could be used to correct aberrant splicing signals in several other CF mutations. Moreover, this targeted gene excision strategy may also be applicable in the study many other genetic disorders where deep-intronic mutations have been identified as a disease cause.

13h45 – Oral Presentation – Communication Orale

S. aureus and *P. aeruginosa* infections in cystic fibrosis: beyond bacteria, to the host response of CFTR-targeting therapies

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Progressive lung damage due to chronic inflammation and bacterial infections, especially with *P. aeruginosa* and *S. aureus*, remains the first cause of morbidity and mortality in cystic fibrosis (CF) patients. Although novel CFTR-targeting therapies have recently emerged, their efficiency remains limited and variable among patients. There is now evidence, including from our laboratory, that *P. aeruginosa* infection down-regulates wt-CFTR and restrains the functional rescue of F508del-CFTR by CFTR correctors. Our previous work also highlighted a link between CFTR function, bacterial infections and epithelial repair mechanisms after injury. Indeed, we discovered that the capability of CF airway epithelia to heal is less efficient than in healthy subjects, most likely due to the basic CFTR defect and bacterial infections. We then discovered that CFTR rescue with correctors enhances epithelial repair in non-pathogenic conditions; this effect is however dampened in the presence of *P. aeruginosa* infection.

Our general objective is to 1) better define the impact of *S. aureus* and *P. aeruginosa* on the response of host epithelial cells to CFTR-targeting therapies and 2) identify therapeutic strategies to improve CFTR rescue and epithelial repair, despite the presence of *S. aureus* or *P. aeruginosa* infection. To achieve our goals, we are using primary cultures of airway epithelial cells (AEC) collected from non-CF and CF patients, exposed to bacterial exoproducts (from *P. aeruginosa* or *S. aureus*) and treated with CFTR modulators (Orkambi (VX-809 corrector + VX-770 potentiator) or Symdeco (VX-661 + VX-770)). CFTR-F508del rescue (CFTR maturation and currents) as well as repair processes (migration/lamellipodia dynamics, cytoskeletal organization, wound healing rates) were then assessed. The effect of various *P. aeruginosa* strains on CFTR was also evaluated in mouse lung tissues collected after chronic *P. aeruginosa* airway infection.

Our data first indicated that the effects of *P. aeruginosa* strains vary as a function of their genotypic/ phenotypic characteristics. Whereas an Early strain (intermittent acute infection) severely dampened CFTR expression *in vitro* and *in vivo*, an engineered mutant of the *lasR gene* (EarlyΔlasR) and a late isolate (from the same patient, when chronic infections are established) did not altered CFTR. We also discovered that interfering with bacterial quorum sensing (QS) with a QS inhibitor (QSI, HDMF) prevented the deleterious effect of *P. aeruginosa* on CFTR rescue by correctors. We then demonstrated that *P. aeruginosa* and *S. aureus* exoproducts significantly impaired airway epithelial repair processes. The deleterious impact of *P. aeruginosa* was prevented by QS inhibition. Our work also showed that the Symdeco or Orkambi combinations elicited a greater beneficial effect, than the corrector alone, on the repair of airway epithelia from F508del/F508del and heterozygous (F508del+another class II mutation). The effects of Orkambi or Symdeco was however dampened in the presence of *P. aeruginosa* or S. aureus exoproducts, indicating that a complementary approach is required to efficiently restore epithelial integrity. Interestingly, *P. aeruginosa* did not affect K^+ channels, which are widely expressed in airway epithelial tissue, including in progenitors cells. Moreover, a treatment with Orkambi or Symdeco, combined with K^+ channel activators, greatly improved airway integrity, despite the presence of infection. Such strategies, targeting *P. aeruginosa* (with QSI) and both CFTR and K^+ channels (with modulators) would deserve further investigation to enhance the efficiency of treatments in cystic fibrosis.

