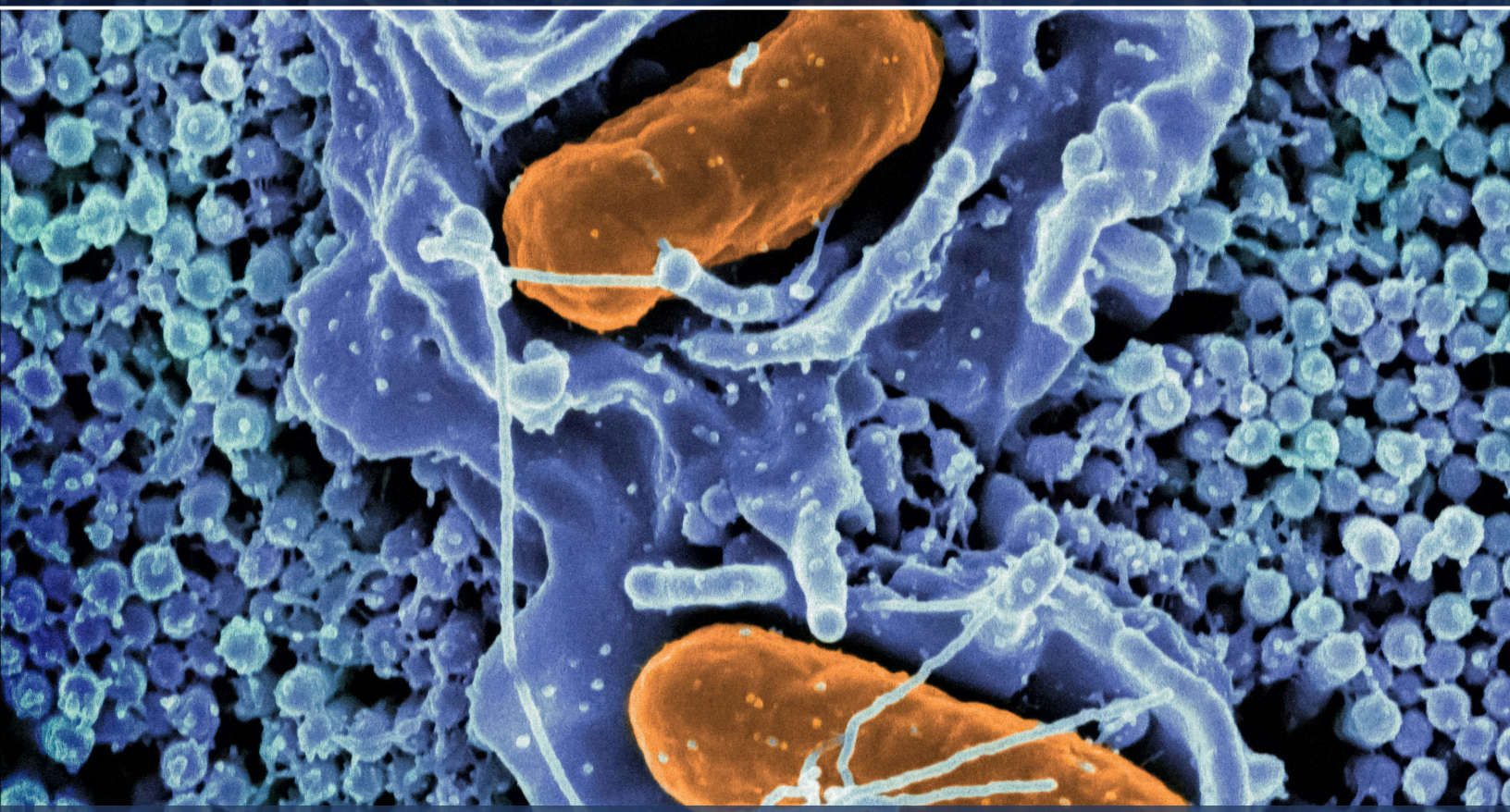
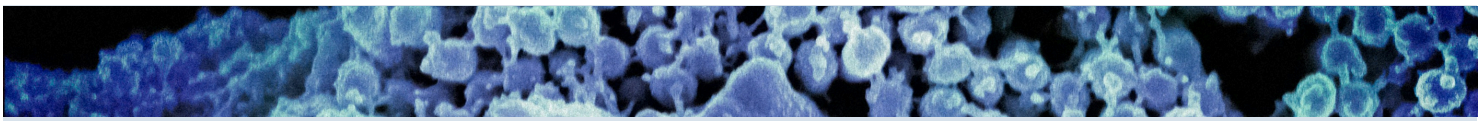
VOL. 5 | NO. 2 | 2026

OSURJ

UNIVERSITY OF OTTAWA SCIENCE UNDERGRADUATE RESEARCH JOURNAL
JOURNAL D'ETUDIANT DE RECHERCHE SCIENTIFIQUE DE L'UNIVERSITE D'OTTAWA





JRSUO
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À PROPOS DE NOUS

Le Journal d'étudiant de recherche scientifique de l'Université d'Ottawa est une revue bilingue, évaluée par les pairs et en libre accès, consacrée à la promotion de la recherche scientifique au premier cycle universitaire. Publiée et dirigée par des étudiants de l'Université d'Ottawa, la revue présente des travaux de recherche originaux, des articles de synthèse et des perspectives couvrant les sciences naturelles, de la santé et quantitatives. Le JÉRSUO conjugue un processus rigoureux d'évaluation par les pairs à une approche de publication académique axée sur le mentorat afin d'offrir une plateforme accessible aux chercheurs émergents du Canada et d'ailleurs.

ÉNONCÉ DE LA MISSION

Le JRSUO cherche à enrichir l'expérience scientifique au premier cycle universitaire en offrant aux étudiants une plateforme leur permettant de rédiger, d'évaluer et de publier des travaux de recherche académique. En impliquant les étudiants dans toutes les étapes du processus de publication scientifique, le JRSUO favorise la rigueur intellectuelle, l'esprit critique et une communication scientifique efficace. Par cette initiative, la revue vise à cultiver un engagement durable envers la recherche, la collaboration et la découverte au sein de la communauté scientifique.

SUR LA COUVERTURE

Couverture adaptée de *Salmonella Typhimurium envahissant une cellule épithéliale humaine – Micrographie électronique à balayage* par le NIAID, sous licence CC-BY-2.0 ; couleurs modifiées.

ABOUT US

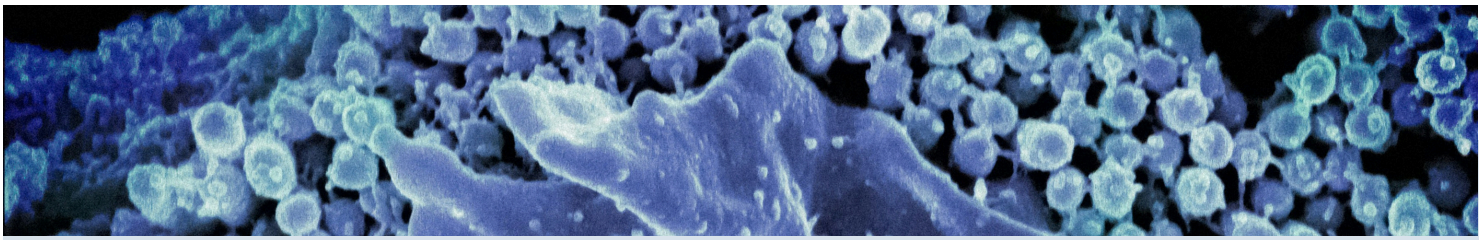
The University of Ottawa Science Undergraduate Research Journal is a bilingual, peer-reviewed, open-access journal dedicated to advancing undergraduate scientific scholarship. Published and managed by students at the University of Ottawa, the journal features original research, reviews, and perspectives spanning the natural, health, and quantitative sciences. OSURJ combines rigorous peer review with mentorship-driven academic publishing to provide an accessible platform for emerging researchers across Canada and beyond.

MISSION STATEMENT

OSURJ seeks to enrich the undergraduate science experience by providing students with a platform to write, review, and publish scholarly research. By engaging students in all stages of the scientific publishing process, OSURJ fosters rigorous inquiry, critical thinking, and effective scientific communication. Through this initiative, the journal aims to cultivate a lasting commitment to research, collaboration, and discovery within the scientific community.

ON THE COVER

Cover adapted from *Salmonella Typhimurium invading a human epithelial cell - Scanning electron micrograph* by NIAID, licensed under CC-BY-2.0; Colours modified.



JRSUO OSURJ

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UNIVERSITY OF OTTAWA SCIENCE UNDERGRADUATE RESEARCH JOURNAL

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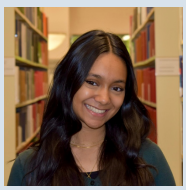

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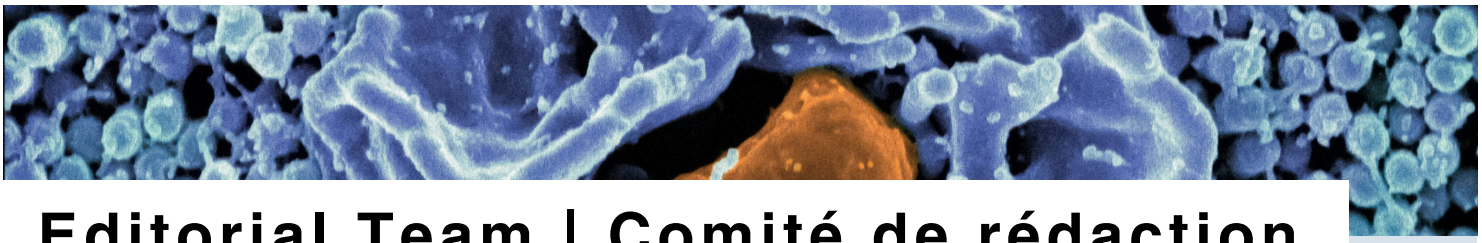

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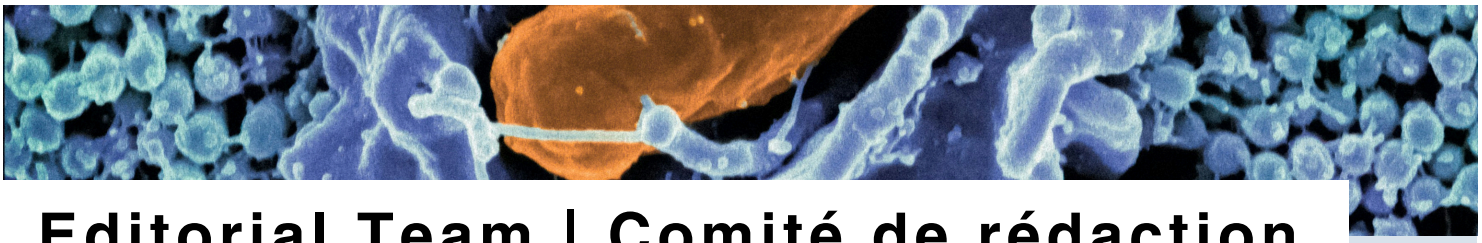

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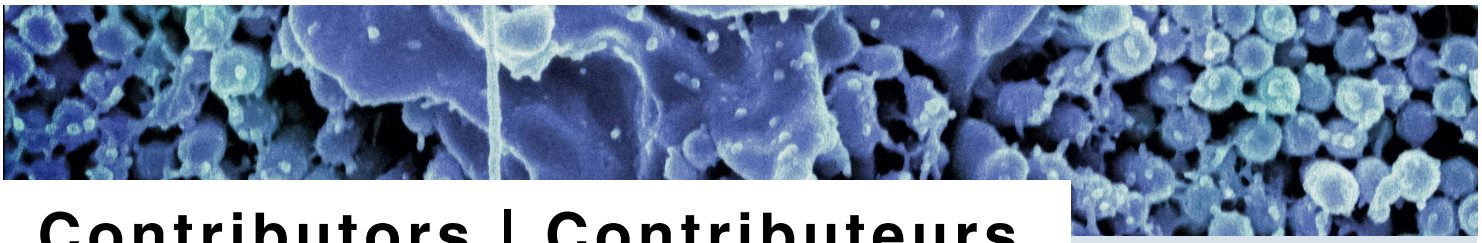

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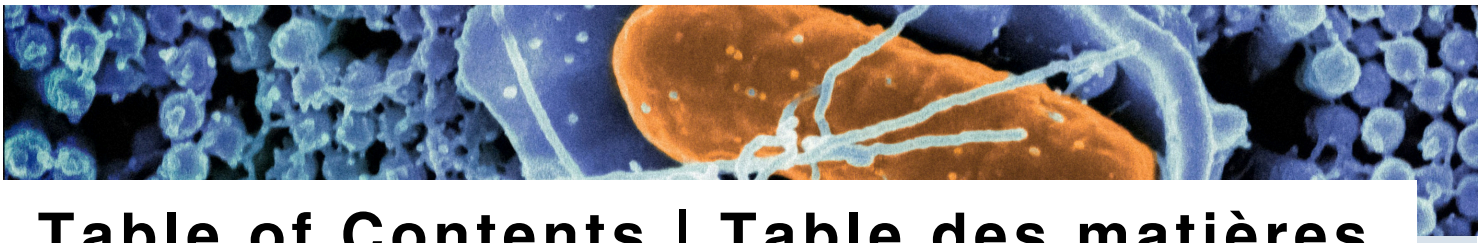

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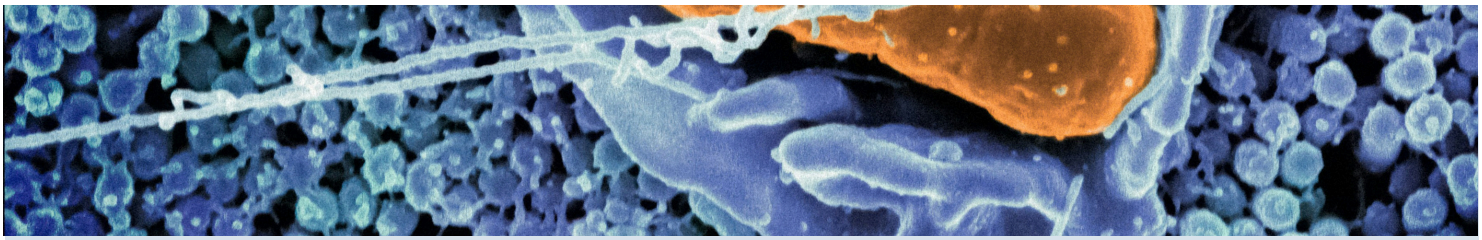
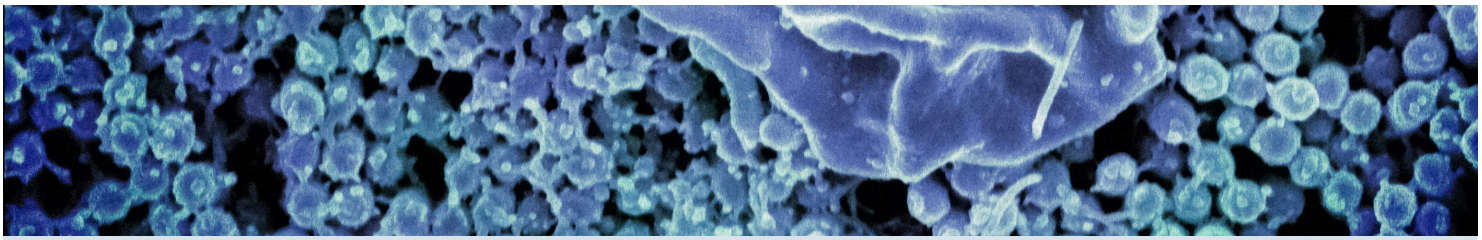


OSIC Research Proposal Abstracts | Résumés des propositions de recherche de l'OSIC



Research Proposals | Propositions de recherche

Foreword

Dear Reader,

Research is driven by an instinct for discovery and exploration. Scientific knowledge advances through continuous scrutiny, as ideas are refined and reshaped in light of new evidence. Opportunities such as the Ottawa Science Innovation Challenge and the University of Ottawa's Undergraduate Science Research Opportunity provide a space where this curiosity begins to take form. Questions about the way the world works are developed into structured inquiry and the first steps toward knowledge creation are made.

This issue of the University of Ottawa Science Undergraduate Research Journal highlights a collection of research abstracts representing both high school research proposals and undergraduate work. The abstracts collected here span disciplines, methodologies, and perspectives, but share a common thread: a commitment to rigorous inquiry and intellectual growth. Each article represents persistence, creativity, and critical thinking held to high standards that define scholarship at the University of Ottawa and beyond.

As a student-run journal, OSURJ exists because of a collective effort. We are deeply grateful to our authors for trusting us with their work, to our reviewers and editors for their care and precision, and to our faculty mentors who continue to support undergraduate research.

Over the past year, OSURJ has evolved into a steadily growing platform for undergraduate scholarship. We have seen a marked increase in submissions, broader disciplinary representation, and an expansion of our editorial team, reflecting a growing confidence in student-led academic publishing. Alongside this expansion, we formalized a double-blind peer-review process, refined our editorial workflows, and strengthened our review team to ensure that growth is matched by rigour. These changes mark an important transition for the journal: from a developing initiative to a more structured and sustainable venue for undergraduate research.

Growth does not simply mean expansion; it demands refinement. As we look ahead, our focus is not only on continuing to expand the reach of OSURJ but on deepening quality and accessibility. We aim to further strengthen our review standards, broaden disciplinary representation, and build a culture in which undergraduate researchers feel supported in bringing their work into the public academic sphere. With a stronger editorial foundation in place, OSURJ is well positioned to continue evolving as a collaborative space for thoughtful, student-driven scholarship.

We hope this volume not only showcases the breadth of undergraduate research conducted at the University of Ottawa and the bright ideas of future scientists but also encourages more students to see their questions as worthy of investigation and publication. Inquiry begins with curiosity, and journals like OSURJ exist to give that curiosity a home. Cover article selections were conducted through a blinded editorial review process. Editorial board members were recused from decisions involving their own submissions.

On behalf of the editorial team, we are proud to present to you Volume V issue ii and thank you for reading and for supporting undergraduate research.

Ishaan Goswami & Sivany Kathir
Co-Editors-in-Chief
University of Ottawa Science Undergraduate Research Journal

Avant-propos

Chère lectrice, cher lecteur,

La recherche est guidée par le désir de découvrir, de comprendre et d'explorer. Les connaissances scientifiques progressent grâce à une remise en question continue : les idées sont constamment précisées, améliorées et transformées à mesure que de nouvelles preuves apparaissent. Des occasions comme le Défi d'innovation scientifique d'Ottawa et le Programme d'occasions de recherche de premier cycle en sciences de l'Université d'Ottawa offrent un espace où cette curiosité peut commencer à prendre forme. Les questions sur le fonctionnement du monde deviennent alors des démarches de recherche structurées, et les premiers pas vers la création de nouvelles connaissances peuvent être entrepris.

Ce numéro du Journal de recherche de premier cycle en sciences de l'Université d'Ottawa met en lumière une collection de résumés de recherche provenant à la fois de propositions d'élèves du secondaire et de travaux de premier cycle. Les résumés présentés ici touchent à plusieurs disciplines, méthodes et perspectives, mais ils ont tous un point commun : ils montrent un engagement envers une recherche rigoureuse et un développement intellectuel. Chaque travail témoigne de la persévérance, de la créativité et de l'esprit critique qui, lorsqu'ils sont maintenus à des normes élevées, définissent la recherche à l'Université d'Ottawa et ailleurs.