14h10 – Flash Presentation – Présentation éclair

Étude des biomarqueurs prédictifs associés à l'ischémie de reperfusion et à la dysfonction primaire du greffon suite à une transplantation pulmonaire chez les patients atteints de fibrose kystique

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Malgré l'amélioration des traitements pour les patients atteints de fibrose kystique (FK), la transplantation pulmonaire demeure la seule option de survie en phase respiratoire terminale.
Toutefois, malgré l'amélioration des méthodes de préservation du greffon, des techniques chirurgicales, des soins péri-opératoires et de l'immunosuppression, le taux de survie à 5 ans (+/- 65%) des greffés pulmonaires reste insuffisant. La dysfonction primaire du greffon (DPG), se développant dans les 72h après la transplantation, est la première cause de décès en période péri-opératoire. Elle est aussi associée à des risques accrus d'infection, de syndrome de détresse respiratoire, et à plus long terme, de rejet chronique et de taux de survie réduits à 5 et 10 ans. Il a été établi que la lésion d'ischémie/reperfusion (I/R) du greffon représente un risque majeur de la DPG. À ce jour, il n'existe aucun traitement pharmacologique efficace pour la DPG. Il est donc crucial d'identifier de nouvelles cibles thérapeutiques.

Nous avons donc proposé l'hypothèse que la dysfonction de l'épithélium alvéolaire joue un rôle majeur dans la pathophysiologie de l'I/R et de la DPG suite à la transplantation pulmonaire chez les patients souffrant de FK.

Les objectifs spécifiques du projet, alliant une composante cellulaire, un volet *in vivo* (EVLP chez le cochon) et translationnel chez les patients transplantés sont de : 1) démontrer l'importance de marqueurs d'intégrité, de dommages et de fonctionnalité de l'épithélium alvéolaire, associés à l'I/R *in vitro* et *in vivo*; 2) valider ces marqueurs prédictifs dans le développement et la sévérité de la DPG chez les transplantés pulmonaires.

En utilisant un protocole mimant l'ischémie/reperfusion (I/R), à partir de culture primaire de cellules épithéliales alvéolaires de rats, nous avons une diminution de l'expression d'un canal sodique (ENaC) jouant un rôle primordial dans la fonction de clairance liquidienne alvéolaire, une baisse de l'expression d'une protéine impliquée dans les jonctions serrées (ZO-1) et de la résistance transépithéliale (RTE), indiquant une altération de l'intégrité alvéolaire. De plus, la capacité de réparation suite aux lésions est affectée.

Grâce à un modèle porcin avec inflammation induite au LPS nous avons pu observer un dommage tissulaire, un œdème pulmonaire, une réaction inflammatoire exacerbée ainsi qu'une baisse de l'expression d'ENaC, qui ne peut être reversée par la procédure de perfusion *ex-vivo* du poumon (EVLP).

Parmi les 53 patients FK transplantés pulmonaires que nous avons recrutés, 22 ont développés par la suite une DPG de grade 2 ou 3 dans les 72h suivant la transplantation pulmonaire. Nos résultats préliminaires, à partir de prélèvements faits lors de ces transplantations, indiquent une réponse inflammatoire, un dommage alvéolaire et une baisse de l'expression d'ENaC et ZO-1 déjà présents dans les greffons des donneurs, parmi les patients greffés ayant par la suite développés une DPG,+ comparé aux receveurs sans développement de la DPG.

Les résultats de nos études *in vitro* et *in vivo* ainsi que du volet translationnel chez l'humain, indiquent une altération de l'épithélium alvéolaire en lien avec l'I/R et la DPG. Compte tenu du dommage précoce observé dans le greffon donneur, notre but est maintenant d'identifier les caractéristiques phénotypiques du donneur (cause de décès, âge, sexe, intervention pré-collecte, *etc*.) associées au développement de la DPG chez les receveurs. Ces données seront cruciales pour développer de nouvelles stratégies thérapeutiques visant à améliorer la survie des transplantés pulmonaires.