En tant que revue dirigée par des étudiants, le JRSUO existe grâce à un effort collectif. Nous sommes profondément reconnaissants envers les auteurs, qui nous ont confié leurs travaux, envers les réviseurs et les éditeurs, qui ont fait preuve de soin et de précision, ainsi qu'envers les mentors professoraux, qui continuent de soutenir la recherche de premier cycle.

Au cours de la dernière année, le JRSUO est devenu une plateforme de plus en plus importante pour la recherche de premier cycle. Nous avons observé une augmentation du nombre de soumissions, une plus grande diversité de disciplines représentées et un élargissement de notre équipe éditoriale. Ces changements montrent une confiance grandissante envers la publication académique dirigée par des étudiants. En parallèle, nous avons officialisé un processus d'évaluation par les pairs en double aveugle, amélioré nos méthodes de travail éditoriales et renforcé notre équipe de révision afin que cette croissance s'accompagne aussi d'un haut niveau de rigueur. Ces progrès marquent une étape importante pour la revue, qui passe d'une initiative en développement à un espace plus structuré et durable pour la recherche de premier cycle.

La croissance ne signifie pas seulement s'agrandir; elle demande aussi de s'améliorer. Pour l'avenir, notre objectif n'est pas seulement d'élargir la portée du JRSUO, mais aussi d'en renforcer la qualité et l'accessibilité. Nous souhaitons continuer à améliorer nos normes de révision, représenter un plus grand nombre de disciplines et créer un environnement où les chercheurs de premier cycle se sentent soutenus lorsqu'ils souhaitent partager leurs travaux dans le milieu académique. Avec une base éditoriale plus solide, le JRSUO est bien placé pour continuer à évoluer comme un espace collaboratif consacré à une recherche réfléchie et menée par des étudiants
.
Nous espérons que ce volume mettra en valeur la diversité des recherches de premier cycle réalisées à l'Université d'Ottawa ainsi que les idées prometteuses de futurs scientifiques. Nous espérons aussi qu'il encouragera davantage d'étudiants à considérer leurs questions comme dignes d'être étudiées et publiées. Toute recherche commence par la curiosité, et des revues comme le JRSUO existent pour offrir un espace à cette curiosité. Les articles sélectionnés pour la couverture ont été choisis dans le cadre d'un processus d'évaluation éditoriale à l'aveugle. Les membres du comité éditorial se sont retirés des décisions concernant leurs propres soumissions.

Au nom de toute l'équipe éditoriale, nous sommes fiers de vous présenter le volume V, numéro ii. Nous vous remercions de votre lecture et de votre soutien à la recherche de premier cycle.

Ishaan Goswami et Sivany Kathir
Co-rédacteurs en chef
Journal d'étudiant de recherche scientifique de l'Université d'Ottawa

Click Chemistry and T3SS Inhibition: Mechanistic Insights into Targeted Anti-Infective Strategies

Chimie clic et inhibition du T3SS : perspectives mécanistiques sur les stratégies anti-infectieuses ciblées

Nhu Nguyen[1*], Christopher Boddy[1]

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Abstract | Résumé

Antimicrobial resistance is a leading cause of death worldwide and only expected to worsen in the coming years. New antimicrobial drugs with new mechanisms of action are desperately needed to address this problem. The Type III Secretion System (T3SS) is a promising new drug target due to its critical role in several bacterial infections including chlamydia, diarrheal disease, plague, and lung infections in cystic fibrosis. The T3SS is a megadalton protein complex in the shape of a syringe that secretes pathogen proteins into the host cell, facilitating the invasion and colonization of the host. Small molecules with T3SS-disrupting activity would block infection without killing the pathogen, making them a promising new class of anti-virulence drugs. A recent high-throughput cell-based screen has identified one of the first compounds known to strongly inhibit T3SS activity. The inhibition is indirect with C5 treatment inducing a 3 to 57 fold downregulation of expression of genes encoding the T3SS. However, the mechanism of downregulation has remained elusive. Click chemistry is a synthetic strategy that relies on a class of highly efficient, selective, and reliable reactions to join smaller molecular building blocks together. A Noble Prize-winning example was the copper-catalyzed aside-alkyne cycloaddition in Chemistry in 2022. Click or bio-orthogonal approaches synchronizes with the Type 3 Secretion System (T3SS) as it reduces the T3SS effector since they allow site-specific, minimal, and non-disruptive tagging of proteins with small chemical handles, such as azide, alkyne, strained alkyne and alkene. After incorporation of the handle, a second step called “click ligation” occurs, therefore, this two-step approach preserves the native function better than direct fusion of bulky tags. Instead of killing bacteria, small molecules can be designed to inhibit T3SS function, blocking the pathogen’s ability to evade the immune system. By designing a narrow spectrum of drugs, this can disarm pathogens with lower resistance pressure.

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La résistance antibiotique est une cause principale de la mort mondialement et est prévue à s'aggraver dans les années à venir. De nouveaux médicaments avec des nouveaux mécanismes d'action sont nécessaires pour addresser ce problème. Le système de sécrétion de type III (SST3) est une nouvelle cible médicamenteuse prometteuse en raison de son rôle essentiel dans plusieurs infections bactériennes, notamment la chlamydia, les maladies diarrhéiques, la peste et les infections pulmonaires associées à la fibrose kystique. Le SST3 est un complexe protéique de l’ordre du mégadalton, en forme de seringue, qui sécrète des protéines pathogènes dans la cellule hôte, facilitant l’invasion et la colonisation de l’hôte. Des petites molécules capables de perturber le SST3 bloqueraient l’infection sans tuer le pathogène, ce qui en fait une nouvelle classe prometteuse de médicaments anti-virulence. Un récent criblage à haut débit basé sur des cellules a identifié l’un des premiers composés connus pour inhiber fortement l’activité du T3SS. Cette inhibition est indirecte, le traitement par C5 induisant une diminution de 3 à 57 fois de l’expression des gènes codant pour le SST3. Toutefois, le mécanisme de cette diminution d’expression reste inconnu. La chimie click est une stratégie de synthèse reposant sur une classe de réactions hautement efficaces, sélectives et fiables permettant d’assembler de plus petites briques moléculaires. Un exemple récompensé par le prix Nobel en chimie en 2022 est la cycloaddition azoture-alcyne catalysée par le cuivre. Les approches de type click ou bio-orthogonales s’alignent avec le système de sécrétion de type III (SST3) en permettant un marquage spécifique, minimal et non perturbateur des protéines à l’aide de petites fonctions chimiques, telles que l’azoture, l’alcyne, l’alcyne contraint et l’alcène. Après l’incorporation de ce groupe fonctionnel, une seconde étape appelée « ligation click » a lieu, alors, cette approche en deux étapes préserve donc mieux la fonction native que la fusion directe de marqueurs volumineux. Plutôt que de tuer les bactéries, des petites molécules peuvent être conçues pour inhiber la fonction du SST3, bloquant ainsi la capacité du pathogène à échapper au système immunitaire. En concevant des médicaments à spectre étroit, il est possible de désactiver les pathogènes tout en exerçant une pression de résistance plus faible.

Developmental Expression of dlx5a and chm in Zebrafish

Expression développementale de dlx5a et chm chez le poisson-zèbre

Maïka Harvey[1*], Ben Harrison[1], Kathleen M. Gilmour[1], Mark Ekker[1]

1. University of Ottawa, Ottawa, ON, Canada
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Abstract | Résumé

Zebrafish (Danio rerio) larvae are a powerful model for visualizing early vertebrate gene expression due to their external development and the conservation of different gene sequences with human counterparts, allowing them to be useful tools for cross-species comparison. The dlx5a gene encodes a homeodomain transcription factor expressed in craniofacial and neural crest-derived regions, whereas chm encodes Rab escort protein 1 (REP-1), which is broadly required for intracellular trafficking and retinal development. We aimed to characterize the baseline developmental expression of dlx5a and chm in wild-type zebrafish embryos at 2 and 3 days post-fertilization (dpf). Whole-mount in situ hybridization was performed on paraformaldehyde-fixed, methanol-stored embryos using digoxigenin-labelled antisense probes for dlx5a or chm, respectively. At 2 dpf, dlx5a staining was detected in distinct domains of the anterior head and along the body axis, consistent with craniofacial and neural crest-related patterning regions. By 3 dpf, the dlx5a signal appeared stronger and more extensive in these domains, reflecting continued development of dlx5a-positive structures. In contrast, chm staining showed a developmental shift in localization. At 2 dpf, expression was predominantly restricted to the head region. By 3 dpf, the chm signal became more apparent in the trunk, indicating an expansion of transcript distribution along the body axis, consistent with its ubiquitous and essential expression pattern in zebrafish, mice and humans. Together, these qualitative expression maps establish a developmental baseline for future studies examining how mutations in chm or dlx5a, or environmental perturbations alter gene localization in relevant zebrafish models.

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Les larves de poisson-zèbre (Danio rerio) constituent un modèle puissant pour visualiser l'expression génique précoce chez les vertébrés en raison de leur développement externe et de la conservation de différentes séquences géniques avec leurs homologues humains, ce qui leur permet d'être des outils utiles pour les comparaisons entre espèces. Le gène dlx5a code un facteur de transcription à homéodomaine exprimé dans les régions craniofaciales et dérivées de la crête neurale, tandis que chm code la protéine d'escorte Rab 1 (REP-1), qui est largement requise pour le trafic intracellulaire et le développement rétinien. Nous avons cherché à caractériser l'expression développementale de base de dlx5a et de chm dans des embryons de poisson-zèbre de type sauvage à 2 et 3 jours post-fécondation (jpf). Une hybridation in situ sur embryon entier a été réalisée sur des embryons fixés au paraformaldéhyde et conservés dans le méthanol à l'aide de sondes antisens marquées à la digoxigénine pour dlx5a ou chm, respectivement. À 2 jpf, le marquage de dlx5a a été détecté dans des domaines distincts de la tête antérieure et le long de l'axe du corps, ce qui est cohérent avec les régions de patterning craniofaciales et liées à la crête neurale. À 3 jpf, le signal de dlx5a semblait plus fort et plus étendu dans ces domaines, reflétant le développement continu des structures positives pour dlx5a. En revanche, le marquage de chm montrait un changement développemental de localisation. À 2 jpf, l'expression était principalement restreinte à la région de la tête. À 3 jpf, le signal de chm devenait plus apparent dans le tronc, indiquant une expansion de la distribution du transcrit le long de l'axe du corps, ce qui est cohérent avec son patron d'expression ubiquitaire et essentiel chez le poisson-zèbre, la souris et l'humain. Ensemble, ces cartes qualitatives d'expression établissent une base développementale pour de futures études examinant comment des mutations dans chm ou dans dlx5a, ou des perturbations environnementales, modifient la localisation génique dans des modèles pertinents de poisson-zèbre.

Does personality influence hypoxia tolerance in zebrafish, Danio rerio?

La personnalité influence-t-elle la tolérance à l'hypoxie chez le poisson-zèbre, Danio rerio?

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Abstract | Résumé

Laboratory-reared zebrafish (Danio rerio) show substantial individual variation in hypoxia tolerance, measured as time to loss of equilibrium (LOE) under low oxygen conditions. In our lab population, some individuals lose equilibrium within minutes, while others tolerate hypoxia for extended periods of time. While physiological traits such as hemoglobin-oxygen binding affinity and gill surface area are known to influence hypoxia tolerance, behavioural differences may also help explain this variation. This project investigated whether personality traits, such as boldness, were associated with hypoxia tolerance in zebrafish. Personality was assessed via two behavioural assays: the novel tank diving test and the emergence test, whichwere repeated one week apart to determine repeatability. Boldness was quantified using latency to reach the top (novel tank test) and latency to emerge (emergence test), where longer latencies indicate a more risk-averse (less bold) personality. Behavioural responses during hypoxia exposure were also measured, including activity levels and aquatic surface respiration (ASR). ASR is a behaviour in which fish ventilate at the air-water interface, where oxygen availability is higher. Use of this behaviour may reflect variation in boldness during low oxygen conditions because it entails going to an exposed area and possibly taking on risk/danger. Behavioural metrics were compared with time-to-LOE to determine whether consistent behavioural profiles correlated with hypoxia tolerance. This project attempts to gain a better understanding of how personality may influence an individual's response to hypoxia by taking into account behavioural and physiological approaches.

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Les poissons-zèbres élevés en laboratoire (Danio rerio) présentent une variation individuelle substantielle de la tolérance à l'hypoxie, mesurée par le temps de survie en conditions de faible teneur en oxygène. Dans notre population de laboratoire, certains individus perdent leur équilibre en quelques minutes, tandis que d'autres tolèrent l'hypoxie pendant de longues périodes. Bien que des traits physiologiques tels que l'affinité hémoglobine-oxygène et la surface branchiale soient connus pour influencer la tolérance à l'hypoxie, des différences comportementales peuvent également contribuer à expliquer cette variation. Ce projet a examiné si des traits de personnalité, tels que l'audace, étaient associés à la tolérance à l'hypoxie chez le poisson-zèbre. La personnalité a été évaluée par deux tests comportementaux : le nouveau test de plongée en bassin et le test d'émergence, qui ont été répétés à une semaine d'intervalle pour déterminer leur répétabilité. L'audace a été quantifiée à l'aide de la latence pour atteindre le sommet (test du nouveau tank) et de la latence pour émerger (test d'émergence), où des latences plus longues indiquent une personnalité plus averse au risque (moins audacieuse). Les réponses comportementales lors de l'exposition à l'hypoxie ont également été mesurées, notamment les niveaux d'activité et la respiration de surface aquatique (RSA). La RSA est un comportement par lequel les poissons ventilent à l'interface air-eau, où la concentration en oxygène est plus élevée. L'utilisation de ce comportement peut refléter une variation de l'audace en cas de faible oxygène, car cela implique de se rendre dans une zone exposée et de prendre un risque ou de se mettre en danger. Les indicateurs comportementaux ont été comparés au temps jusqu'à la perte d'équilibre afin de déterminer si des profils comportementaux cohérents corréleraient avec la tolérance à l'hypoxie. Ce projet vise à mieux comprendre comment la personnalité peut influencer la réponse d'un individu à l'hypoxie, en tenant compte des approches comportementales et physiologiques.

Effect of Laboratory Adaptation on Male Harm in Drosophila melanogaster

Effet de l'adaptation en laboratoire sur les dommages masculins chez Drosophila melanogaster

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Abstract | Résumé

Sexual conflict arises when males and females have differing reproductive interests, often resulting in traits that enhance male reproductive success while reducing female fitness. In Drosophila, male harm has been widely documented, in which traits that increase male reproductive success can also reduce female fitness. This study investigates whether laboratory adaptation influences the expression of male harm by comparing lab adapted and recent descendants of wild collected Drosophila melanogaster. A 2x2x2 factorial experimental design was used, manipulating male exposure (low vs. high), male type (lab-adapted vs. wild), and female type (lab-adapted vs. wild). Virgin females were exposed to males for several days under controlled conditions and then isolated to lay eggs in groups. Female fitness was quantified by counting the number of eclosing offspring. Male harm was calculated as the relative reduction in female reproductive output under high compared to low male exposure. Results indicate that male harm was significant overall, with increased male exposure reducing female fitness. Harm was greater when females were exposed to lab males than to wild males (H_lab = 0.54, H_wild = 0.34), supported by a significant interaction between male exposure and male type (F = 9.12, df = 1, 149, P = 0.003). These findings suggest that laboratory adapted males are more harmful than wild males, highlighting the importance of ecological context in studies of sexual conflict. These results are consistent with previous work showing that structurally complex environments reduce the expression and evolution of male harm compared to simplified laboratory environments (see Yun et al., 2017; Osijo et al., 2026). Together, these results suggest that standard laboratory conditions may overestimate the strength of male harm relative to natural populations.

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Les conflits sexuels surviennent lorsque les mâles et les femelles ont des intérêts reproductifs différents, ce qui aboutit souvent à des traits qui améliorent le succès reproducteur des mâles tout en réduisant la condition physique des femelles. Dans Drosophila, un dommage masculin a été largement documenté, dans lequel des traits augmentant le succès reproducteur masculin peuvent également réduire la condition physique féminine. Cette étude étudie si l'adaptation laboratoire influence l'expression des blessures masculines en comparant les descendants adaptés en laboratoire et récents de Drosophila melanogaster collectés à l'état sauvage. Un plan expérimental factoriel 2x2x2 a été utilisé, manipulant l'exposition masculine (faible vs. élevée), le type mâle (adapté en laboratoire vs. sauvage) et le type femelle (adapté en laboratoire vs. sauvage). Les femelles vierges étaient exposées aux mâles pendant plusieurs jours dans des conditions contrôlées, puis isolées pour pondre des œufs en groupes. La condition physique des femelles a été quantifiée en comptant le nombre de petits en eclosion. Le préjudice masculin a été calculé comme la réduction relative de la production reproductive féminine sous une exposition élevée par rapport à une faible exposition masculine. Les résultats indiquent que les blessures masculines étaient globalement significatives, l'exposition accrue réduisant la condition physique féminine. Le préjudice était plus important lorsque les femelles étaient exposées à des mâles de laboratoire qu'à des mâles sauvages (H_lab = 0,54, H_wild = 0,34), soutenu par une interaction significative entre l'exposition des mâles et le type de mâle (F = 9,12, df = 1 149, P = 0,003). Ces résultats suggèrent que les mâles adaptés en laboratoire sont plus dangereux que les mâles sauvages, soulignant l'importance du contexte écologique dans les études sur les conflits sexuels. Ces résultats sont conformément aux travaux antérieurs montrant que des environnements structurellement complexes réduisent l'expression et l'évolution des dommages masculins par rapport aux environnements de laboratoire simplifiés (voir Yun et al., 2017 ; Osijo et al., 2026). Ensemble, ces résultats suggèrent que les conditions de laboratoire standard peuvent surestimer la force des blessures masculines par rapport aux populations naturelles.

A Protease-Based System to Release Protein from the Surface of *Escherichia coli*

Un système à base de protéase pour libérer les protéines de la surface d'Escherichia coli

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Abstract | Résumé

We are developing a novel secretion system that uses two autotransporter constructs, which self-transport their passenger domain cargo across the outer membrane, to transport and cleave a protein of interest (POI). The tobacco etch virus protease (TEVp) is transported to the surface by the E. coli autotransporter protein YfaL. Here, it cleaves a POI from a second autotransporter construct, facilitating its secretion. Currently, premature cytosolic cleavage prevents efficient secretion. In previous work, the araBAD promoter system and theophylline riboswitch were added to allow the POI to reach the cell surface before induction of the TEVp construct. While this strategy yielded a tightly inducible system, it did not prevent premature cleavage. Therefore, we sought additional strategies to reduce cytosolic cleavage.

In this project, we implemented solutions to this challenge by controlling cytosolic expression, stability, and activity. First, the TEVp construct was expressed in a lower copy plasmid, pUdO4s. From this, we first added a C-terminus proteolysis tag to reduce the half-life of the protein. In E. coli, this tag is added to truncated proteins by the ssrA transcript, facilitating recognition and degradation by intracellular proteases. This reduces cytosolic stability and concentration, favouring surface-bound TEVp.

We expressed these constructs in E. coli and measured relative protein expression and presence of cleavage products in comparison to previous work. As expected, we observed a significant reduction in the expression of membrane-bound TEVp in the new constructs. While premature cleavage still occurred in the original construct, presence of cleavage products was minimal in the current strain, NEBExpress Iq, and little to none was present pre- or post-induction in either novel construct. This warrants further investigation into factors influencing the formerly observed premature cleavage, as well as the apparent lack of adequate cell surface-level protease activity.

Nous développons un nouveau système de sécrétion utilisant deux constructs d'autotransporteurs, qui transportent elles-mêmes leur domaine passager transporté à travers la membrane externe, afin de transporter et de cliver une protéine d'intérêt (PI). La protéase du virus de la gravure du tabac (TEVp) est transportée à la surface par la protéine autotransporteuse YfaL d'Escherichia coli. À cet endroit, elle clive une PI provenant d'une deuxième construction d'autotransporteur, facilitant ainsi sa sécrétion. Actuellement, un clivage cytosolique prématuré empêche une sécrétion efficace. Dans des travaux précédents, le système de promoteur araBAD et le riborégulateur à théophylline ont été ajoutés afin de permettre à la PI d'atteindre la surface cellulaire avant l'induction de la construction TEVp . Bien que cette stratégie ait permis d'obtenir un système à induction étroitement contrôlée, elle n'a pas empêché le clivage prématuré. Nous avons donc recherché des stratégies supplémentaires pour réduire le clivage cytosolique.

Dans ce projet, nous avons mis en œuvre des solutions à ce problème en contrôlant l'expression, la stabilité et l'activité cytosoliques. Premièrement, la construction TEVp a été exprimée dans un plasmide à faible nombre de copies, pUdO4s. À partir de celui-ci, nous avons d'abord ajouté une étiquette de dégradation en C-terminus afin de réduire la demi-vie de la protéine. Chez Escherichia coli, cette étiquette est ajoutée aux protéines tronquées par le transcrit ssrA, facilitant leur reconnaissance et leur dégradation par les protéases intracellulaires. Cela réduit la stabilité et la concentration cytosoliques, favorisant la TEVp liée à la surface.

Nous avons exprimé ces constructions chez Escherichia coli et mesuré l'expression protéique relative ainsi que la présence de produits de clivage en comparaison avec les travaux précédents. Comme prévu, nous avons observé une réduction significative de l'expression de la TEVp liée à la membrane dans les nouvelles constructions. Bien qu'un clivage prématuré se produisait encore dans la construction originale, la présence de produits de clivage était minimale dans la souche actuelle, NEBExpress Iq, et peu ou aucun produit n'était présent avant ou après l'induction dans les deux nouvelles constructions. Cela justifie des recherches supplémentaires sur les facteurs influençant le clivage prématuré précédemment observé, ainsi que sur le manque apparent d'une activité protéasique suffisante au niveau de la surface cellulaire.

Rising to the top: Surfacing Behaviour Reflects Swimbladder Filling in Zebrafish

En haut : Le comportement de la surface reflète le remplissage de la vessie natatoire chez le poisson-zèbre

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Abstract | Résumé

In zebrafish, the swim bladder is filled by swallowing air at the water's surface. Adjusting the volume of the swim bladder allows the fish to achieve neutral buoyancy at a given depth. Zebrafish lacking functional expression of the serotonin (5-HT) reuptake transporter Sertb (sertb-knockout, sertb-KO; slc6a4b) exhibit "surfacing behaviour", spending essentially all of their time at the top of the water column. In the current study, we aimed to determine whether surfacing behaviour in sertb-KO fish is associated with swim bladder filling and buoyancy. We measured buoyancy by placing anesthetized sertb-KO zebrafish larvae at 7 days post-fertilization (dpf) in a water column to assess the rate and direction of movement, and calculated swim bladder volume from photographs. The sertb-KO larvae displayed higher buoyancy than wildtype (WT) larvae, and higher buoyancy was associated with larger swim bladder volume. Treatment of WT larvae with fluoxetine, a selective inhibitor of Sert, was also associated with higher buoyancy and swim bladder volume. Conversely, treatment of sertb-KO larvae with the serotonin synthesis inhibitor p-chlorophenylalanine (pCPA) resulted in decreased buoyancy and smaller swim bladder volume. Further, WT larvae treated with an agonist of the 5-HT1A receptor displayed higher buoyancy and swim bladder volume. These results implicate elevated swim bladder volume in surfacing behaviour, and confirm a link between elevated serotonin levels and swim bladder volume, likely mediated by the 5-HT1A receptor. A key remaining question is whether surfacing behaviour is caused by serotonin-mediated swim bladder over-filling, or whether sertb-KO larvae choose to be at the surface and over-fill the swim bladder to help them remain there.

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Chez le poisson-zèbre, la vessie natatoire se remplit en avalant de l'air à la surface de l'eau. Ajuster le volume de la vessie natatoire permet au poisson d'atteindre une flottabilité neutre à une profondeur donnée. Les poissons-zèbres dépourvus d'expression fonctionnelle du transporteur de recapture de la sérotonine (5-HT) Sertb (sertb-knockout, sertb-KO ; slc6a4b) présentent un « comportement de remontée », passant pratiquement tout son temps au sommet de la colonne d'eau. Dans la présente étude, nous avons cherché à déterminer si le comportement de remontée à la surface chez les poissons sertb-KO est associé à un remplissage de la vessie natatoire et à la flottabilité. Nous avons mesuré la flottabilité en plaçant des larves de poisson-zèbre Sertb-KO anesthésiées à 7 jours après la fécondation (DPF) dans une colonne d'eau pour évaluer la vitesse et la direction des mouvements, et a calculé le volume de la vessie natatoire à partir de photographies. Les larves sertb-KO présentaient une flottabilité plus élevée que les larves de type sauvage (WT), et une flottabilité plus élevée était associée à un volume de vessie natatoire plus important. Le traitement des larves de WT avec la fluoxétine, un inhibiteur sélectif du Sert, était également associé à une flottabilité et un volume de vessie natatoire plus élevés. Inversement, le traitement des larves de sertb-KO avec l'inhibiteur de la synthèse de la sérotonine p-chlorophénylalanine (pCPA) a entraîné une diminution de la flottabilité et un volume de vessie natatoire plus réduit. De plus, les larves WT traitées avec un agoniste du récepteur 5-HT1A ont montré une flottabilité et un volume de vessie natatoire plus élevés. Ces résultats impliquent une natation élevée en volume de la vessie dans le comportement de sortie et confirment un lien entre des niveaux élevés de sérotonine et le volume de la vessie natatoire, probablement médiés par le récepteur 5-HT1A. Une question clé restante est si le comportement de remontée à la surface est causé par un surremplissage de la vessie natatoire médiée par la sérotonine, ou si les larves de sertb-KO choisissent d'être à la surface et de remplir trop la vessie natatoire pour y rester.

Synthesis of α-Methylated Amino Acids for Buchwald-Hartwig Cross-Coupling

Synthèse d'acides aminés α-méthylés pour le couplage croisé de Buchwald-Hartwig

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Abstract | Résumé

α-Methylated amino acids are valuable components in peptide drugs due to their ability to protect peptides from enzymatic degradation. The objective of this work was to demonstrate that an α methylated amino acid, bearing an alkyl amine side chain, could undergo cross-coupling with an aryl halide via Buchwald-Hartwig amination. Progress has been made toward the synthesis of these amino acids from N-benzyloxycarbonyl-D-alanine.

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Les acides aminés α-méthylés sont des composantes précieuses pour les médicaments peptidiques dû à leur habileté à protéger les peptides de la dégradation enzymatique. L'objectif de ce travail était de démontrer qu'un acide aminé α-méthylé portant une chaîne latérale alkylamine pourrait subir un couplage croisé avec une halogénure d'aryle via l'amination de Buchwald-Hartwig. Des progrès ont été réalisés en vue de la synthèse de ces acides aminés à partir de la N-benzyloxycarbonyl-D-alanine.

Targeting PolyP Accumulation in E. coli Using PPK Inhibitors to Disrupt Antibiotic Resistance Mechanisms

Cibler l'accumulation de polyp chez E. coli à l'aide d'inhibiteurs de PPK pour perturber les mécanismes de résistance aux antibiotiques

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Abstract | Résumé

Polyphosphates (polyP) are linear chains of inorganic phosphate (Pi) that are highly conserved among various organisms. In Escherichia coli, PolyP is synthesized primarily by polyphosphate kinase 1 (PPK1, encoded by ppk) in response to environmental stressors. PolyP contributes to bacterial survival by regulating biofilm formation, stress responses, and antibiotic resistance. The mechanisms controlling PolyP accumulation and its effects on bacterial physiology and resistance remain incompletely understood. Notably, PPK-mediated PolyP accumulation modulates lipopolysaccharide (LPS) structure, contributing to polymyxin resistance during starvation stress. Compared to the wild-type (WT) strain, Δppk mutants exhibit significant growth defects when transferred to MOPS minimal medium, whereas WT cells show minimal growth defects under this nutrient-starved condition. This differential phenotype serves as a useful readout for evaluating PPK inhibitors. Deletion of the low-affinity phosphate transporter PitA rescues the growth defect of Δppk mutants; this interaction is still under investigation. Different repurposed drugs act as PPK inhibitors, including mesalamine (widely used to treat inflammatory bowel diseases such as Crohn's disease and ulcerative colitis) and gallein (originally a histological dye and Gβγ inhibitor). Both compounds inhibit bacterial PPK enzymes. In this study, we evaluated the effects of mesalamine and gallein on the growth phenotypes of four E. coli strains (WT, Δppk, ΔpitA, and the double mutant ΔppkΔpitA) under nutrient-starved conditions (MOPS minimal medium). Both inhibitors produced only mild, non-specific growth impairment across strains, and the result was not improved by higher concentrations of drug use. Gallein treatment showed no clear effect on PolyP levels, and polyP levels following mesalamine treatment has not been evaluated. These preliminary results highlighted the need for optimized drug delivery or alternative inhibition strategies to fully recapitulate the phenotypes of genetic PolyP deficiency.

Les polyphosphates (polyP) sont des chaînes linéaires de phosphate inorganique (Pi) hautement conservées chez divers organismes. Chez Escherichia coli, le PolyP est synthétisé principalement par la polyphosphate kinase 1 (PPK1, codée par ppk) en réponse aux facteurs de stress environnementaux. Le PolyP contribue à la survie bactérienne en régulant la formation de biofilms, les réponses au stress et la résistance aux antibiotiques. Les mécanismes contrôlant l'accumulation de polyP et leurs effets sur la physiologie et la résistance bactériennes restent incomplètement compris. Notamment, l'accumulation de polyP médiée par PPK module la structure des lipopolysaccharides (LPS), contribuant à la résistance à la polymyxine pendant le stress de famine. Comparées à la souche de type sauvage (WT), les mutants Δppk présentent des défauts de croissance significatifs lorsqu'ils sont transférés dans un milieu minimal MOPS, tandis que les cellules WT présentent des défauts de croissance minimes dans cette condition carente en nutriments. Ce phénotype différentiel sert de lecture utile pour évaluer les inhibiteurs PPK. La délétion du transporteur de phosphate à faible affinité PitA sauve le défaut de croissance des mutants Δppk ; Cette interaction fait toujours l'objet d'une enquête. Différents médicaments réutilisés agissent comme inhibiteurs de la PPK, notamment la mésalamine (largement utilisée pour traiter les maladies inflammatoires de l'intestin telles que la maladie de Crohn et la colite ulcéreuse) et la galléine (à l'origine un colorant histologique et un inhibiteur de Gβγ). Les deux composés inhibent les enzymes PPK bactériennes. Dans cette étude, nous avons évalué les effets de la mésalazine et de la gallée sur les phénotypes de croissance de quatre souches d'E. coli (WT, Δppk, ΔpitA, et le double mutant ΔppkΔpitA) dans des conditions de manque de nutriments (milieu minimal MOPS). Les deux inhibiteurs n'ont produit qu'une légère altération de la croissance non spécifique entre souches, et cet effet n'a pas été amélioré par des concentrations plus élevées de médicaments. Le traitement par galléine n'a montré aucun effet clair sur les niveaux de polyP, et les niveaux de polyP après un traitement à la mésalaline n'ont pas été évalués. Ces résultats préliminaires ont mis en lumière la nécessité d'optimiser la délivrance de médicaments ou de stratégies alternatives d'inhibition pour reproduire pleinement les phénotypes d'une déficience génétique en polyP.

Targeting Cancer Stem Cell Survival in Triple-Negative Breast Cancer Through Bacterial Effector-Mediated NF-κB Inhibition

Cibler la survie des cellules souches cancéreuses dans le cancer du sein triple négatif grâce à l'inhibition de NF-κB par des effecteurs bactériens

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Abstract | Résumé

Triple-Negative Breast Cancer (TNBC) is an aggressive breast cancer subtype characterized by the absence of estrogen, progesterone, and HER2 receptors, resulting in limited responsiveness to standard therapies. Chemotherapy is the primary treatment and induces initial tumour regression; however, TNBC frequently recurs due to the persistence of Cancer Stem Cells (CSCs), a subpopulation capable of self-renewal, tumour regeneration, and therapeutic resistance. A central driver of CSC survival is Nuclear Factor Kappa B (NF-κB), a transcriptional regulator that promotes inflammation, anti-apoptotic signalling, and resistance to cytotoxic stress. In TNBC, NF-κB signalling is chronically active, reinforcing CSC-mediated chemoresistance and disease relapse. Current treatment strategies do not effectively eliminate CSCs or directly suppress NF-κB signalling, highlighting a critical therapeutic gap. This study aims to evaluate whether a panel of bacterial effector proteins can be repurposed as targeted molecular tools to inhibit NF-κB signalling in TNBC. These effectors were selected based on their ability to disrupt NF-κB activation. TNBC models were engineered to enable controlled expression of these effectors across a variety of cell lines. NF-κB activity was assessed using reverse transcription quantitative polymerase chain reaction (RT-qPCR) and flow cytometry, while flow cytometry was also used to evaluate apoptosis and CSC marker expression. This work provides mechanistic insight into targeting CSC-driven resistance pathways and informs the development of more precise therapeutic strategies for aggressive TNBC.

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Le cancer du sein triple négatif est une forme agressive de cancer du sein. Il se caractérise par l'absence des récepteurs aux œstrogènes, à la progestérone et à HER2, ce qui limite l'efficacité de plusieurs traitements habituellement utilisés contre le cancer du sein. Dans ce contexte, la chimiothérapie demeure le traitement principal. Elle peut d'abord réduire la taille de la tumeur, mais le cancer triple négatif revient souvent après le traitement. Cette récidive est en grande partie liée à la présence de cellules souches cancéreuses. Ces cellules représentent une petite population de cellules tumorales capables de se renouveler, de reformer une tumeur et de résister aux traitements. Un facteur important qui favorise leur survie est NF-κB, une protéine régulatrice qui contrôle l'expression de plusieurs gènes liés à l'inflammation, à la survie cellulaire et à la résistance au stress causé par les traitements. Dans le cancer du sein triple négatif, la voie NF-κB est souvent activée de façon constante, ce qui renforce la résistance des cellules souches cancéreuses à la chimiothérapie et augmente le risque de rechute. Les traitements actuels ne permettent pas d'éliminer efficacement ces cellules souches cancéreuses ni de bloquer directement la voie NF-κB. Cela représente donc une limite importante dans la prise en charge de ce type de cancer. Cette étude vise à déterminer si certaines protéines effectrices bactériennes peuvent être réutilisées comme outils moléculaires ciblés pour inhiber la signalisation NF-κB dans le cancer du sein triple négatif. Ces protéines ont été choisies parce qu'elles sont capables de perturber l'activation de NF-κB. Pour tester cette approche, des modèles de cancer du sein triple négatif ont été modifiés afin de permettre l'expression contrôlée de ces protéines effectrices dans différentes lignées cellulaires. L'activité de NF-κB a ensuite été évaluée par RT-qPCR et par cytométrie en flux. La cytométrie en flux a aussi été utilisée pour mesurer l'apoptose, c'est-à-dire la mort cellulaire programmée, ainsi que l'expression de marqueurs associés aux cellules souches cancéreuses. Dans l'ensemble, ce travail permet de mieux comprendre comment cibler les mécanismes qui rendent les cellules souches cancéreuses résistantes aux traitements. Il pourrait aussi contribuer au développement de stratégies thérapeutiques plus précises pour traiter les formes agressives du cancer du sein triple négatif.

Testing of Novel Prodrugs in Nitroreductase Chemogenetic Ablation of Dopaminergic Neurons for Parkinson's Disease Modelling

Évaluation de nouvelles prodrogues dans l'ablation chimiogénétique des neurones dopaminergiques par nitroreductase pour modéliser la maladie de Parkinson

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Abstract | Résumé

Parkinson's disease is a progressive neurodegenerative disorder highlighted by the loss of midbrain dopaminergic neurons, resulting in tremors, stiffness, slowness of movement (bradykinesia) as well as non-motor symptoms. Affecting over six million people globally, current treatments are largely symptomatic and do not halt disease progression. Developing a reliable, scalable model of dopaminergic neuron ablation is therefore critical for therapeutic discovery. With its close neurofunctional and behavioural similarities to humans, the zebrafish (Danio rerio) is an excellent model for neurodegenerative, neurodevelopmental, and neuropharmacological studies. Chemogenetic ablation is preferred because it enables highly specific, temporally controlled, and minimally toxic ablation of dopaminergic neurons, allowing accurate modelling of neurodegeneration and facilitating studies of neuronal regeneration, which are not possible with purely chemical or genetic approaches. This study utilizes a chemogenetic ablation method based on the bacterial nitroreductase (NTR) expressed in dopaminergic neurons of zebrafish larvae, specifically the Tg(dat:CFP-NTR) zebrafish line. This allows for dopaminergic neuron ablation assessment across different brain regions. In this system, NTR converts otherwise non-toxic prodrugs, metronidazole and ronidazole into cytotoxic compounds, enabling spatial and temporally controlled ablation. Dopaminergic neuron survival was quantified in the olfactory bulb, telencephalon, and diencephalon using tyrosine hydroxylase and cyan fluorescent protein , the latter expressed under regulatory elements of the dopamine transporter gene. Behavioural assays assessed locomotor function at one and two days post-treatment. No significant decreases in dopaminergic neuron counts or behavioural deficits were detected relative to vehicle controls. This outcome likely arose from several limitations, including poor prodrug solubility and bioavailability, the limited catalytic efficiency of first-generation NTR relative to NTR 2.0, and potential rapid neuronal regeneration in larval organisms.

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La maladie de Parkinson est une maladie neurodégénérative progressive, principalement caractérisée par la perte de neurones dopaminergiques dans le mésencéphale. Cette perte entraîne des symptômes moteurs comme les tremblements, la rigidité musculaire et la lenteur des mouvements, aussi appelée bradykinésie, ainsi que plusieurs symptômes non moteurs. La maladie touche plus de six millions de personnes dans le monde, mais les traitements actuels servent surtout à soulager les symptômes. Ils ne permettent pas encore d'arrêter la progression de la maladie. Pour cette raison, il est important de développer des modèles fiables et faciles à reproduire afin d'étudier la perte des neurones dopaminergiques et de tester de nouvelles approches thérapeutiques. Le poisson-zèbre, ou Danio rerio, est un modèle très utile dans ce contexte, car il présente plusieurs similarités neurofonctionnelles et comportementales avec l'humain. Il est aussi largement utilisé dans les études sur les maladies neurodégénératives, le développement du système nerveux et les effets des médicaments sur le cerveau. Dans cette étude, l'ablation chimiogénétique a été utilisée comme approche pour éliminer de façon contrôlée les neurones dopaminergiques. Cette méthode est intéressante, car elle permet de cibler précisément certains neurones, au moment voulu, tout en limitant la toxicité générale. Elle permet donc de mieux modéliser la neurodégénérescence et d'étudier la régénération neuronale, ce qui est plus difficile avec des approches uniquement chimiques ou génétiques. L'étude repose sur un système d'ablation chimiogénétique utilisant la nitroreductase bactérienne, ou NTR, exprimée dans les neurones dopaminergiques de larves de poisson-zèbre. Plus précisément, la lignée Tg(dat:CFP-NTR) a été utilisée. Dans ce modèle, la NTR transforme des prodrogues normalement peu toxiques, comme le métronidazole et le ronidazole, en composés toxiques capables de provoquer la mort des cellules ciblées. Cela permet une ablation des neurones dopaminergiques contrôlée dans l'espace et dans le temps. La survie des neurones dopaminergiques a été mesurée dans différentes régions du cerveau, notamment le bulbe olfactif, le télencéphale et le diencéphale. Pour cela, deux marqueurs ont été utilisés : la tyrosine hydroxylase, un marqueur des neurones dopaminergiques, et la protéine fluorescente cyan, exprimée sous le contrôle d'éléments régulateurs du gène du transporteur de la dopamine. Des tests comportementaux ont aussi été réalisés afin d'évaluer la locomotion des larves un et deux jours après le traitement. Les résultats n'ont pas montré de diminution significative du nombre de neurones dopaminergiques ni de déficit comportemental par rapport aux groupes témoins. Ce résultat pourrait s'expliquer par plusieurs limites, notamment la faible solubilité et la faible biodisponibilité des prodrogues utilisées, l'efficacité catalytique limitée de la première génération de NTR par rapport à NTR 2.0, ainsi que la possibilité d'une régénération neuronale rapide chez les larves de poisson-zèbre.