14h15 – Flash Presentation – Présentation éclair

Les canaux potassiques : nouvelles stratégies thérapeutiques dans la réparation de l'épithélium des voies aériennes fibrose kystique en présence d'exoproduits infectieux de *S. aureus*

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Introduction: Chez les patients atteints de fibrose kystique (FK), la présence d'infection/inflammation chroniques entraîne une atteinte pulmonaire progressive, qui est la cause principale de morbidité et mortalité. Bien que la prévalence des pathogènes respiratoires soit variable d'un patient à l'autre et en fonction de son âge, la bactérie *Staphylococcus aureus* (*SA*) prédomine chez les jeunes patients, tandis que les infections à *Pseudomonas aeruginosa* (*PA*) deviennent plus fréquentes chez les patients adultes. Nous avons précédemment démontré que l'infection, par des exoproduits sécrétés par *PA*, altère la réparation de l'épithélium des voies aériennes FK ainsi que l'efficacité des correcteurs de CFTR. Notre but est de développer des stratégies efficaces favorisant la réparation de l'épithélium des voies aériennes FK en ciblant les canaux potassiques K^+(K_vLQT1 et K_{ATP}) et le canal CFTR (Symdeco®)en présence d'exoproduits de *SA*.

Méthodes: Nous avons collecté les cellules épithéliales des voies aériennes humaines FK (hAEC) à partir des poumons provenant de patients FK transplantés homozygotes F508del/F508del, mutation la plus fréquente. La vitesse de réparation a été évaluée en réalisant des tests de blessure à partir de cultures primaires d'hAEC. Les cellules ont été soumises à des traitements pharmacologiques : un activateur de K_vLQT1 (ML-277, 4 µM) et/ou de K_{ATP} (pinacidil. 50 µM); et/ou des modulateurs de CFTR utilisés en clinique (Symdeko® : combinaison du correcteur Tezacaftor VX-661, 4 µM et du potentiateur Ivacaftor VX-770, 100 nM) en présence ou en absence d'exoproduits bactériens de *SA*.

Résultats: Nous avons tout d'abord mis en évidence que les canaux potassiques K_vLQT1 et K_{ATP}, étaient exprimés dans les hAEC FK et que leurs expressions n'étaient pas affectées suite à une exposition d'exoproduits bactériens de *SA*. Ensuite, nous avons pu également confirmer l'effet délétère de l'infection par *SA* sur la réparation épithéliale FK. Nous avons pu noter un faible effet stimulateur du Symdeko sur la réparation épithéliale dans des conditions infectieuses. Toutefois, la vitesse de réparation est nettement améliorée par les activateurs ML-277 et pinacidil. De façon intéressante, un effet synergique est observé avec des co-traitements aux activateurs des canaux K^+ (ML-277 et pinacidil) et modulateurs de CFTR (VX-661 + VX-770), malgré la présence d'exoproduits de SA.

Conclusion: Nos résultats démontrent que des traitements combinés avec des modulateurs des canaux CFTR et K^+ pourraient ainsi être une stratégie efficace pour favoriser la réparation de l'épithélium respiratoire FK et ce malgré la présence d'infection bactérienne. Cette stratégie pourrait être non seulement testée sur des cellules issues de patients FK homozygotes pour la mutation F508del mais également sur d'autres classes de mutations.

Financements: Fonds de Recherche du Québec en Santé (FRQS), Instituts de recherche en santé du Canada (IRSC) et le Réseau en Santé Respiratoire (RSR).