Treatment of Triple Negative Breast Cancer Using RNA-based Nanotherapeutics

Traitement du cancer du sein triple négatif à l'aide de nanothérapies à base d'ARN

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Abstract | Résumé

Triple-negative breast cancer (TNBC) is an aggressive and therapeutically challenging subtype of breast cancer, accounting for a disproportionate number of breast cancer–related deaths. The absence of ER, PR and HER2 receptors limits targeted treatment options, leaving chemotherapy as the primary therapeutic strategy. However, chemotherapy is often associated with significant toxicity, rapid development of drug resistance, and tumour recurrence due to the enrichment of cancer stem cells (CSCs). These CSCs exist in interconvertible mesenchymal-like and epithelial like states, which lead to the excessive growth of one type of CSC and disease relapse when targeting the other.

This study aimed to develop and evaluate RNA-based nanotherapeutics targeting regulatory molecules involved in TNBC survival and CSC maintenance. Specifically, the roles of a specific miRNA and cancer-suppressive mRNA were investigated for their potential to suppress oncogenic signaling and reduce CSC populations. RNA-loaded nanoparticles were prepared and characterized to enable efficient delivery into TNBC cells.

The efficacy of these RNA-nanoparticles was assessed through multiple *in vitro* approaches. Gene expression changes were quantified using RT-qPCR, while protein-level and CSC associated markers, including CD44 and ALDH, were analyzed using flow cytometry. Bulk tumor cell viability was assessed by MTT and PrestoBlue.

It is expected that RNA-nanotherapeutics will reduce both bulk TNBC cell viability and CSC associated phenotypes, thereby limiting tumour progression and recurrence. This work aims to contribute to the development of targeted therapies for TNBC by addressing "undruggable targets" through RNA-based approaches to reduce CSC-driven resistance.

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Le cancer du sein triple négatif est une forme particulièrement agressive de cancer du sein, qui demeure difficile à traiter. Même s'il ne représente qu'une partie des cas de cancer du sein, il est responsable d'un nombre important de décès liés à cette maladie. Cette difficulté s'explique en partie par l'absence des récepteurs ER, PR et HER2, qui sont normalement utilisés comme cibles dans certains traitements. Comme ces récepteurs sont absents, les options de thérapies ciblées sont limitées, et la chimiothérapie reste souvent le principal traitement utilisé.

Cependant, la chimiothérapie présente plusieurs limites. Elle peut entraîner des effets toxiques importants, et les cellules cancéreuses peuvent rapidement développer une résistance aux médicaments. De plus, même après un traitement, la tumeur peut réapparaître, notamment à cause de la présence de cellules souches cancéreuses. Ces cellules ont la capacité de survivre aux traitements et de contribuer à la rechute de la maladie. Elles peuvent aussi exister sous différents états, notamment un état de type mésenchymateux et un état de type épithélial. Comme ces états peuvent se transformer l'un en l'autre, cibler seulement un type de cellule souche cancéreuse peut permettre à l'autre type de se développer, ce qui favorise la progression ou le retour du cancer.

Cette étude vise donc à développer et à évaluer des nanothérapies à base d'ARN capables de cibler certaines molécules régulatrices impliquées dans la survie du cancer du sein triple négatif et dans le maintien des cellules souches cancéreuses. Plus précisément, le projet examine le rôle d'un microARN particulier ainsi que d'un ARNm ayant des propriétés suppressives contre le cancer. L'objectif est de déterminer si ces molécules peuvent réduire les signaux oncogéniques, c'est-à-dire les signaux qui favorisent le développement du cancer, et diminuer la population de cellules souches cancéreuses. Pour permettre leur entrée efficace dans les cellules cancéreuses, ces ARN sont incorporés dans des nanoparticules, qui sont ensuite préparées et caractérisées.

L'efficacité de ces nanoparticules contenant de l'ARN est évaluée à l'aide de plusieurs méthodes en laboratoire. Les changements dans l'expression des gènes sont mesurés par RT-qPCR. Les niveaux de protéines ainsi que certains marqueurs associés aux cellules souches cancéreuses, comme CD44 et ALDH, sont analysés par cytométrie en flux. La viabilité des cellules tumorales est aussi évaluée à l'aide de tests comme MTT et PrestoBlue, qui permettent de mesurer la survie et l'activité métabolique des cellules.

Dans l'ensemble, on s'attend à ce que ces nanothérapies à base d'ARN réduisent à la fois la viabilité des cellules tumorales du cancer du sein triple négatif et les caractéristiques associées aux cellules souches cancéreuses. Cela pourrait aider à limiter la progression de la tumeur et à réduire le risque de récidive. Ce travail pourrait donc contribuer au développement de traitements plus ciblés contre le cancer du sein triple négatif, en utilisant des approches basées sur l'ARN pour atteindre des cibles souvent considérées comme difficiles à traiter avec les médicaments classiques.

The Effect of Antibiotic-Altered Maternal Gut Microbiome Composition on Autism Spectrum Disorder Related Behaviours in Offspring

L'Effet de la modification du microbiome intestinal maternel par les antibiotiques sur les comportements associés au trouble du spectre de l'autisme chez la descendance

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Abstract | Résumé

Autism Spectrum Disorder (ASD) is a prevalent neurodevelopmental condition that is caused by a multitude of factors, many of which are not predictable or trackable (1) Compared to neurotypical individuals, those with ASD have gut dysbiosis affecting the bidirectional communication between the gut microbiome and brain, or the gut-microbiota-brain axis (2). Specifically, people with ASD typically have a decreased concentration of certain bacteria like gram positive *Bifidobacterium, Lactobacillus* and *Firmicutes* (2,3). Based on this trend, it is plausible that variations in microbiome compositions can have a direct impact on the development of ASD (4). Additionally, current research concludes that mothers with gut microbiomes exhibiting similar microbial imbalances more commonly have children with ASD (5). However, this does not prove the direct causation between the presence of certain gut bacteria in mothers and their ability to affect fetal neurological development in their offspring. The aim of the following research is to understand how the disruption of the maternal gut microbiome through vancomycin antibiotic exposure increases dysbiosis and influences neurodevelopmental outcomes in offspring. Although there are many factors that affect gut microbiome composition such as diet, exercise, and other lifestyle factors, vancomycin antibiotic intake allows for a controlled change to the gut microbiome as it specifically reduces the presence of gram-positive bacteria. In this study, C57BL/6J mice will be used where half the mice will be administered vancomycin, while the other half will be mock treated with a placebo, serving as a control group (6) Tests will then be performed to assess repetitive behaviours, sociability and cognitive function. Overall, this research will help clarify the development and heritability of ASD, as well as contribute to improved detection and prevention of the disorder.

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Le trouble du spectre de l'autisme (TSA) est une condition neurodéveloppementale fréquente qui peut être influencée par de nombreux facteurs, dont plusieurs sont difficiles à prévoir ou à suivre. Comparativement aux personnes neurotypiques, les personnes ayant un TSA présentent souvent une dysbiose intestinale, c'est-à-dire un déséquilibre dans la composition des bactéries intestinales. Ce déséquilibre peut affecter la communication bidirectionnelle entre le microbiome intestinal et le cerveau, aussi appelée l'axe intestin-microbiote-cerveau. Plus précisément, les personnes ayant un TSA présentent généralement une diminution de certaines bactéries, comme les bactéries Gram positives *Bifidobacterium*, *Lactobacillus* et *Firmicutes*. À partir de cette observation, il est possible que des variations dans la composition du microbiome aient un effet direct sur le développement du TSA. De plus, les recherches actuelles montrent que les mères ayant un microbiome intestinal présentant des déséquilibres similaires ont plus souvent des enfants ayant un TSA. Cependant, cela ne prouve pas qu'il existe un lien de causalité direct entre la présence de certaines bactéries intestinales chez la mère et leur capacité à influencer le développement neurologique du fœtus. L'objectif de cette recherche est donc de mieux comprendre comment la perturbation du microbiome intestinal maternel causée par une exposition à l'antibiotique vancomycine augmente la dysbiose et influence les résultats neurodéveloppementaux chez la descendance. Même si plusieurs facteurs peuvent modifier la composition du microbiome intestinal, comme l'alimentation, l'activité physique et d'autres habitudes de vie, l'utilisation de la vancomycine permet de provoquer un changement contrôlé du microbiome, puisqu'elle réduit spécifiquement la présence de bactéries Gram positives. Dans cette étude, des souris C57BL/6J seront utilisées. La moitié des souris recevra de la vancomycine, tandis que l'autre moitié recevra un placebo et servira de groupe témoin. Des tests seront ensuite réalisés afin d'évaluer les comportements répétitifs, la sociabilité et les fonctions cognitives. Dans l'ensemble, cette recherche permettra de mieux comprendre le développement et la transmission possible du TSA, tout en contribuant à améliorer sa détection et sa prévention.

References

1. H. Hodges, C. Fealko, N. Soares, Autism spectrum disorder: Definition, epidemiology, causes, and clinical evaluation. Translational Pediatrics. 9, 55–65 (2020).
2. G. C. Wong, J. M. Montgomery, M. W. Taylor, The Gut-Microbiota-Brain Axis in Autism Spectrum Disorder. Autism Spectrum Disorders, 95–114 (2021).
3. M. De Angelis, M. Piccolo, L. Vannini, S. Siragusa, A. De Giacomo, D. I. Serrazzanetti, F. Cristofori, M. E. Guerzoni, M. Gobbetti, R. Francavilla, Fecal Microbiota and Metabolome of Children with Autism and Pervasive Developmental Disorder Not Otherwise Specified. PLoS ONE. 8, e76993 (2013).
4. L. W. J. Van Den Elsen, J. Garssen, R. Burcelin, V. Verhasselt, Shaping the Gut Microbiota by Breastfeeding: The Gateway to Allergy Prevention? Frontiers in Pediatrics. 7 (2019), doi:https://doi.org/10.3389/fped.2019.00047.
5. N. Li, J. Yang, J. Zhang, C. Liang, Y. Wang, B. Chen, C. Zhao, J. Wang, G. Zhang, D. Zhao, Y. Liu, L. Zhang, J. Yang, G. Li, Z. Gai, L. Zhang, G. Zhao, Correlation of Gut Microbiome Between ASD Children and Mothers and Potential Biomarkers for Risk Assessment. Genomics, Proteomics & Bioinformatics. 17, 26–38 (2019).
6. S. Isaac, J. U. Scher, A. Djukovic, N. Jiménez, D. R. Littman, S. B. Abramson, E. G. Pamer, C. Ubeda, Short- and long-term effects of oral vancomycin on the human intestinal microbiota. Journal of Antimicrobial Chemotherapy. 72, 128–136 (2017).

Effects of Early Bisphenol A Exposure on Cortical Excitatory and Inhibitory Balance and Behaviour in Mice

Effets de l'exposition précoce au bisphénol A sur l'équilibre et le comportement excitateurs et inhibiteurs corticales chez la souris

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Abstract | Résumé

Exposure to endocrine-disrupting chemicals such as 4-[2-(4-hydroxyphenyl)propan-2-yl]phenol (BPA) during development has been linked to altered neurodevelopment and increased risk for autism spectrum disorder (ASD) (1-5). During pregnancy, BPA exposure occurs through ingestion of contaminated food or beverages from polycarbonate plastics and epoxy resin-lined containers, allowing it to enter maternal circulation and cross the placenta (6-7). A proposed neurobiological mechanism underlying ASD involves disruption of cortical excitatory/inhibitory (E/I) synaptic balance, critical for neural network function and behavioural regulation (3, 8-12). However, it remains unclear whether combined prenatal and early postnatal BPA exposure alters cortical E/I organization in a dose-dependent and sex-specific manner. This study will investigate how early-life BPA exposure influences cortical synaptic markers and ASD-relevant behaviours in C57BL/6J mice. Eighteen pregnant dams (n = 6 per group) will be randomly assigned to control (water), low-dose BPA, or high-dose BPA groups. Through oral gavage, BPA will be administered from gestational day 0 through postnatal day 21 (PND21), targeting a critical window of synaptogenesis. This study will include only full-term offspring. BPA doses will model environmentally relevant human exposure (13-15). The experiment will divide offspring into molecular and behavioural cohorts. Molecular analyses will quantify excitatory (VGLUT1, PSD-95) and inhibitory (GAD67) synaptic markers using immunohistochemistry and western blotting to calculate cortical E/I ratios at PND21. Adolescent offspring will undergo behavioural testing between PND35 and PND60, including the three-chamber social interaction and open-field tests to assess sociability and anxiety-related behaviours. The study expects to determine that early-life BPA exposure produces dose-dependent and sex-specific alterations in cortical E/I balance accompanied by ASD-relevant behavioural changes (1, 3, 16-21). By integrating molecular and behavioural outcomes, this study will clarify whether early-life BPA exposure disrupts cortical E/I balance, a neurobiological feature implicated in ASD. This link would strengthen mechanistic evidence that environmental chemicals influence neurodevelopment and guide public health strategies to reduce exposure during critical developmental periods

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L'exposition à des substances perturbatrices endocriniennes telles que le 4-[2-(4-hydroxyphényl)propane-2-yl]phénol (BPA) au cours du développement a été associée à un neurodéveloppement altéré et à un risque accru de trouble du spectre de l'autisme (TSA) (1-5). Pendant la grossesse, l'exposition au BPA se produit par l'ingestion d'aliments ou de boissons contaminés provenant de plastiques polycarbonates et de contenants doublés de résine époxy, ce qui lui permet d'entrer dans la circulation maternelle et de traverser le placenta (6-7). Un mécanisme neurobiologique proposé sous-jacent au TSA implique la perturbation de l'équilibre synaptique cortical-excitateur/inhibiteur (E/I), essentiel au fonctionnement des réseaux neuronaux et à la régulation comportementale (3, 8-12). Cependant, il reste incertain si l'exposition combinée prénatale et précoce au BPA postnatal modifie l'organisation corticale de l'E/I de manière dépendant de la dose et spécifique au sexe. Cette étude étudiera comment l'exposition au BPA au début de la vie influence les marqueurs synaptiques corticales et les comportements liés au TSA chez les souris C57BL/6J. Dix-huit mères gestantes (n = 6 par groupe) seront assignées au hasard aux groupes témoins (eau), à faible dose de BPA ou à forte dose de BPA. Par voie orale, le BPA sera administré du jour 0 gestationnel au jour postnatal 21 (PND21), ciblant une fenêtre critique de synaptogenèse. Cette étude n'inclura que les enfants à terme. Les doses de BPA modélisent l'exposition humaine environnementale (13-15). L'expérience divisera la descendance en cohortes moléculaires et comportementales. Les analyses moléculaires quantifieront les marqueurs synaptiques excitateurs (VGLUT1, PSD-95) et inhibiteurs (GAD67) en utilisant l'immunohistochimie et le transfert de protéines pour calculer les rapports corticaux E/I à PND21. Les enfants adolescents subiront des tests comportementaux entre PND35 et PND60, incluant les tests d'interaction sociale à trois chambres et des tests en champ ouvert pour évaluer la sociabilité et les comportements liés à l'anxiété. L'étude s'attend à déterminer que l'exposition au BPA au début de la vie entraîne des altérations dépendantes de la dose et spécifiques au sexe dans l'équilibre cortical/E/I, accompagnées de changements comportementaux liés au TSA (1, 3, 16-21). En intégrant les résultats moléculaires et comportementaux, cette étude clarifiera si l'exposition au BPA au début de la vie perturbe l'équilibre cortical/E/I, une caractéristique neurobiologique impliquée dans le TSA. Ce lien renforcerait les preuves mécanistes que les substances chimiques environnementales influencent le neurodéveloppement et guiderait les stratégies de santé publique pour réduire l'exposition durant les périodes critiques du développement.

References

1. **C**. Welch, K. Mulligan, Does bisphenol A confer risk of neurodevelopmental disorders? What we have learned from developmental neurotoxicity studies in animal models. International Journal of Molecular Sciences 23, 2894 (2022).
2. T. P. Stein, M. D. Schluter, R. A. Steer, L. Guo, X. Ming, Bisphenol A exposure in children with autism spectrum disorders. Autism Research 8, 272–283 (2015).
3. S. Kanlayaprasit, T. Saeliw, S. Thongkorn, P. Panjabud, K. Kasitipradit, P. Lertpeerapan, K. Songsritaya, W. Yuwattana, T. Jantheang, D. Jindatip, V. W. Hu, T. Kikkawa, N. Osumi, T. Sarachana, Sex-specific impacts of prenatal bisphenol A exposure on genes associated with cortical development, social behaviors, and autism in the offspring's prefrontal cortex. Biology of Sex Differences 15, 40 (2024).
4. H. E. Costa, E. Cairrao, Effect of bisphenol A on the neurological system: A review update. Archives of Toxicology 98, 1–73 (2023).
5. E. Quiñones-Medina, F. Galleguillos, V. Parra, G. Arriagada, F. J. Bustos, Gene–environment interactions in autism spectrum disorders: The role of bisphenol A in modulating genetic susceptibility. Autism Research 10.1002/aur.70186 (2026).
6. H. F. S. Abulehia, N. S. Mohd Nor, S. H. Sheikh Abdul Kadir, The current findings on the impact of prenatal BPA exposure on metabolic parameters: In vivo and epidemiological evidence. Nutrients 14, 2766 (2022).
7. V. Volberg, K. Harley, A. M. Calafat, V. Davé, J. McFadden, B. Eskenazi, N. Holland, Maternal bisphenol A exposure during pregnancy and its association with adipokines in Mexican-American children. Environmental and Molecular Mutagenesis 54, 621–628 (2013).
8. V. S. Sohal, J. L. R. Rubenstein, Excitation-inhibition balance as a framework for investigating mechanisms in neuropsychiatric disorders. Molecular Psychiatry 24, 1248–1257 (2019).
9. H. Bruining, R. Hardstone, E. L. Juarez-Martinez, J. Sprengers, A. Avramiea, S. Simpraga, S. J. Houtman, S. Poil, E. Dallares, S. Palva, B. Oranje, J. M. Palva, H. D. Mansvelder, K. Linkenkaer-Hansen, Measurement of excitation-inhibition ratio in autism spectrum disorder using critical brain dynamics. Scientific Reports 10, 9195 (2020).
10. M. C. D. Bridi, N. Luo, G. Kim, B. J. Menarchek, R. A. Lee, B. Rodriguez, D. Severin, C. Wesselborg, C. O'Ferrall, R. Patel, S. Bertrand, S. Kannan, A. Kirkwood, C. Moreno, A. Contreras, Daily oscillation of the excitation/inhibition ratio is disrupted in two mouse models of autism. iScience 28, 111494 (2024).
11. E. Lee, J. Lee, E. Kim, Excitation/inhibition imbalance in animal models of autism spectrum disorders. Biological Psychiatry 81, 838–847 (2017).
12. L. Colomar, A. S. J. Cáceres, J. Álvarez-Linera, J. González-Peñas, A. H. Patón, D. M. De Blas, A. P. P. Arrondo, A. Solís, E. Jones, M. Parellada, Role of cortical excitatory/inhibitory imbalance in autism spectrum disorders from a symptom severity trajectories framework: A study protocol. BMC Psychiatry 23, 213 (2023).
13. E. Kimura, C. Matsuyoshi, W. Miyazaki, S. Benner, M. Hosokawa, K. Yokoyama, M. Kakeyama, C. Tohyama, Prenatal exposure to bisphenol A impacts neuronal morphology in the hippocampal CA1 region in developing and aged mice. Archives of Toxicology 90, 691–700 (2015).
14. R. N. Sadowski, P. Park, S. L. Neese, D. C. Ferguson, S. L. Schantz, J. M. Juraska, Effects of perinatal bisphenol A exposure during early development on radial arm maze behavior in adult male and female rats. Neurotoxicology and Teratology 42, 17–24 (2014).
15. L. M. Wise, D. Hernández-Saavedra, S. M. Boas, Y. Pan, J. M. Juraska, Perinatal high-fat diet and bisphenol A: Effects on behavior and gene expression in the medial prefrontal cortex. Developmental Neuroscience 41, 1–16 (2018).
16. D. Rebolledo-Solleiro, L. Y. C. Flores, H. Solleiro-Villavicencio, Impact of BPA on behavior, neurodevelopment and neurodegeneration. Frontiers in Bioscience 26, 363–400 (2020).
17. D. Kumar, M. K. Thakur, Anxiety-like behavior due to perinatal exposure to bisphenol A is associated with decrease in excitatory to inhibitory synaptic density of male mouse brain. Toxicology 378, 107–113 (2017).
18. C. Symeonides, K. Vacy, S. Thomson, S. Tanner, H. K. Chua, S. Dixit, T. Mansell, M. O'Hely, B. Novakovic, J. B. Herbstman, S. Wang, J. Guo, J. Chia, N. T. Tran, S. E. Hwang, K. Britt, F. Chen, T. H. Kim, C. A. Reid, W. C. Boon, Male autism spectrum 6 disorder is linked to brain aromatase disruption by prenatal BPA in multimodal investigations and 10HDA ameliorates the related mouse phenotype. Nature Communications 15, 6367 (2024).
19. M. Kundakovic, K. Gudsnuk, B. Franks, J. Madrid, R. L. Miller, F. P. Perera, F. A. Champagne, Sex-specific epigenetic disruption and behavioral changes following low-dose in utero bisphenol A exposure. Proceedings of the National Academy of Sciences 110, 9956–9961 (2013).
20. D. Nesan, K. M. Feighan, M. C. Antle, D. M. Kurrasch, Gestational low-dose BPA exposure impacts suprachiasmatic nucleus neurogenesis and circadian activity with transgenerational effects. Science Advances 7, eabd1159 (2021).
21. C. Jiang, J. Guan, X. Tang, Y. Zhang, X. Li, Y. Li, Z. Chen, J. Zhang, J. Li, Prenatal low-dose bisphenol A exposure impacts cortical development via cAMP-PKA-CREB pathway in offspring. Frontiers in Integrative Neuroscience 18, 1419607 (2024)

Investigating Autism-linked Mitochondrial and Social Behavioural Deficits: MFN2 Knockdown in Zebrafish and Rescue by Human MFN2 Genes

Étude des déficits mitochondriaux et comportementaux sociaux liés à l'autisme : inactivation du gène MFN2 chez le poisson-zèbre et restauration par les gènes MFN2 humains

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Abstract | Résumé

Autism spectrum disorder (ASD) is a complex neurodevelopmental condition defined by impaired social interaction and repetitive behaviors (1). Emerging evidence suggests that mitochondrial dysfunction may be a contributor to ASD. Proper neuronal energy production is critical for synaptic function, which facilitates pro-social behavior. Mitofusin-2 (MFN2), a key regulator of mitochondrial fusion, shows reduced expression in ASD patients, and is associated with abnormal mitochondrial morphology that impairs ATP production. However, the direct causal link between MFN2-mediated mitochondrial dysfunction and autism-related social behavior remains unclear (2).

This study investigates the effects of MFN2 knockdown on neuronal energy and social behavior in zebrafish (Danio rerio), and whether human MFN2 expression can rescue these deficits. Zebrafish are widely used as a neurodevelopmental model because they possess conserved genetic pathways related to human neurological disorders and display quantifiable social behaviours such as shoaling. Additionally, their transparent embryos and rapid development allow efficient genetic manipulation and real-time observation of developmental effects. Zebrafish embryos will be divided into four groups: wild-type, sham-injected, MFN2 knockdown, and a knockdown group receiving human MFN2 mRNA for rescue. MFN2 knockdown will be achieved via antisense morpholino injection at the one-cell stage, with expression levels validated through quantitative polymerase chain reaction and Western blot. To assess the impact on cellular energy and sociality, neuronal ATP levels will be measured using a luminescent assay while social cohesion will be evaluated using shoaling behavioral assay with automated tracking software. The Average Inter-Individual Distance will be quantified as the primary metric of group cohesion.

It is hypothesized that MFN2 knockdown will impair mitochondrial fusion, leading to reduced neuronal ATP availability and disrupted pro-social behavior, while human MFN2 expression will restore both energy production and shoaling. By establishing MFN2 as a critical link between mitochondrial dynamics and sociality, this research will clarify the role of metabolic dysfunction in ASD, providing insights into potential molecular pathways for therapeutic intervention.

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Le trouble du spectre de l'autisme (TSA) est une condition neurodéveloppementale complexe définie par l'interaction sociale affaiblie et des comportements répétitifs (1). Des données émergentes suggèrent qu'un dysfonctionnement mitochondrial pourrait contribuer aux TSA. Une production d'énergie neuronale adéquate est essentielle au bon fonctionnement des synapses, ce qui favorise les comportements prosociaux. La mitofusine-2 (MFN2), un régulateur clé de la fusion mitochondriale, présente une expression réduite chez les patients atteints de TSA et est associée à une morphologie mitochondriale anormale qui altère la production d'ATP. Cependant, le lien de causalité direct entre le dysfonctionnement mitochondrial induit par MFN2 et les comportements sociaux liés à l'autisme reste incertain (2).

Cette étude examine les effets de l'inactivation de la MFN2 sur l'énergie neuronale et le comportement social chez le poisson-zèbre (Danio rerio) et si l'expression de la MFN2 humaine peut remédier à ces déficits. Le poisson-zèbre est largement utilisé comme modèle de développement neurologique car il possède des voies génétiques conservées liées aux troubles neurologiques humains et présente des comportements sociaux quantifiables tels que le regroupement en bancs. De plus, la transparence de leurs embryons et leur développement rapide permettent une manipulation génétique efficace et l'observation en temps réel des effets sur le développement. Les embryons de poisson-zèbre seront divisés en quatre groupes : de type sauvage, ayant reçu une injection simulée, présentant une inactivation de la MFN2 et un groupe présentant une inactivation recevant de l'ARNm MFN2 humain. L'inhibition de la MFN2 sera réalisée par injection de morpholino antisens au stade unicellulaire, les niveaux d'expression étant validés par réaction en chaîne par polymérase quantitative et par un test Western Blot. Pour évaluer l'impact sur l'énergie cellulaire et la sociabilité, les niveaux d'ATP neuronal seront mesurés par un test de luminescence, tandis que la cohésion sociale sera évaluée par un test comportemental de regroupement avec un logiciel de suivi automatisé. La distance interindividuelle moyenne sera quantifiée comme principal indicateur de la cohésion du groupe.

L'hypothèse est que l'inhibition de la MFN2 altère la fusion mitochondriale, entraînant une diminution de la disponibilité d'ATP neuronal et une perturbation des comportements prosociaux, tandis que l'expression de la MFN2 humaine rétablit la production d'énergie et le regroupement en bancs. En établissant la MFN2 comme un lien essentiel entre la dynamique mitochondriale et la sociabilité, cette recherche permettra de mieux comprendre le rôle des dysfonctionnements métaboliques dans les TSA, et d'identifier des voies moléculaires potentielles pour des interventions thérapeutiques.

References

1. American Psychiatric Association, What Is Autism Spectrum Disorder? Psychiatry.org (2024); https://www.psychiatry.org/patients-families/autism/what-is-autism-spectrum-disorder.
2. L. Citrigno, M. Muglia, A. Qualtieri, P. Spadafora, F. Cavalcanti, G. Pioggia, A. Cerasa, The Mitochondrial Dysfunction Hypothesis in Autism Spectrum Disorders: Current Status and Future Perspectives. International Journal of Molecular Sciences. 21, 5785 (2020). doi:10.3390/ijms21165785.

Rôle du gène MeCP2 sur la mort subite inattendue en épilepsie dans le syndrome de Rett

Role of the MeCP2 Gene in Sudden Unexpected Death in Epilepsy in Rett Syndrome

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Résumé | Abstract

La déficience intellectuelle et l'épilepsie sont fréquemment associées (1-2), augmentant considérablement le risque de mort subite inattendue en épilepsie (1). Le syndrome de Rett, causé par une mutation du gène MeCP2, est un modèle d'étude pertinent, car il conjugue déficience intellectuelle, crises épileptiques et dysautonomie cardiorespiratoire (3-5). Bien que le rôle de MeCP2 dans l'homéostasie neuronale soit documenté (6-7), les mécanismes liant sa déficience à la mort subite inattendue en épilepsie demeurent mal élucidés. Cette recherche examine si la perte de MeCP2 perturbe la coordination entre le cortex cérébral et les centres autonomes du tronc cérébral, accentuant la dysautonomie cardiaque post-ictale. Quatre groupes murins seront comparés pour refléter la variabilité phénotypique du syndrome : I) témoin sain, II) épileptique sain, III) mâles hémizygotes (MeCP2-/Y) et IV) femelles hétérozygotes (MeCP2+/-). Étant donné que le gène est lié au chromosome X, la distinction des groupes III et IV est nécessaire pour offrir une représentation réaliste du syndrome tout en recueillant des données concluantes. L'activité corticale et cardiaque sera mesurée par électroencéphalogramme et électrocardiogramme. L'excitabilité neuronale sera visualisée par imagerie avec un indicateur calcique génétiquement codé (GCaMP6) (8), tandis que les crises seront induites par injection répétée de pentylènetétrazol (8-9) pour simuler un état épileptique chronique chez tous les murins à l'exception du groupe I. L'analyse des paramètres avant, pendant et après les crises permettra d'évaluer la dynamique post-ictale. Il est prévu que les souris déficientes en MeCP2 présenteront une hyperactivité des centres autonomes et un déséquilibre excitation/inhibition, entraînant des crises plus sévères et un ralentissement de la récupération cardiorespiratoire, comme le suggèrent des études antérieures (5). En clarifiant ces mécanismes, cette étude vise à mieux comprendre la physiopathologie de la mort subite inattendue en épilepsie et à orienter le développement de stratégies thérapeutiques ciblées pour le syndrome de Rett.

Intellectual disability and epilepsy are frequently associated (1-2), significantly increasing the risk of sudden unexpected death in epilepsy (1). Rett syndrome, caused by a mutation in the MeCP2 gene, is a relevant study model as it combines intellectual disability, epileptic seizures, and cardiorespiratory dysautonomia (3-5). Although the role of MeCP2 in neuronal homeostasis is documented (6-7), the mechanisms linking its deficiency to sudden unexpected death in epilepsy remain poorly elucidated. This research examines whether the loss of MeCP2 disrupts coordination between the cerebral cortex and autonomic centers of the brainstem, exacerbating post-ictal cardiac dysautonomia. Four murine groups will be compared to reflect the phenotypic variability of the syndrome: I) healthy control, II) healthy epileptic, III) hemizygous males (MeCP2-/Y), and IV) heterozygous females (MeCP2+/-). Since the gene is X-linked, the distinction between groups III and IV is necessary to provide a realistic representation of the syndrome while collecting conclusive data. Cortical and cardiac activity will be measured by electroencephalogram and electrocardiogram. Neuronal excitability will be visualized through imaging with a genetically encoded calcium indicator (GCaMP6) (8), while seizures will be induced by repeated pentylenetetrazole injection (8-9) to simulate a chronic epileptic state in all mice except group I. Analysis of parameters before, during, and after seizures will allow evaluation of post-ictal dynamics. It is expected that MeCP2-deficient mice will exhibit hyperactivity of autonomic centers and an excitation/inhibition imbalance, leading to more severe seizures and slower cardiorespiratory recovery, as suggested by previous studies (5). By clarifying these mechanisms, this study aims to better understand the pathophysiology of sudden unexpected death in epilepsy and guide the development of targeted therapeutic strategies for Rett syndrome.

Références

1. S. Jansen, L.E.L.M. Vissers, B.B.A. de Vries, The Genetics of Intellectual Disability. Brain Science 13, 231 (2023).
2. R. Shankar, Managing epilepsy in people with intellectual disabilities - creating capable communities. BJPsych Advances 29, 305-307 (2023).
3. J.M. Bissonnette, S.J. Knopp, J. Maylie, T. Thong, Autonomic cardiovascular control in methyl-CpG-binding protein (Mecp2) deficient mice. Autonomic Neuroscience: Basic and Clinical 136, 82-89 (2007).
4. L.M. Lombardi, S.A. Baker, H.Y. Zoghbi, MECP2 disorders: from the clinic to mice and back. Journal of Clinical Investigation 125, 2914-2923 (2015).
5. A. Percy, V. Cuddapah, S. Nwaobi, M. Olsen, Rett syndrome pathophysiological perspectives. Degenerative Neurological and Neuromuscular Disease 5, 103-116 (2015).
6. M.L. Gonzales, J.M. LaSalle, The Role of MeCP2 in Brain Development and Neurodevelopmental Disorders. Current Psychiatry Reports 12, 127-134 (2010).
7. Y. M. Vuu, C.-T. Roberts, M. Rastegar, MeCP2 Is an Epigenetic Factor That Links DNA Methylation with Brain Metabolism. International Journal of Molecular Sciences 24, 4218 (2023).
8. F. M. Simoes de Souza, R. Williamson, C.McCullough, A.Teel, G.Futia, M. Ma, A. True, J. P. Crimaldi, E. Gibson, D. Restrepo, Miniscope Recording Calcium Signals at Hippocampus of Mice Navigating an Odor Plume. Journal of Visualized Experiments 211, e67039 (2024).
9. A. J. Rocha, A. A. C. Rangel, C. M. Queiroz, Behavioral Characterization of Pentylenetetrazole-induced Seizures: Moving Beyond the Racine Scale. Journal of Visualized Experiments 221, e68112 (2025).

Targeted demethylation of the Gamma-Aminobutyric Acid Type A Receptor Beta-1 Subunit gene using CRISPR–dCas9–TET to restore Gamma-Aminobutyric Acid type A receptor function in epilepsy

Déméthylation ciblée du gène de la sous-unité bêta-1 du récepteur gamma-aminobutyrique type A à l'aide de CRISPR–dCas9–TET pour restaurer la fonction du récepteur gamma-aminobutyrique type A dans l'épilepsie

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Abstract | Résumé

Epilepsy is a neurodevelopmental disorder characterized by recurring, unprovoked seizures due to abnormal imbalances between excitatory and inhibitory neurotransmission (1). Affecting nearly 1 in 100 Canadians (2), epilepsy significantly reduces quality of life through physical risks, cognitive impairment, and clinically significant anxiety and depression (3). Gamma-Aminobutyric Acid type A Receptor (GABAAR) is an ionotropic receptor that mediates inhibitory neurotransmission by binding to the GABA neurotransmitter (4). The methylation of the GABAAR Beta-1 Subunit (GABRB1) gene has been shown to reduce GABAAR function in individuals with epilepsy, thereby contributing to neuronal hyperexcitability and highlighting a potential epigenetic target for therapeutic intervention aimed at restoring inhibitory signaling (5). This study proposes administering CRISPR-dCas9-TET to selectively demethylate cytosines in GABRB1 promoter regions of mice using the GABRB1 sgRNA sequence (5' to 3') CACCg GCCGCGAGGGCTTCGGGCGT and its complementary 3' to 5' sequence to reactivate GABRB1 expression (6). FVB/N mouse models with kainic acid-induced epilepsy are used due to neurological sensitivity and similarities to homo sapiens (7). Calcium ion imaging of genetically encoded fluorescent indicators using two-photon microscopy through a surgically implanted cranial window will be conducted alongside qualitative analysis of seizure frequency to determine the treatment effects on neuronal inhibition. Limitations to this approach include potential cerebral trauma due to cranial windowing or CRISPR-dCas9-TET administration, potentially resulting in a systematic decrease in inhibitory signaling (8). This proposed epigenetic approach is important because of its potential as an alternative to anti-seizure medications (ASMs). Notably, ASMs require frequent dosing and are often ineffective in treating patients with drug-resistant epilepsy (DRE) (9). The proposed treatment could reduce seizure susceptibility, offering a long-term, single-intervention treatment for both epileptic and DRE epileptic patients. Overall, this study will provide insight into the effectiveness of targeted demethylation in restoring inhibitory neurotransmission.

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L'épilepsie est un trouble neurodéveloppemental caractérisé par des crises récurrentes non provoquées dues à des déséquilibres anormaux entre la neurotransmission excitatrice et inhibitrice (1). Touchant près d'un Canadien sur 100 (2), l'épilepsie réduit significativement la qualité de vie par des risques physiques, des troubles cognitifs et une anxiété et dépression cliniquement significatives (3). Le récepteur gamma-aminobutyrique de type A (GABAAR) est un récepteur ionotrope qui médiatise la neurotransmission inhibitrice en se liant au neurotransmetteur GABA (4). La méthylation du gène de la sous-unité GABAAR Bêta-1 (GABRB1) a démontré réduire la fonction du GABAAR chez les personnes épileptiques, contribuant ainsi à l'hyperexcitabilité neuronale et mettant en lumière une cible épigénétique potentielle pour une intervention thérapeutique visant à restaurer la signalisation inhibitrice (5). Cette étude propose d'administrer CRISPR-dCas9-TET pour déméthyler sélectivement les cytosines dans les régions promoteurs GABRB1 chez la souris en utilisant la séquence d'ARNg GABRB1 (5' à 3') CACCg GCCGCGGGGCTTCGGGTGT et sa séquence complémentaire 3' à 5' afin de réactiver l'expression de GABRB1 (6). Des modèles murins FVB/N avec épilepsie induite par l'acide kainique sont utilisés en raison de leur sensibilité neurologique et de similitudes avec l'homo sapiens (7). L'imagerie des ions calcium d'indicateurs fluorescents codés génétiquement à l'aide d'une microscopie à deux photons via une fenêtre crânienne implantée chirurgicalement sera réalisée parallèlement à une analyse qualitative de la fréquence des crises afin de déterminer les effets du traitement sur l'inhibition neuronale. Les limites de cette approche incluent un traumatisme cérébral potentiel dû à un fenêtrement crânien ou à l'administration de CRISPR-dCas9-TET, pouvant entraîner une diminution systématique de la signalisation inhibitrice (8). Cette approche épigénétique proposée est importante en raison de son potentiel en tant qu'alternative aux médicaments antiépileptiques (ASM). Notamment, les ASM nécessitent des doses fréquentes et sont souvent inefficaces pour traiter les patients atteints d'épilepsie pharmaco-résistante (DRE) (9). Le traitement proposé pourrait réduire la susceptibilité aux crises, offrant un traitement à long terme à intervention unique pour les patients épileptiques et DRE. Dans l'ensemble, cette étude apportera un éclairage sur l'efficacité de la déméthylation ciblée pour restaurer la neurotransmission inhibitrice.