14h20 – Flash Presentation – Présentation éclair

***Pseudomonas aeruginosa* evasion of neutrophil antibacterial functions in early cystic fibrosis lung infection**

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Pseudomonas aeruginosa (PA) is the predominant pathogen which causes chronic lung infection in patients with cystic fibrosis (CF). Failure to clear PA by the host innate immune responses leads to persistent PA infections in the CF airways, which has been associated with lung function decline and worse clinical outcomes. Although inhaled tobramycin treatment has demonstrated efficacy in improving eradication of PA in CF children with new onset infections, 40% patients still failed inhaled tobramycin eradication therapy. The adequate recruitment and antibacterial functions of neutrophils (PMNs) upon initial PA infection is likely a key step required for successful PA eradication even when antibiotics are used. Interestingly, microbiological analysis from clinical trials of PA eradication therapy suggested that certain PA bacterial phenotypes (loss of flagellar and pilus-mediated motilities, mucoidy or wrinkly colony morphology) are associated with PA eradication failure, but results vary between studies and the mechanism remains to be determined. Thus, we hypothesize that PA isolates that persist after inhaled tobramycin in CF patients with initial PA infection elicit impaired PMN mediated antibacterial functions. To examine this, we tested a collection of PA strains isolated from initial PA infection from CF children followed at the Sick Kids as part of an "Early PA eradication" study. We compared *in vitro* phagocytosis and intracellular bacterial killing by PMN-like cells (differentiated HL-60) in response to persistent clinical PA isolates (N = 10 persistent patients with 18 isolates) vs eradicated clinical PA isolates (N = 32 eradicated patients with 53 isolates). So far, we observed a significantly lower PMN phagocytosis ($p < 0.01$) and intracellular bacterial killing ($p < 0.05$) of persistent PA compared to eradicated PA. To identify which bacterial phenotypes have impacts on PMN antibacterial functions, we compared various bacterial phenotypes (Type IV pilus mediated twitching and flagellum mediated swimming motility, overproduction of alginate or Psl exopolysaccharides, biofilm production, pyocyanin and protease secretion) using univariate and multivariable regression. We found that PMN phagocytosis is significantly associated with twitching motility ($r = 0.26$, $p = < 0.01$) and mucoidy ($r = -0.28$, < 0.01). Furthermore, our preliminary data in a subset of PA isolates, persistent PA (n = 7) produce a remarkably higher levels of Psl ($p < 0.01$) compared to eradicated PA (n = 7). In order to determine whether Psl produced by CF clinical PA isolates plays a role in PMN phagocytosis, we compared the effect of an anti-Psl monoclonal antibody (mAb) Psl0096 in *in vitro* PMN phagocytosis of persistent and eradicated PA with high and low Psl production respectively. We observed that PMN phagocytosis of PA isolates was significantly increased in a Psl dependent manner by the treatment of Psl0096 compared to its isotype control. Our results to date therefore suggest that Psl may be a determinant which significantly contributes to impaired PMNs phagocytosis in addition to mucoidy and lack of twitching, and may be associated with PA eradication failure in CF patients. We will extend our preliminary findings by first validating our *in vitro* results using PA clinical isolates from another independent patient cohort. We will further test the relevance of our *in vitro* findings in a murine pulmonary infection model for *in vivo* PMN phagocytosis and bacterial clearance in response to persistent and eradicated PA isolates.

14h25 – Oral Presentation – Communication Orale

Predicting and tuning the dynamics of microbial evolution to delay drug resistance

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The rise of antimicrobial resistance (AMR) has compromised our ability to manage chronic diseases, such as cystic fibrosis. To circumvent AMR, we need to discover new antibiotics. However, this will not be enough. We also need to conserve the efficacy and maximize the duration of usefulness of both current and future antibiotics. Antibiotic resistance will always arise, but if we are able to predict how they evolve, we can delay their emergence. At the fundament level, the evolution of AMR, like any other biological system, is governed by processes at several scales of biological organization—molecular, cellular, organismal, and population level. Although the immediate effects of mutations are on the properties of proteins or fitness of individual cells, the eventual evolutionary success of these mutations is affected by population dynamics.

In this talk, I describe our efforts to develop a quantitative and predictive model of the emergence of AMR by bridging these multiple scales in bacterial evolution. First, we determined the comprehensive "mutational landscape" of gene targets that are crucial target of antimicrobials. We find that the overall survival of mutations that eventually become clinical isolates is strongly determined by the fitness of the bacteria in the presence of the drug (the "resistance level") and by the fitness of the bacteria without the drug (the "fitness cost"). Using simulation and population genetics theory, we then predict the survival probability of these mutations under different selective regimes defined by drug concentration and population structure of the bacteria. These predictions are validated by lab evolution and data from clinical isolates. Altogether, by driving the bacterial populations into a region of the landscape where they are unable to grow, we can delay drug resistance.