References

1. National Institute of Neurological Disorders and Stroke. (2025, April 7). Epilepsy and Seizures. https://www.ninds.nih.gov
2. Public Health Agency of Canada. (2018, March 23). Epilepsy in Canada. https://www.canada.ca/en/public-health/services/publications/diseases-conditions/epilepsy.html
3. Pugh, R., Vaughan, D. N., Jackson, G. D., Ponsford, J., & Tailby, C. (2024). Cognitive and psychological dysfunction is present after a first seizure, prior to epilepsy diagnosis and treatment at a First Seizure Clinic. Epilepsia Open.
4. Jewett, B., & Sharma, S. (2021). Physiology, GABA. StatPearls. National Library of Medicine. https://www.ncbi.nlm.nih.gov/books/NBK513311/
5. Tao, H., Chen, Z., Wu, J., Chen, J., Chen, Y., Fu, J., Sun, C., Zhou, H., Zhong, W., Zhou, X., & Li, K. (2021). DNA methylation signature of epileptic encephalopathy-related pathogenic genes encoding ion channels in temporal lobe epilepsy. Front. Neurol. 12. https://doi.org/10.3389/fneur.2021.692412
6. Duan, J., Pandey, S., Li, T., Castellano, D., Gu, X., Li, J., Tian, Q., Lu, W. (2019). Genetic deletion of GABAA receptors reveals distinct requirements of neurotransmitter receptors for GABAergic and glutamatergic synapse development. Front. Cell. Neurosci. 13. https://www.frontiersin.org/journals/cellular-neuroscience/articles/10.3389/fncel.2019.00217/full
7. Kohnken, R. A., & Schwahn, D. J. (2016). Lack of chronic histologic lesions supportive of sublethal spontaneous seizures in FVB/N mice. Comp. Med. 66(2), 105–111. https://pmc.ncbi.nlm.nih.gov/articles/PMC4825959/
8. Parga Becerra, A., Logsdon, A. F., Banks, W. A., & Ransom, C. B. (2021). Traumatic brain injury broadly affects GABAergic signaling in dentate gyrus granule cells. eNeuro 8(3), ENEURO.0055-20.2021. https://doi.org/10.1523/ENEURO.0055-20.2021
9. Fattorusso, A., Matricardi, S., Mencaroni, E., Dell'Isola, G. B., Di Cara, G., Striano, P., & Verrotti, A. (2021). The pharmacoresistant epilepsy: An overview on existent and new emerging therapies. Front. Neurol. 12. https://doi.org/10.3389/fneur.2021.674483

Chronic Circadian Disruption as a Driver of Microglial NLRP3-Mediated Tau Pathology in Alzheimer's Disease: A Research Proposal

La perturbation chronique du rythme circadien comme moteur de la pathologie tau médiée par l'inflammasome NLRP3 microglial dans la maladie d'Alzheimer : une proposition de recherche

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Abstract | Résumé

Alzheimer's disease (AD) affects more than 55 million people worldwide and has no approved disease-modifying treatment. One known risk factor is disruption of the circadian rhythm — the body's internal 24-hour clock — but exactly how this disruption contributes to the buildup of toxic tau protein in the brain is unknown. Brain immune cells called microglia have their own internal clocks, controlled by the BMAL1/CLOCK protein complex. When this clock is disrupted, microglia become chronically inflamed. Separately, activation of a microglial inflammatory pathway called the NLRP3 inflammasome causes tau to become abnormally phosphorylated — a key step in forming the neurofibrillary tangles (NFTs) that kill neurons in AD. This proposal identifies an untested mechanistic link between these findings. It is hypothesized that chronic jet lag (CJL), a laboratory model of circadian disruption, would impair microglial BMAL1 rhythmicity and thereby drive unchecked NLRP3 activation and accelerated tau pathology in P301S tauopathy mice. Five groups would be compared, using the pharmacological NLRP3 blocker MCC950 as a rescue approach to confirm causality. These findings would identify a modifiable lifestyle factor as a direct driver of AD and point to microglial NLRP3 as a promising therapeutic target.

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La maladie d'Alzheimer (MA) touche plus de 55 millions de personnes dans le monde, et il n'existe toujours pas de traitement approuvé capable de modifier directement l'évolution de la maladie. Un facteur de risque connu est la perturbation du rythme circadien, c'est-à-dire l'horloge interne de 24 heures du corps. Cependant, on ne sait pas encore exactement comment cette perturbation contribue à l'accumulation de la protéine tau toxique dans le cerveau. Les cellules immunitaires du cerveau, appelées microglies, possèdent aussi leur propre horloge interne. Cette horloge est contrôlée par le complexe de protéines BMAL1/CLOCK. Lorsque cette horloge est perturbée, les microglies peuvent rester dans un état d'inflammation chronique. De façon séparée, l'activation d'une voie inflammatoire microgliale appelée inflammasome NLRP3 peut rendre la protéine tau anormalement phosphorylée. Cette étape est importante dans la formation des enchevêtrements neurofibrillaires, qui contribuent à la mort des neurones dans la MA. Cette proposition met donc en évidence un lien mécanique qui n'a pas encore été testé directement. L'hypothèse est que le décalage horaire chronique, utilisé en laboratoire comme modèle de perturbation circadienne, pourrait affaiblir le rythme de BMAL1 dans les microglies. Cela pourrait ensuite entraîner une activation non contrôlée de NLRP3 et accélérer la pathologie tau chez des souris P301S, un modèle de tauopathie. Cinq groupes seraient comparés, et le bloqueur pharmacologique de NLRP3, MCC950, serait utilisé comme approche de "sauvetage" afin de confirmer le lien de causalité. Ces résultats pourraient montrer qu'un facteur de mode de vie modifiable contribue directement à la MA et que NLRP3 dans les microglies est une cible thérapeutique prometteuse.

Keywords: Alzheimer's disease; circadian rhythm disruption; NLRP3 inflammasome; microglia; tau hyperphosphorylation; MCC950; tauopathy

Background and Rationale

AD accounts for 60–70% of all dementia cases globally (1). As shown in Figure 2, World Bank data on the proportion of people aged 65 and over shows growth from 6.9% in 2000 to 9.8% in 2022. Statistical modelling projects this will reach 17.8% globally — and 28.5% in high-income countries — by 2050. Because AD risk rises sharply with age, this demographic shift means the disease burden is accelerating rapidly, yet there is still no way to slow or reverse it.

Disrupted circadian rhythms are an increasingly recognized and potentially modifiable risk factor for AD. Sleep disruption and circadian misalignment in midlife consistently raise the risk of developing AD, and circadian behavioural changes — including sundowning — appear years before obvious memory loss (2). Tau protein has been found to accumulate in neurons connecting to the suprachiasmatic nucleus (SCN), the brain's master clock, creating a self-reinforcing loop where tau pathology worsens circadian function (2). Whether the reverse is also true — whether circadian disruption itself accelerates tau buildup — remains unanswered.

Figure 1. Proposed mechanistic pathway from chronic circadian disruption to tau pathology via microglial NLRP3 inflammasome activation. MCC950 pharmacological rescue (green) confirms NLRP3 as the causal node. Supporting literature indicated above each node.

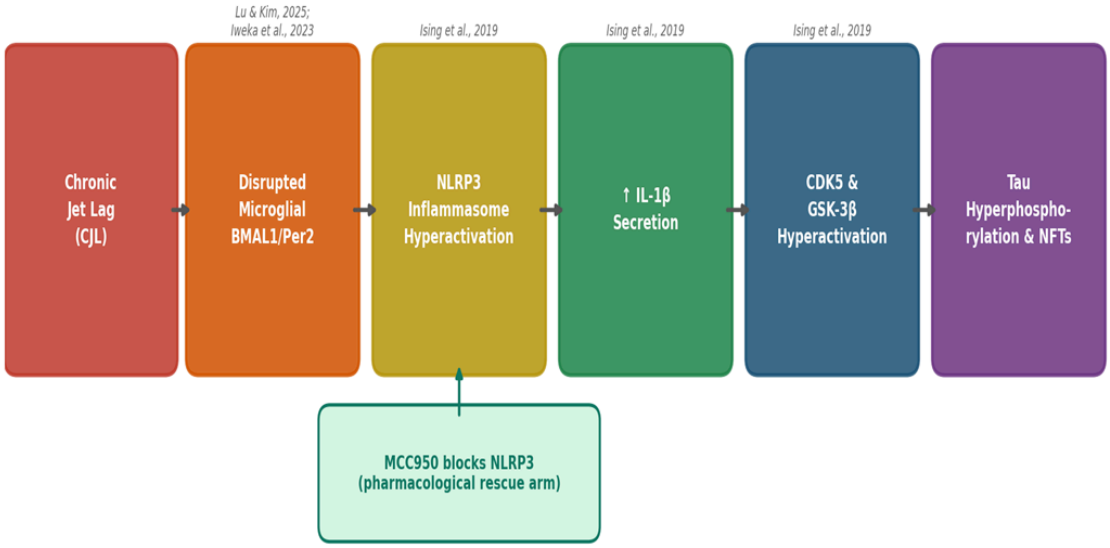

Figure 1. Proposed mechanistic pathway from chronic circadian disruption to tau pathology via microglial NLRP3 inflammasome activation. Each arrow represents a proposed causal step supported by the cited literature. The green box shows where MCC950 would intervene to confirm NLRP3 as the key causal node. BMAL1 = brain and muscle ARNT-like 1; Per2 = period circadian regulator 2; NLRP3 = NOD-like receptor protein 3; IL-1β = interleukin-1 beta; CDK5 = cyclin-dependent kinase 5; GSK-3β = glycogen synthase kinase 3 beta; NFTs = neurofibrillary tangles.

Microglia have their own internal circadian clocks driven by the brain and muscle ARNT-like 1 (BMAL1)/CLOCK molecular feedback loop. Deleting BMAL1 specifically in immune cells accelerates cognitive aging in mice through chronic inflammatory activation and impaired synaptic pruning (3). External neuroinflammation has also been shown to shift the phase of microglial BMAL1, locking microglia in a pro-inflammatory state (4). This raises the possibility that circadian disruption could constitutively activate microglia.

Crucially, activation of the microglial NLRP3 inflammasome drives tau hyperphosphorylation by triggering release of interleukin-1 beta (IL-1β), which activates the tau kinases cyclin-dependent kinase 5 (CDK5) and glycogen synthase kinase 3 beta (GSK-3β) while suppressing the phosphatase PP2A in the Tau22 tauopathy model (5). Together with Figure 1, these findings converge on an untested chain — CJL → impaired microglial BMAL1 → uncontrolled NLRP3 → IL-1β → accelerated tau phosphorylation and NFT formation — that this proposal is designed to test directly

Research Question and Objectives

Primary Research Question: Would chronic circadian disruption worsen microglial NLRP3 inflammasome activation and tau phosphorylation in a tauopathy mouse model, and would blocking NLRP3 pharmacologically reverse these effects?

Specific Aim 1
To determine whether CJL disrupts BMAL1 and period circadian regulator 2 (Per2) expression rhythms in microglia of P301S mice compared to normally entrained controls.

Hypothesis 1: CJL-exposed P301S mice would show significantly reduced BMAL1 amplitude, and disrupted Per2 oscillation timing compared to normally entrained P301S controls, reflecting loss of microglial circadian gating.

Specific Aim 2
To assess whether disrupted microglial clock function is associated with elevated NLRP3 inflammasome activation and IL-1β release in the hippocampus of CJL-treated P301S mice.

Hypothesis 2: NLRP3, apoptosis-associated speck-like protein containing a CARD (ASC), and cleaved IL-1β would be significantly higher in P301S+CJL mice than in normally entrained P301S controls, correlating inversely with BMAL1 amplitude.

Specific Aim 3
To test whether CJL-driven NLRP3 activation accelerates tau phosphorylation and memory impairment in P301S mice, and whether MCC950 reverses these effects.

Hypothesis 3: P301S+CJL mice would show greater AT8 (phospho-Ser202/Thr205) and PHF1 (phospho-Ser396/Ser404) tau phosphorylation and worse Morris Water Maze (MWM) performance than P301S+NLC mice, with both outcomes significantly reduced by MCC950.

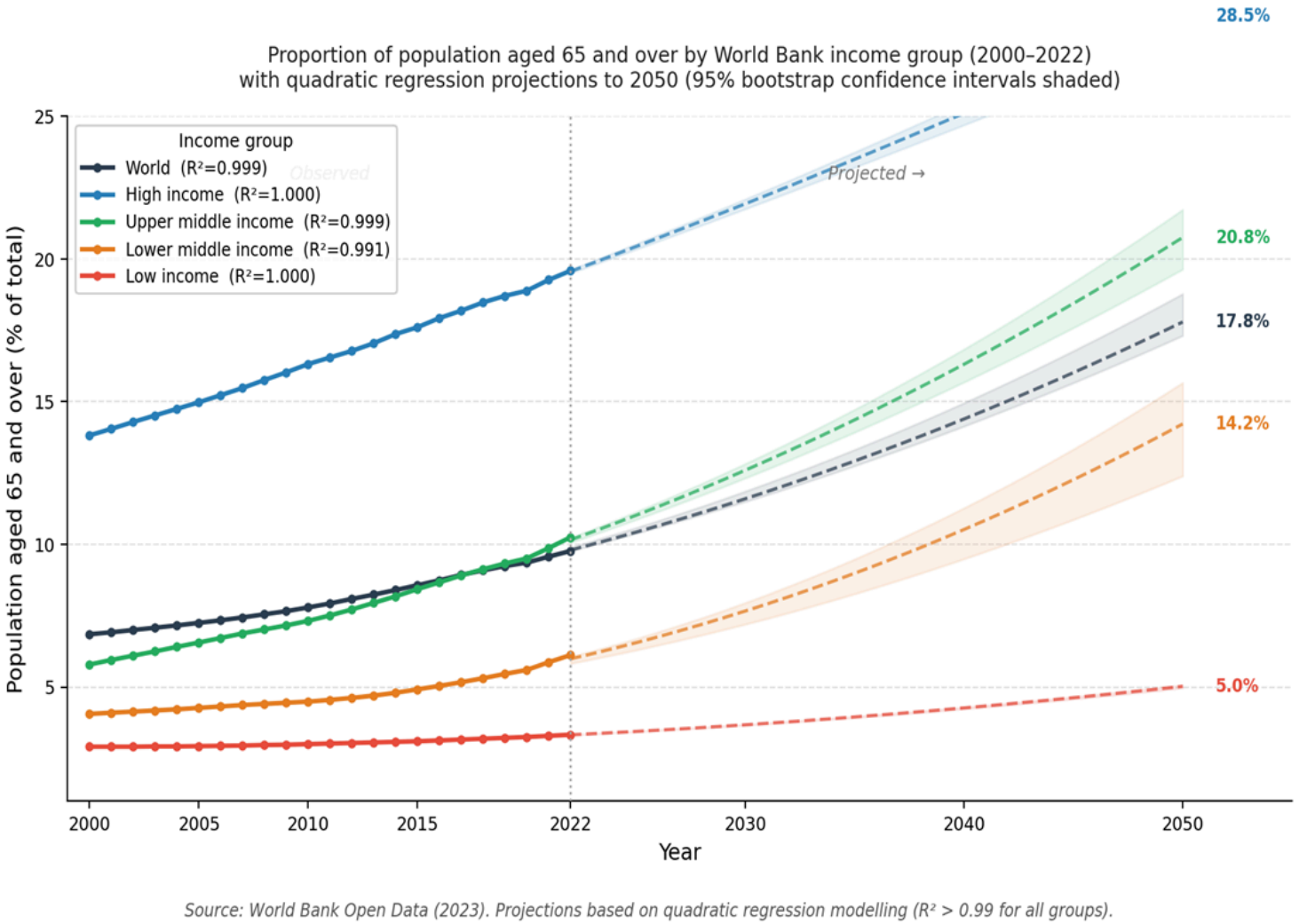

Figure 2. Proportion of population aged 65 and over by World Bank income group (2000–2022), with quadratic regression projections to 2050 and 95% bootstrap confidence intervals (shaded). Solid lines = observed data; dashed lines = model-based projections. $R^2 > 0.99$ across all income groups. Projections assume continuation of historical aging trends and do not account for potential future disruptions. Source: World Bank Open Data (2023) (8).

Proposed Methodology

Study design

Five groups of P301S (PS19) transgenic mice (Jackson Laboratories #008169) would be established at 3 months of age, before overt tau pathology: (1) wild-type (WT) + normal light-dark cycle (NLC); (2) WT + CJL; (3) P301S + NLC; (4) P301S + CJL; (5) P301S + CJL + MCC950. A total of n=55 animals (n=11 per group with equal sex distribution, including 10% over-recruitment for attrition) would be used. Power calculations indicate n=10 per group achieves 82% power to detect a medium effect size (Cohen's d=0.8) at α=0.05, based on published tau phosphorylation variability in this model. Animals would be randomly assigned using a computer-generated sequence stratified by sex. Any animal meeting pre-defined exclusion criteria (>20% body weight loss unrelated to the experiment) would be excluded and documented.

Circadian disruption

CJL would be induced by shifting the light phase forward by 8 hours every 3 days for 12 weeks — an established protocol that disrupts both central and peripheral circadian synchrony without total sleep deprivation (6). Locomotor activity would be continuously recorded by infrared actigraphy to confirm entrainment disruption.

Pharmacological rescue

Group 5 would receive MCC950 (50 mg/kg i.p., every other day), a selective NLRP3 inhibitor with established central nervous system (CNS) penetrance in mice (7). Group 4 would receive an equivalent vehicle.

Primary endpoints

At endpoint (15 months of age), microglia would be isolated from

brain tissue by CD11b+ magnetic-activated cell sorting (MACS). Clock gene expression (BMAL1 and Per2) would be measured by quantitative reverse transcription polymerase chain reaction (qRT-PCR) from cells collected at Zeitgeber Time (ZT) 0, 6, 12, and 18; cosinor rhythmometry would quantify amplitude and phase. NLRP3, ASC, and cleaved IL-1β would be measured by Western blot and Luminex multiplex immunoassay in brain tissue and cerebrospinal fluid (CSF). Tau phosphorylation (AT8, PHF1 antibodies) and upstream kinase activity (active GSK-3β at pTyr216; CDK5/p25 complex) would be assessed by Western blot. NFT burden would be quantified by AT8 immunohistochemistry and Gallyas silver staining. MWM and novel object recognition (NOR) tasks would assess spatial and recognition memory, conducted blind to group between ZT4–ZT8. Histological analyses would be performed by investigators blinded to group assignment throughout.

Statistical analysis

One-way ANOVA with Tukey's post-hoc test; Bonferroni correction applied across multiple endpoints. Sex was included as a covariate throughout. Within-group Pearson correlations (or Spearman's if normality is violated per the Shapiro-Wilk test) would assess the mechanistic linkage between IL-1β and tau phosphorylation. Missing data would be reported transparently; sensitivity analyses would be conducted if attrition exceeds 15%.

Feasibility and Justification

All proposed methods are well established in the field. P301S mice are commercially available, widely used, and well characterized. The CJL protocol requires only programmable lighting cabinets and has been implemented in numerous published studies. MACS isolation, qRT-PCR, Western blot, and Luminex assays are routine techniques in university neuroscience facilities. MCC950 is commercially available at an appropriate purity. An 18-month timeline — 3 months of colony setup, 12 months for the main cohort, and 3 months for analysis — is realistic under faculty supervision.

Expected Outcomes and Impact

P301S+CJL mice would likely show stronger NLRP3 activation, increased tau phosphorylation, and poorer memory performance compared with P301S+NLC controls. If MCC950 were able to largely reverse these effects, this would suggest that circadian disruption plays a direct pathomechanistic role in Alzheimer's disease, rather than simply being associated with it. This would represent an important conceptual shift. From a clinical perspective, identifying microglial NLRP3 activation as the causal link would support future trials of NLRP3 inhibitors in AD patients with documented sleep problems, a large group that remains relatively underserved. Several NLRP3-targeting therapies are already moving into Phase II testing, making this a realistic therapeutic direction.

Limitations and Risk Assessment

The P301S model carries a rare inherited tau gene mutation (MAPT) absent in most sporadic AD cases; however, the CDK5/GSK-3β kinase pathway under study is mutation-independent and expected to generalize across tauopathies. The CJL protocol disrupts peripheral circadian rhythms — including the hypothalamic-pituitary-adrenal (HPA) axis stress response — in addition to brain clocks, which could confound CNS-specific interpretation. Plasma corticosterone measurements and continuous actigraphy monitoring would be used to characterize systemic effects. Because MCC950 inhibits NLRP3 across all cell types, a conditional microglial Nlrp3 knockout (Cx3cr1-Cre; Nlrp3fl/fl) is planned as a confirmatory follow-up. Sex-stratified analysis is incorporated throughout, given documented differences in tau pathology progression between male and female P301S mice.

Future Directions

Confirmatory experiments using microglial-specific Bmal1 conditional knockout mice (Cx3cr1-Cre; Bmal1fl/fl) would establish whether the CJL effect is specifically driven by the microglial clock rather than peripheral inflammatory signals. Validation in the MAPT H1-GR humanized knock-in model would extend findings beyond the overexpression system. Translational studies measuring plasma p-tau217, serum IL-1β, and objective sleep metrics in early-stage AD patients stratified by circadian dysfunction severity would provide the first human biomarker evidence for this mechanistic axis and inform the design of NLRP3-targeting clinical trials.

Ethical Considerations

Any study of this design would require Institutional Animal Care and Use Committee (IACUC) approval and compliance with Canadian Council on Animal Care (CCAC) guidelines. The 3Rs principles would guide all decisions. Replacement: *in vitro* microglial models would be used first to refine hypotheses before moving to animal work. Reduction: group sizes are set by power analysis to minimize animal use. Refinement: the CJL protocol avoids total sleep deprivation; humane endpoints would be defined in advance (>20% body weight loss; severe motor impairment); animals would be euthanized by approved methods. No human participants or biological materials would be involved.

Abbreviations

AD = Alzheimer's disease; ASC = apoptosis-associated speck-like protein containing a CARD; BMAL1 = brain and muscle ARNT-like 1; CCAC = Canadian Council on Animal Care; CDK5 = cyclin-dependent kinase 5; CJL = chronic jet lag; CNS = central nervous system; CSF = cerebrospinal fluid; GSK-3β = glycogen synthase kinase 3 beta; HPA = hypothalamic-pituitary-adrenal; IACUC = Institutional Animal Care and Use Committee; IL-1β = interleukin-1

beta; MACS = magnetic-activated cell sorting; MAPT = microtubule-associated protein tau; MWM = Morris Water Maze; NFT = neurofibrillary tangle; NLC = normal light-dark cycle; NLRP3 = NOD-like receptor protein 3; NOR = novel object recognition; Per2 = period circadian regulator 2; PP2A = protein phosphatase 2A; p-tau217 = phosphorylated tau-217; qRT-PCR = quantitative reverse transcription polymerase chain reaction; SCN = suprachiasmatic nucleus; WT = wild-type; ZT = Zeitgeber Time

References

1. World Health Organization. (2023). Dementia: Key facts. https://www.who.int/news-room/fact-sheets/detail/dementia
2. Warfield, A. E., Gupta, P., Ruhmann, M. M., Jeffs, Q. L., Guidone, G. C., Rhymes, H. W., Thompson, M. I., & Todd, W. D. (2023). A brainstem to circadian system circuit links tau pathology to sundowning-related disturbances in an Alzheimer's disease mouse model. Nature Communications 14(1), 5027. https://doi.org/10.1038/s41467-023-40546-w
3. Iweka, C. A., Seigneur, E., Hernandez, A. L., Peters, L., Rosarda, J. D., Quinti, L., … Bhatt, D. L. (2023). Myeloid deficiency of the intrinsic clock protein BMAL1 accelerates cognitive aging by disrupting microglial synaptic pruning. Journal of Neuroinflammation 20(1), 48. https://doi.org/10.1186/s12974-023-02727-8
4. Lu, Q., & Kim, J. Y. (2025). Microglial clock dysfunction during neuroinflammation impairs oligodendrocyte progenitor cell recruitment and disrupts neuroimmune homeostasis. Frontiers in Immunology 16, 1620343. https://doi.org/10.3389/fimmu.2025.1620343
5. Ising, C., Venegas, C., Zhang, S., Scheiblich, H., Schmidt, S. V., Vieira-Saecker, A., … Heneka, M. T. (2019). NLRP3 inflammasome activation drives tau pathology. Nature, 575(7784), 669–673. https://doi.org/10.1038/s41586-019-1769-z
6. Castanon-Cervantes, O., Wu, M., Ehlen, J. C., Paul, K., Gamble, K. L., Johnson, R. L., … Davidson, A. J. (2010). Dysregulation of inflammatory responses by chronic circadian disruption. Journal of Immunology, 185(10), 5796–5805. https://doi.org/10.4049/jimmunol.1001026
7. Coll, R. C., Robertson, A. A. B., Chae, J. J., Higgins, S. C., Muñoz-Planillo, R., Inserra, M. C., … O'Neill, L. A. J. (2015). A small-molecule inhibitor of the NLRP3 inflammasome for the treatment of inflammatory diseases. Nature Medicine, 21(3), 248–255. https://doi.org/10.1038/nm.3806
8. World Bank. (2023). Population ages 65 and above (% of total population) [Data set]. https://data.worldbank.org/indicator/SP.POP.65UP.TO.ZS

GLP-1 Follicular Extracellular Vesicles: β-Cell Regenerative Therapy in Type 2 Diabetes Mellitus and PMOS Dysfunction

GLP-1 Vésicules folliculaires extracellulaires : thérapie régénérative à cellules β dans le diabète sucréel de type 2 et les dysfonctionnements du PMOS

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Abstract | Résumé

Type 2 Diabetes Mellitus (T2DM) is characterized by β-cell dysfunction and loss of insulin-producing mass in the pancreas (1). Current glucose-lowering therapies slow disease progression but fail to reverse β-cell decline (2). Polyendocrine Metabolic Ovarian Syndrome (PMOS) and T2DM share underlying metabolic dysfunction, including insulin resistance and chronic inflammatory signaling. Restoring β-cell health may represent a promising strategy to improve metabolic regulation, improve glycemic management, and potentially alleviate PMOS-related infertility (3).

This study proposes engineering ovarian follicular-fluid-derived extracellular vesicles (FF-EVs) to support pancreatic β-cell regeneration. FF-EVs are intended to enhance β-cell mass, improve glucose-stimulated insulin secretion, and reduce T2DM-linked reproductive dysfunction like PMOS (4).

Follicular fluid from hormonally stimulated female C57BL/6 mice will be used to isolate EVs enriched in reproductive microRNAs and insulin-like growth factors (5). These are engineered to display glucagon-like-peptide-1 (GLP-1) on their membrane surface through fusion with EV-anchoring Lysosome-Associated Membrane Protein 2B (LAMP2B), enabling selective pancreatic β-cell uptake through GLP-1 receptor binding. The engineered EVs will be loaded with pro-regenerative cargo proteins (harmine and mRNAs encoding PDX1, NGN3, and MAFA), promoting β-cell proliferation and β-cell mass restoration (6).

Optimal EV formulations will be tested in C57BL/6 mice (no-STZ, STZ-induced). β-cell viability, diabetic markers (Glut2, Glut4, INSR, IRS1/2) were assessed *in vitro* through glucose-stimulated insulin secretion, then *in vivo* evaluation of pancreatic β-cell proliferation (7).

Translational evaluation will use human β-cell lines and *in vitro* human organoids (pancreas-ovarian follicles) to validate β-cell recovery and improvements of metabolic-reproductive parameters.

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Le diabète sucré de type 2 (DT2) se caractérise par un dysfonctionnement des cellules β et une perte de masse productrice d'insuline dans le pancréas (1). Les thérapies actuelles de réduction de la glycémie ralentissent la progression de la maladie mais ne parviennent pas à inverser le déclin des cellules β (2). Le syndrome ovarien métabolique polyendocrine (PMOS) et le DT2DM partagent des dysfonctionnements métaboliques sous-jacents, notamment la résistance à l'insuline et la signalisation inflammatoire chronique. Restaurer la santé des cellules β pourrait représenter une stratégie prometteuse pour améliorer la régulation métabolique, améliorer la gestion glycémique et potentiellement atténuer l'infertilité liée au PMOS (3).

Cette étude propose d'ingénier des vésicules extracellulaires dérivées du liquide folliculaire ovarien (FF-EV) pour soutenir la régénération pancréatique β-cellulaire. Les FF-EV visent à augmenter la masse cellulaire β, à améliorer la sécrétion d'insuline stimulée par le glucose et à réduire les dysfonctionnements reproductifs liés au DT2DM comme le PMOS (4).

Le liquide folliculaire de souris C57BL/6 stimulées hormonalement sera utilisé pour isoler les EV enrichies en microARN reproducteurs et en facteurs de croissance de type insuline (5). Ces appareils sont conçus pour afficher du peptide similaire-glucagon-1 (GLP-1) à leur surface membranaire par fusion avec la protéine membranaire lysosome-associée 2B (LAMP2B) ancrant les EV, permettant une captation sélective des cellules pancréatiques β via la liaison aux récepteurs GLP-1. Les VE conçus seront chargés de protéines cargo pro-régénératives (harmine et ARNm codant pour PDX1, NGN3 et MAFA), favorisant la prolifération des cellules β et la restauration de masse des cellules β (6).

Les formulations optimales de la cellule électrique seront testées chez des souris C57BL/6 (sans STZ, induite par STZ). La viabilité des β-cellules et les marqueurs diabétiques (Glut2, Glut4, INSR, IRS1/2) ont été évalués *in vitro* via la sécrétion d'insuline stimulée par le glucose, puis une évaluation *in vivo* de la prolifération des β pancréas (7). L'évaluation translationnelle utilisera des lignées β humaines et des organoïdes humains *in vitro* (follicules pancréas-ovaires) pour valider la récupération des cellules β et l'amélioration des paramètres métabolo-reproducteurs.

Keywords: Follicular fluid, pancreatic β-cell regeneration, EV-based therapeutics, LAMP2B fusion proteins, EV cargo loading (siRNA, mRNA, miRNA), vesicle-mediated RNA delivery, ovarian microenvironment, Type 2 Diabetes Mellitus

Background Information

T2DM is a prevalent metabolic disorder, and while current therapies control hyperglycemia, they do not address underlying β-cell dysfunction, making β-cell preservation and regeneration critical therapeutic goals. T2DM intersects with reproductive disorders (PMOS), where affected women have a 4-8.8-fold higher risk of developing T2DM (8). This shared metabolic-hormonal dysfunction highlights the need to improve pancreatic β-cell functions. EVs are biocompatible with therapeutic carriers capable of transporting proteins and bioactive molecules. FF-EVs are appealing because they contain developmental and endocrine signals that support cell survival. For β-cell targeting, FF-EVs can be engineered to display GLP-1 fused to the EV membrane protein LAMP2B, enabling receptor-specific delivery.
FF-EVs is expected to reverse β-cell loss, addressing T2DM and its intersection with reproductive metabolic disorders.

Research Idea

Two therapeutic benefits are offered by FF-EV-GLP-1-LAMP2B construct. First, the GLP-1 cargo helps maintain β-cell identity while promoting β-cell regeneration and enhancing insulin secretion. Second, the inherent bioactivity of FF-EVs, including growth signaling factors and developmental microRNAs, further supports metabolic regulation and glycemic control (9).
When combined, this produces an EV platform that can improve β-cell function in diabetic conditions and resolve T2DM-mediated reproductive issues in females.

Rationale

Conventional T2DM therapies are limited by poor β-cell targeting and low durability *in vivo* (10). FF-EVs transport developmental miRNAs, metabolic regulators, and proteins directly to endocrine targets. This minimizes off-target exposure and improves β-cell-specific therapeutic action (11). FF-EVs attach to pancreatic β-cells when designed with GLP-1R-targeting LAMP2B fusion peptide, enabling selective docking and transmission of regeneration signals (6). This study will also examine reproductive markers owing to correlations between female reproductive disorders and T2DM (3). The MIN6 mouse cell line is utilized as it transports glucose and secretes insulin similarly to pancreatic β-cells (12)

Hypothesis

FF-EVs engineered with a GLP-1R β-cell–homing peptide fused to LAMP2B are hypothesized to enhance β-cell targeting and improve glycemic regulation in T2DM. Furthermore, the resulting improvements in metabolic function may contribute to alleviating reproductive imbalances associated with PMOS.

Methodology

For β-cell specificity, engineered FF-EV-GLP-1-LAMP2B fusion protein is used (13). C-terminus GLP-1 will be fused to the N-terminus of LAMP2B through glycine–serine flexible linker (G_4S), preventing steric hindrance, allowing proper folding and surface presentation (13, 14). Granulosa cells will be isolated from hormonally stimulated adult female mice, cultured *in vitro*. Cells are transfected with fusion plasmid GLP-1-LAMP2B construct (CMV promoter and SV40 polyA terminator), enabling secretion of FF-EVs displaying GLP-1 on the surface for GLP-1R–mediated β-cell homing (15, 16). Purified FF-EVs will be loaded with β-cell cargo proteins such as harmine, a selective DYRK1A inhibitor, to promote β-cell proliferation (17). Furthermore, mRNAs encoding PDX1, NGN3, and MAFA will be included to induce pancreatic endocrine fate through NGN3, establish β-cell lineage identity through PDX1, and enhance glucose responsiveness through MAFA (18). miR-132 will also be incorporated to enhance β-cell survival and proliferation through PTEN/AKT/FOXO3 signaling pathways (19). Additionally, betacellulin, a growth factor involved in β-cell differentiation, survival, and insulin secretion, will be included within the FF-EVs. The mRNA and miRNA will be incorporated using electroporation; harmine, and Betacellulin through sonication (20).

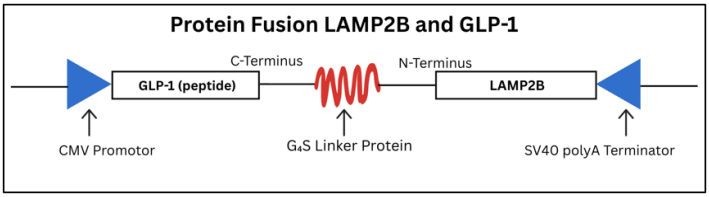

Figure 1. Structure of GLP-1-LAMP2B fusion protein. Figure 1 shows a schematic representation of the modified fusion protein utilized for β-cell selectivity in extracellular vesicles formed from follicular fluid (FF-EVs). A flexible glycine–serine linker (G_4S) fuses the C-terminus of glucagon-like peptide-1 (GLP-1) to the N-terminus of lysosome-associated membrane protein 2B (LAMP2B), reducing steric hindrance and facilitating appropriate protein folding and surface display. A cytomegalovirus (CMV) promoter drives the construct, while an SV40 polyadenylation signal ends. GLP-1's surface presentation makes it easier for EVs to target and home to pancreatic β-cells via the GLP-1 receptor (GLP-1R).

Experimental Design

In the proposed study, the independent variable is the type of treatment administered to MIN6 cells, mice, human cells, and organoids. Dependent variables include β-cell functional outcomes and expression levels of diabetic markers (*D) and reproductive markers (*R). The control variables include cell line (MIN6), standardized EV isolation procedures, uniform culture conditions (DMEM composition, temperature, CO_2 levels, and confluence at treatment), EV dosing (1×10^8–1×10^9 particles/mL), treatment duration (24-hour incubation), controlled 0.5-1mM STZ exposure conditions.

Table 1. Diabetic Molecular Markers

Experimental Model	Diabetic Markers (*D)
In-Vitro MIN6 Cell Line	Ins1/Ins2
In-Vivo C57BL/6 Mice Model	Serum markers: insulin, glucose, C-peptide, leptin, adiponectin, TGs Tissue markers: Pdx1, MAFA, Pparγ
Human β-Cell Line	INS, C-peptide
Pancreatic Organoids	Glut1, Glut2, Glut4, INSR, IRS1/2

Table 1. shows a summary of key metabolic markers used to evaluate β-cell function and glucose homeostasis *in vitro* and *in vivo* models. MIN6 cells will be analyzed for insulin gene expression through Ins1/Ins2, while the C57BL/6 mouse model includes both serum markers (insulin, glucose, C-peptide, leptin, adiponectin, triglycerides) and pancreatic tissue markers (Pdx1, MAFA, Pparγ). Human β-cell lines were assessed for insulin (INS) and C-peptide levels, and pancreatic organoids were evaluated for glucose transport and insulin signaling markers (GLUT1, GLUT2, GLUT4, INSR, IRS1/2).

Table 2. Reproductive Endocrine Markers

Experimental Model	Reproductive Markers (*R)
In-Vitro MIN6 Cell Line	ESR1, ESR2, FSHR
In-Vivo C57BL/6 Mice Model	Esr1/2, Fshr Serum: Estradiol, Progesterone, LH, FSH,
Human β-Cell Line	ESR1, ESR2, FSHR
Ovarian Follicle Organoids	ESR1/ESR2, FSHR

Table 2. shows a summary of reproductive and hormonal markers used to assess endocrine function across experimental systems. MIN6 and human β-cell lines will be evaluated for estrogen receptor expression (ESR1, ESR2) and follicle-stimulating hormone receptor (FSHR). *In vivo* C57BL/6 mice will be analyzed for both gene expression (Esr1/2, Fshr) and circulating reproductive hormones (estradiol, progesterone, luteinizing hormone [LH], and follicle-stimulating hormone [FSH]. Ovarian follicle organoids will be assessed for ESR1/ESR2 and FSHR expression.

In Vitro

Cell culture: MIN6 cells will be grown in DMEM with 10% heat-inactivated FBS, 100 U/mL penicillin, 100 µg/mL streptomycin, and 2.0 mM glutamine in CO_2 incubator at 37°C with 5% CO_2. Cells seeded into 12-or 24-well plates to reach 70-80% confluence over 24h before treatment (21).

Induction of β-cell injury (STZ): The normal β-cell will receive no STZ. MIN6 cells will be treated to STZ (0.5-1mM) for 24h to elicit T2D-like/β-cell damage. The wells will be cleansed and reintroduced to fresh medium before treatment. Insertion of EVs into MIN6 cells will be accomplished by directly adding EV-containing media to wells at 1×10^8, 5×10^8, 1×10^9 particles/mL dosage. EVs will be incubated with MIN6 cells for 24h and absorbed by cells through endocytosis.