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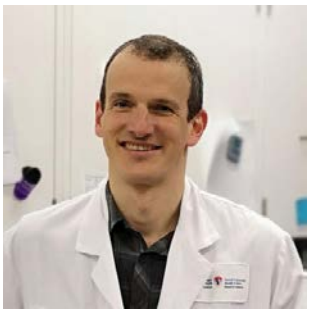
14h50 – Oral Presentation – Communication Orale



The potential roles of the Aryl-Hydrocarbon Receptor in the host defense response of airway epithelial cells exposed to *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa are gram-negative bacteria that frequently infect the lungs of cystic fibrosis (CF) patients. This bacterium is highly responsive to changes in its environment, resulting in the expression of a diverse array of bacterial genes that may contribute to host-pathogen interactions. *P. aeruginosa* is well-known to induce neutrophilic inflammation via the activation of Toll-Like Receptors (TLRs). Recently, it was shown that pyocyanin, a phenazine produced by *P. aeruginosa*, binds to the aryl hydrocarbon receptor (AhR), leading to neutrophilic inflammation. AhR is a ligand-dependent transcription factor involved in the regulation of innate immunity, which also has non-genomic functions. Understanding the role of the AhR in the *P. aeruginosa*-induced epithelial infection and its subsequent immune response could help better understand the complexity of CF lung disease. In this presentation, the differential contribution of AhR and TLRs to neutrophilic inflammation will be presented as well as a potential role of AhR in the *P. aeruginosa* "detoxifying" response.

15h45 – Flash Presentation – Présentation éclair

Understanding how pathoadaption of *Pseudomonas aeruginosa* in cystic fibrosis alters host-microbe interactions in the airway epithelium

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Chronic *Pseudomonas aeruginosa* (PA) infections occur in 60-80% of all adult Cystic Fibrosis (CF) patients and are associated with an accelerated lung function decline and increased mortality. Due to host and bacterial factors that allow PA to rapidly adapt to the host environment and cause impaired bacterial clearance, PA is nearly impossible to eradicate once it has established a chronic infection. PA infections cause progressive lung disease by inducing an exuberant and non-resolving lung inflammation. While PA is traditionally considered an extracellular pathogen, our lab has recently demonstrated the presence of intracellular PA within airway epithelial cells (AECs) of CF lung explant tissues, indicating the possible presence of an intracellular reservoir *in vivo*. In this project, we investigate how chronic PA pathoadaptations modulate host-microbe interactions in the CF airway, including intracellular survival in airway epithelial cells, a novel mechanism of PA persistence. We hypothesize that genetic adaptations commonly observed in PA isolates from chronic infections promote increased lung inflammation and an intracellular PA lifestyle which promote bacterial persistence.

Our first aim is to analyze the impact of LasR (major quorum sensing transcriptional activator in PA) loss of function on ICAM-1, an important pro-inflammatory mediator expressed by AEC in response to PA extracellular diffusible bacterial products and intracellular bacteria. Preliminary results show that expression of membrane-bound ICAM-1 in AEC is upregulated in response to *lasR* mutant filtrates compared to filtrates from wild-type PA and that this upregulation enhances neutrophil binding to AECs *in vitro*, suggesting that it might promote neutrophilic lung inflammation in the CF airway.

Our second aim is to examine whether LasR and Type 3 secretion system (T3SS) loss of function, two common pathoadaptations, modulate internalization of PA into AECs as well as its intracellular persistence *in vitro*. So far, we have observed that *lasR* mutation promotes internalization into CFBE Δ*F508* cells, an immortalized cell line homozygous for the most common CFTR mutation observed in CF patients, as well as primary CF AECs. We have further found that loss of T3SS injectisome function enhances bacterial persistence in AECs for up to 120h, and this may be unrelated to T3SS-induced cytotoxicity or secreted effectors.

In conclusion, our results suggest that loss of LasR might increase lung inflammation and thus contribute to CF lung function decline. Furthermore, loss of LasR and T3SS function, two phenotypic adaptations commonly observed in PA strains from chronic CF infections, promote an intracellular PA lifestyle, potentially allowing bacteria to avoid clearance and persist. Consequently, further examination of the involved mechanisms might provide insight into how common pathoadaptations contribute to disease progression and PA persistence.

Future experiments will further explore how the T3SS modulates PA intracellular persistence and whether our ICAM-1 *in vitro* data translates to increased membrane-bound ICAM-1 induced by *lasR* mutants compared to wild-type PA *in vivo*.

15h50 – Flash Presentation – Présentation éclair

The opposing effect of cigarette smoke on CFTR activity is dependent on the channel's phosphorylation state

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Defective CFTR function is a major risk factor in CF, COPD, and other lung diseases. Chronic cigarette smoke (CS) exposure, a leading cause of COPD, inhibits CFTR activity at transcriptional, biochemical, and functional levels. The organic components of CS, the condensate (CSC), however, transiently activate the resting CFTR channel in respiratory epithelia. The comparable phospho-occupancy of ten PKA consensus sites, after CSC and forskolin exposure, determined by affinity-enriched tandem mass spectrometry, suggest that PKA stimulation causes the channel activation. This is accomplished by subcompartmentalised elevation of cAMP concentration due to inhibition of the MRP4, a cAMP export pump, which is a constituent of the CFTR macromolecular signalling complex at the cell surface. In sharp contrast, CSC reversibly inhibits the phosphorylated CFTR channel activity *in vivo* and in phospholipid bilayers, without altering its phospho-occupancy and cell surface expression. Put together, we posit that CS may also elicit a dual acute effect on CFTR in the airways; an acute activation of the resting channel that enhances the protective mucociliary clearance efficiency, and a subsequent channel inactivation that contributes to the COPD lung pathology.

15h55 – Flash Presentation – Présentation éclair

Role of the proteasome in the biosynthetic arrest of SLC26A9 by F508del-CFTR

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Introduction: Currently available drugs that correct F508del-CFTR misfolding provide only modest clinical benefit for most CF patients, therefore alternative anion channels and other potentially druggable targets are being explored. SLC26A9 is a constitutively active anion channel expressed in human airways that modifies the severity of CF airway disease in patients with the G551D-CFTR mutation. The functional expression of complex glycosylated SLC26A9 is reduced in cells that express F508del-CFTR mediated in part by a PDZ- and CAL-dependent mechanism. SLC26A transporters also interact with the regulatory (R) domain of WT-CFTR through its Sulfate Transporter AntiSigma factor antagonist (STAS) domain, however the role of this interaction in SLC26A9 biosynthetic arrest is uncertain. Indeed, it is not known if the interaction with immature F508del-CFTR in the endoplasmic reticulum (ER) contributes to the retention and proteasomal degradation of SLC26A9.

Aim: to understand the interaction between SLC26A9 and CFTR and its impact on SLC26A9 expression.

Methods: BHK cells overexpressing wild-type (WT) or F508del-CFTR and parental BHK cells lacking CFTR were transiently transfected with SLC26A9 cDNA. SLC26A9 protein was quantified in lysates by immunoblotting and at the plasma membrane by cell surface biotinylation. SLC26A9 levels were assessed in well-differentiated CF and non-CF primary human bronchial epithelial cells (pHBEs) by immunofluorescence staining and confocal microscopy.

Results: Total and plasma membrane SLC26A9 expression were both lower in BHK cells when co-expressed with F508del-CFTR than when expressed alone or with WT-CFTR. Similar results were obtained when well-differentiated CF and non-CF primary human bronchial epithelial cells (pHBEs) were studied by confocal imaging. Since F508del-CFTR misfolding leads to its retention in the ER and subsequent proteasomal degradation, we examined the effects of proteasome inhibitors on SLC26A9 degradation. Inhibiting the proteasome increased SLC26A9 immunofluorescence in F508del-CFTR homozygous pHBE cells but not in non-CF pHBEs, suggesting there is enhanced proteasomal degradation of SLC26A9 with F508del-CFTR in pHBEs. This difference in proteasome inhibitor sensitivity was not observed in non-epithelial cells overexpressing SLC26A9 with F508del-CFTR or WT-CFTR. Apical SLC26A9 levels increased in pHBEs and in non-epithelial cells when F508del-CFTR was partially corrected using low temperature or VX-809, and this rescue was mimicked by co-transfecting cells with WT-CFTR. The rate of SLC26A9 degradation was measured when expressed alone or with WT-CFTR or F508del-CFTR. In the presence of the protein synthesis inhibitor cycloheximide, degradation of immature SLC26A9 was enhanced in F508del-expressing cells suggesting there is CFTR-dependent, proteasome-mediated degradation of SLC26A9 at the ER.

Conclusions: These results suggest that ER retention of F508del-CFTR and SLC26A9 leads to premature degradation of both proteins and SLC26A9 biosynthetic arrest is due in part to an interaction between them. Disrupting this interaction will make SLC26A9 an exciting therapeutic target for most CF patients.

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16h00 – Oral Presentation – Communication Orale

Divergent functions and selective interactions of the Hsp70 chaperone system with CFTR

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Chaperones assist the folding and quality control degradation of the CFTR channel, both its wild-type form and the misfolded ΔF508 cystic fibrosis mutant. The Hsc70/Hsp70 chaperone promotes folding, but also ubiquitination by the E3 ligase CHIP, leading to ER-associated degradation, or internalization and lysosomal degradation from the plasma membrane. We addressed the overall role of Hsc70/Hsp70 and co-chaperones DNAJA1 and DNAJA2 in cells. Unexpectedly, the net effect of Hsp70 was to suppress CFTR trafficking. While DNAJA1 supported biosynthetic folding, DNAJA2 specifically enhanced ER-associated degradation through Hsp70 and CHIP. Excess Hsp70 also promoted CFTR degradation, but this occurred through the lysosomal pathway and required CHIP but not Hsp90 chaperone complex formation. Notably, the Hsp70 inhibitor MKT077 enhanced levels of mature CFTR and ΔF508-CFTR, by slowing turnover and allowing delayed maturation, respectively. MKT077 also boosted the channel activity of ΔF508-CFTR when combined with the corrector compound VX809. To further understand the divergent effects of chaperones, we screened a synthetic peptide library to identify binding sites within CFTR for Hsc70, DNAJA1 and DNAJA2. While many sites were shared between Hsc70 and DNAJA2, DNAJA1 binding was more restrictive. Moreover, the sites mapped to regions critical for the folding of CFTR. These results suggest how the chaperones act in coordination on these structurally labile regions for different outcomes. Modulation of the chaperone system may offer ways to relieve the misfolding phenotype.

16h25 – Oral Presentation – Communication Orale

Behavior of the CF disease modifier: SLC26A9

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The diversity in CF pathology makes the SLC26A family of anion transporters and ion channels attractive candidates to investigate as interacting partners with CFTR due to their diverse anion transport functions, tissue distributions and reciprocal regulation. Genome-wide association studies (GWAS) have implicated SLC26A9, originally identified in the lung, as a modifier of several CF-associated pathologies, including meconium ileus (1), CF-related diabetes (2), and prenatal exocrine pancreatic damage (3). Recently, GWAS-identified a non-coding, single nucleotide polymorphism in SLC26A9 (A9) as a modulator of the airway response to potentiator VX-770 in CF patients with the gating mutation G551D CFTR (4).

We found previously that primary, human bronchial epithelia (HBE) from non-CF donors exhibit constitutive anion secretion attributable to A9 (5); however, this secretory process was absent in HBE from CF donors. Therefore, we asked whether changes in A9 constitutive activity could be attributed to a loss of CFTR trafficking, and what role PDZ interactions played. HEK293 cells co-expressing A9 with the trafficking mutant F508del CFTR exhibited a significant reduction in constitutive current compared to cells co-expressing A9 with either WT or G551D CFTR, and the expression of A9 at the plasma membrane was reduced. A9 interacted with NHERF-1 and CAL, and its interaction with both PDZ proteins significantly increased with co-expression of WT CFTR. However, expression with F508del CFTR only increased A9's interaction with CAL, and core-glycosylated F508del CFTR co-immunoprecipitated endogenous CAL. Mutation of A9's PDZ motif restored its constitutive activity when co-expressed with F508del; also, correcting F508del CFTR trafficking in CF HBE with VX-809 restored A9 activity. Thus, CFTR can modify A9's interactions with PDZ-domain proteins in different cellular compartments along the protein maturation pathway (6). A9 has the potential to restore anion secretion to CF airway epithelia as it is farther along the maturation pathway than F508del CFTR, thus having therapeutic implications.

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