Experimental groups: Three treatment groups will be created for STZ and No STZ. The control group contains MIN6 cells with no EV. FF-EV-GLP1 group will contain MIN6 cells in medium containing FF-EVs with GLP-1. FF-EV-GLP1-LAMP2B group will contain MIN6 in medium containing FF-EVs with GLP-1-LAMP2B.

Detection: Following EV exposure, qPCR/Western blot for β-cell markers (PDX1, MAFA, INS, GLUT2) will be conducted. Following those studies, best EV conditions for restoring/enhancing β-cell activity in STZ-injured MIN6 cells will be chosen for mouse studies. *D markers will be validated using ELISAs (22).

In Vivo

Selection process; n=10: The in-vivo mice profile includes: C57BL/6 female mice aged 8-10 weeks, 20-25g, kept in standardized settings (12h light/dark cycle, controlled temperature/humidity, consistent food/water) (23). Before treatment, baseline fasting blood glucose (FBG) levels, body weight, and health condition recorded. Mice randomly assigned to no-STZ or STZ-induced groups (24). Diabetes in STZ group produced using low-dose STZ (50 mg/kg intraperitoneally, 5 days), hyperglycemia confirmed before EV therapy (25).

Experimental groups: Experimental groups will consist of both no-STZ and STZ-induced conditions, each further divided into three treatment groups. The control group will contain normal β-cells receiving vehicle treatment only. The FF-EV-GLP1 group will receive GLP-1-enriched FF-EVs administered through subcutaneous injections twice per week for four weeks (26). Similarly, the FF-EV-GLP1-LAMP2B group will receive LAMP2B-engineered GLP-1 EVs through subcutaneous injections twice per week for four weeks.

Monitoring/Detection: Weekly measurements of body weight and fasting blood glucose (FBG) will be collected throughout the study. Intraperitoneal glucose tolerance tests will be conducted at designated intervals, while ELISA assays will be used to quantify plasma insulin levels (27). At the conclusion of the study, pancreatic tissues will be collected for gene expression analysis and histological assessment of β-cell mass and apoptotic indicators. Ovarian tissues will also be analyzed to assess follicle morphology, with *D and *R levels detected through ELISA assays.

Translational study with human β-cell and organoids: Both diabetic and non-diabetic human β-cell lines will contain identical experimental groups, including a control group receiving vehicle only, an FF-EV-GLP1 group treated with GLP-1 enriched FF-EVs, and an FF-EV-GLP1-LAMP2B group treated with LAMP2B-engineered GLP-1 EVs. *D screened conditions will also be included. Similarly, human pancreatic and follicular organoid models will undergo the same treatment conditions. Under non-diabetic conditions, the study will assess EV penetration into three-dimensional tissue structures. Under diabetic conditions, the study will evaluate whether EV mediated GLP-1 delivery improves β-cell function within 3D tissue models mimicking Type 2 Diabetes Mellitus (28). Both *D and *R screened conditions will be included

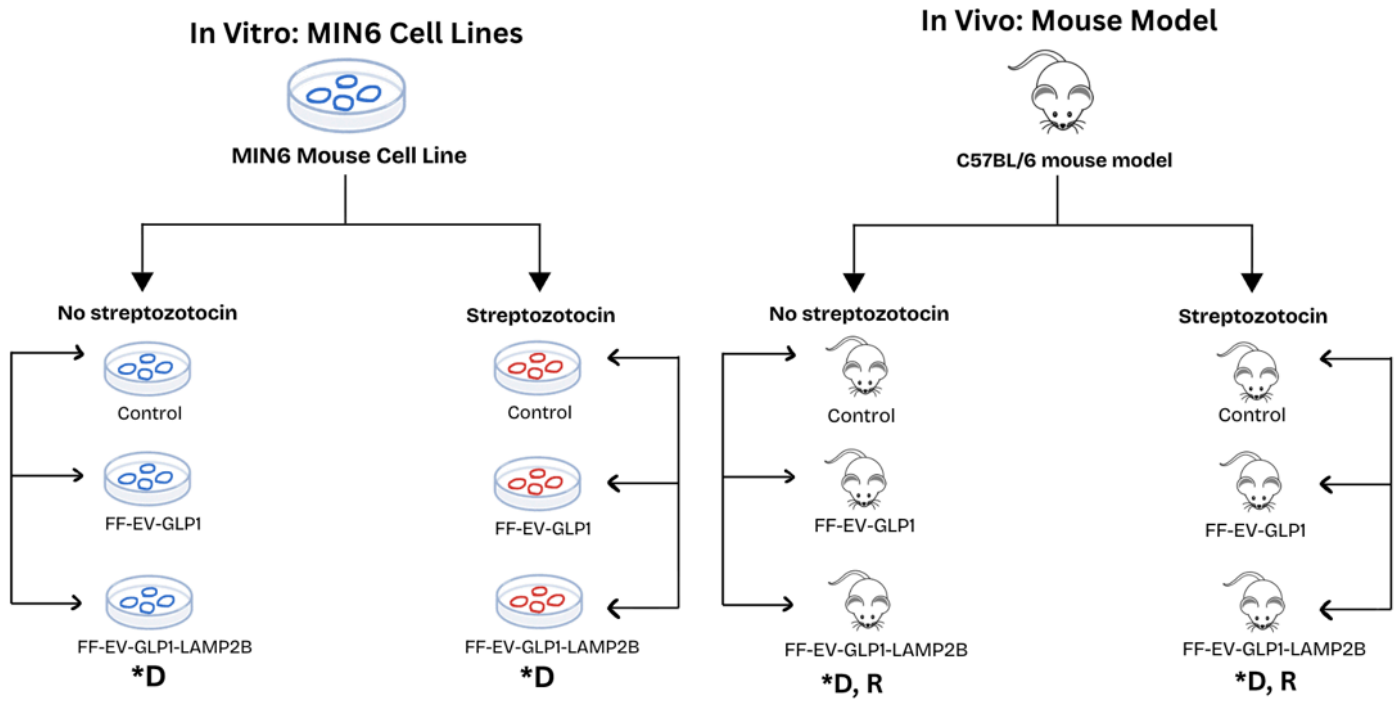

Figure 2. FF-EV based β-cell targeting β MIN6 cells and C57BL/6 mouse models. Figure 2. shows a schematic overview of experimental design in MIN6 β-cell lines and C57BL/6 mouse models. Cells and animals were divided into control, FF-EV-GLP1, and FF-EV-GLP1-LAMP2B treatment groups under both basal (no streptozotocin) and diabetic (streptozotocin-induced) conditions. Outcomes were assessed using diabetic metabolic markers (*D) *in vitro* and both metabolic and reproductive markers (*D, R) *in vivo*.

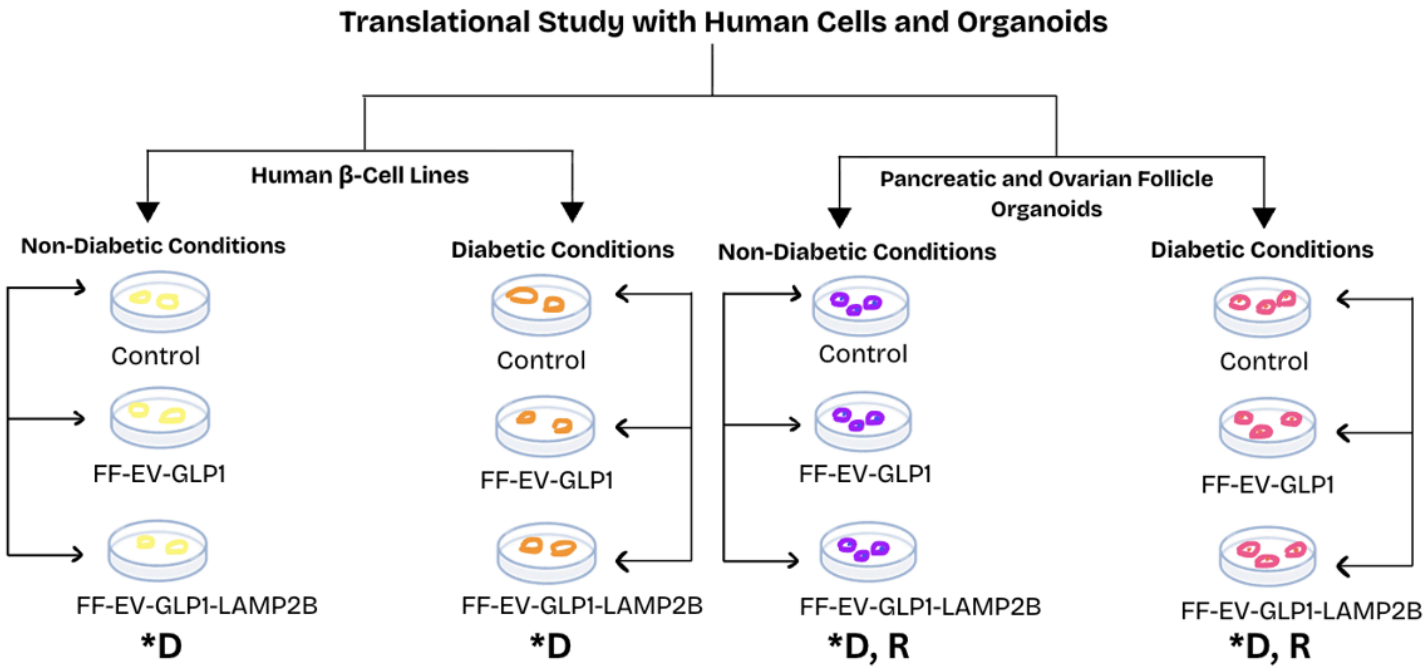

Figure 3. Translational validation of FF-EV–based therapy in human β-cell lines and pancreatic and ovarian follicle organoid systems. Figure 3 shows a translational validation assessing therapeutic efficacy under non-diabetic and diabetic conditions in human β-cell lines and pancreatic and ovarian follicle organoids. Treatment groups included control, FF-EV-GLP1, and FF-EV-GLP1-LAMP2B. Outcomes were evaluated using diabetic metabolic markers (*D) and reproductive endocrine markers (*R) to assess both metabolic and endocrine function

within the organoid studies.

Expected Outcomes & Impact

Engineered follicular extracellular vesicles (FF-EV-GLP-1-LAMP2B) are expected to improve pancreatic β-cell survival, proliferation, and glucose-stimulated insulin secretion in both *in vitro* and *in vivo* models, along with improved glycemic control and metabolic marker normalization. Additionally, because T2DM and reproductive dysfunction share metabolic pathways, EV treatment may improve endocrine signaling and reproductive markers. If effective, this method has the potential to extend regenerative therapies beyond symptom management and into disease-modifying medicines for T2DM and associated disorders.

Limitations & Risk Assessment

There may be some restrictions and risks associated with this proposed study. Reproducibility and therapeutic dependability may be impacted by variations in EV isolation, cargo loading efficiency, and batch consistency. While the goal of GLP-1-LAMP2B surface modification is to improve β-cell targeting, variations in EV uptake among cell types may decrease targeting specificity and result in off-target consequences. Furthermore, the intricacy of human T2DM may not be fully captured by widely used diabetes models, such as STZ-induced systems, which limit translational applicability.

Conclusion

If the alternative hypothesis is accepted, FF-EV-GLP1-LAMP2B will target pancreatic β-cells, deliver regenerative cargo, enhance β-cell proliferation, and reduce metabolic dysfunction in diabetes and PMOS. This would support EV-based therapeutics as a biocompatible strategy for reversing β-cell loss in T2DM and improving reproductive health.

Future studies will use letrozole-induced C57BL/6 mice to model PMOS-associated dysfunction, as letrozole is a potent aromatase inhibitor that interferes with the conversion of androgens to estrogens, resulting in hyperandrogenemia and key metabolic and reproductive characteristics of PMOS (32). This model makes it possible to look into metabolic and endocrine issues related to the relationship between T2DM and PMOS. Future research in this area will test the therapeutic efficiency of modified follicular extracellular vesicles (FF-EV-GLP-1-LAMP2B) *in vivo*, particularly their capacity to improve PMOS-related and reproductive dysfunction while simultaneously promoting β-cell regeneration. By confirming treatment effects across interrelated physiological systems, this strategy will increase the study's translational value.

Definitions

1. Betacellulin: A growth factor from the epithelial growth factor family that promotes pancreatic β-cell proliferation, survival, and maturation; often used to enhance insulin-producing cell development (29).
2. DSPE-PEG–Peptide Insertion: A membrane-engineering method where a lipid–PEG–peptide conjugate (DSPE-PEG–peptide) is spontaneously inserted into the extracellular vesicle (EV) membrane. DSPE is a hydrophobic phospholipid that anchors into the EV lipid bilayer, while PEG provides a flexible spacer that displays the peptide on the EV surface. This allows targeted ligands (GLP-1) to be added to EVs without genetic modification (30).
3. Electroporation: A laboratory method that uses short electrical pulses to temporarily open pores in cell or EV membranes, allowing DNA, RNA, or drugs to enter (31).
4. ELISA: A biochemical assay used to quantify proteins by using antigen–antibody binding coupled with a colorimetric or fluorescent readout (32).
5. GLP1: An incretin hormone that enhances glucose-stimulated insulin secretion and slows gastric emptying; β-cells express its receptor (GLP-1R), enabling targeted therapies (33).
6. Harmine: A small-molecule inhibitor of DYRK1A that stimulates robust proliferation of human pancreatic β-cells by releasing cell-cycle suppression (34).
7. Hyperglycemia: A condition characterized by abnormally high blood glucose levels, typically resulting from impaired insulin secretion, insulin resistance, or both (35).
8. Incretin: A class of gut-derived hormones (such as GLP-1 and GIP) released after eating that enhance glucose-dependent insulin secretion and help regulate blood sugar levels (36).
9. LAMP2B: An EV-associated membrane protein commonly used as a scaffold for displaying targeting peptides on extracellular vesicles through fusion-protein engineering (6).
10. PMOS: Polyendocrine Metabolic Ovarian Syndrome, a prevalent reproductive endocrine disorder in women characterized by hormonal imbalance, irregular ovulation, metabolic dysfunction, and an increased risk of insulin resistance and Type 2 diabetes (8).
11. Pancreatic β-cell: A specialized endocrine cell located in the islets of Langerhans responsible for producing, storing, and secreting insulin in response to glucose (37).
12. Follicular Extracellular vesicles: Nanoparticles naturally present in ovarian follicular fluid that carry proteins, RNAs, and signaling molecules involved in reproductive and metabolic regulation (38).
13. qPCR: A laboratory technique used to quantify gene expression by measuring mRNA levels (39).
14. Sonification: A technique that uses high-frequency sound waves to break apart membranes or mix biological samples; in EV work, can be used to load cargo into vesicles (40).
15. Streptozotocin: A chemical β-cell toxin that induces diabetes *in vitro* and *in vivo* by causing DNA alkylation and selective β-cell death; used to create diabetic models (7).
16. Western blot: A protein-based technique used to detect and quantify specific proteins via antibody binding (41).

References

1. S. Kassem, A. Rajpal, M. V. Barreiro, F. Ismail-Beigi, Beta-cell function in type 2 diabetes (T2DM): Can it be preserved or enhanced? Journal of Diabetes 15, 817–837 (2023)
2. D. Porte, S. E. Kahn, Beta-cell dysfunction and failure in type 2 diabetes: potential mechanisms. Diabetes 50 (suppl. 1), S160–S163 (2001).
3. "The Link Between Diabetes and Reproductive Health" (Gynecology Associates of Gwinnett, 2017); https://gyngwinnett.com/the-link-between-diabetes-and-reproductive-health/.
4. J. Qian, R. Zhu, R. Yan, X. Long, F. Guo, Isolation of mouse ovarian follicles for single-cell RNA-seq and in vitro culture. STAR Protocols 3, 101537 (2022).
5. P. Wang, J. C. Alvarez-Perez, D. F. Felsenfeld, H. Liu, S. Sivendran, A. Bender, et al., A high-throughput chemical screen reveals that harmine-mediated inhibition of DYRK1A increases human pancreatic beta cell replication. Nature Medicine 21, 383–388 (2015).
6. R. Sakai, S. Aizawa, H. C. Lee-Okada, K. Hase, H. Fujita, H. Kikuchi, et al., The lysosomal membrane protein LAMP2B mediates microlipophagy to target obesity-related disorders. Cell Reports 44, 115829 (2025); https://pubmed.ncbi.nlm.nih.gov/40503939/.
7. Ghasemi, S. Jeddi, Streptozotocin as a tool for induction of rat models of diabetes: a practical guide. EXCLI Journal 22, 274–294 (2023); https://www.excli.de/index.php/excli/article/view/5720/4520.
8. Krans, "What's the Connection Between Polycystic Ovarian Syndrome (PCOS) and Diabetes?" (Healthline Media, 2015); https://www.healthline.com/health/diabetes/are-pcos-and-diabetes-connected.
9. M. Ezzati, M. Izadpanah, Extracellular vesicles in monitoring and modulation of oocyte competence: focus on exosomes. Journal of Ovarian Research 18, 1 (2025).
10. B. Gieroba, A. Kryska, A. Sroka-Bartnicka, Type 2 diabetes mellitus – conventional therapies and future perspectives in innovative treatment. Biochemistry and Biophysics Reports 42, 102037 (2025); https://www.sciencedirect.com/science/article/pii/S2405580825001244.
11. M. Pournourali, N. Mizban, R. Ehsani, S. Ebrahimian, T. Nadri, N. Azari-Dolatabad, Extracellular vesicles: key mediators in in vitro embryo production. Frontiers in Veterinary Science 12, 12405430 (2025); https://pmc.ncbi.nlm.nih.gov/articles/PMC12405430/.
12. U. G. Bhat, V. Ilievski, T. G. Unterman, K. Watanabe, Porphyromonas gingivalis lipopolysaccharide upregulates insulin secretion from pancreatic β cell line MIN6. Journal of Periodontology 85, 1629–1636 (2014); https://dx.doi.org/10.1902%2Fjop.2014.140070.
13. Z. Li, X. Zhou, X. Gao, D. Bai, Y. Dong, W. Sun, et al., Fusion protein engineered exosomes for targeted degradation of specific RNAs in lysosomes: a proof-of-concept study. Journal of Extracellular Vesicles 9, 1816710 (2020); https://pubmed.ncbi.nlm.nih.gov/33133429/.
14. V. P. Reddy Chichili, V. Kumar, J. Sivaraman, Linkers in the structural biology of protein–protein interactions. Protein Science 22, 153–167 (2013); https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3588912/.
15. N. S. Yew, D. M. Wysokenski, K. X. Wang, R. J. Ziegler, J. Marshall, D. McNeilly, et al., Optimization of plasmid vectors for high-level expression in lung epithelial cells. Human Gene Therapy 8, 575–584 (1997).
16. Z. Zheng, Y. Zong, Y. Ma, Y. Tian, Y. Pang, C. Zhang, et al., Glucagon-like peptide-1 receptor: Mechanisms and advances in therapy. Signal Transduction and Targeted Therapy9, 1–29 (2024).
17. A.C. Title, M. Karsai, J. Mir-Coll, Ö. Y. Grining, C. Rufer, S. Sonntag, et al., Evaluation of the effects of harmine on β-cell function and proliferation in standardized human islets using 3D high-content confocal imaging and automated analysis. Frontiers in Endocrinology 13 (2022).
18. Y. Zhu, Q. Liu, Z. Zhou, Y. Ikeda, PDX1, Neurogenin-3, and MAFA: critical transcription regulators for beta cell development and regeneration. Stem Cell Research & Therapy 8(2017).
19. C. Bai, Q. Ren, H. Liu, X. Li, W. Guan, Y. Gao, miR-212/132-enriched extracellular vesicles promote differentiation of induced pluripotent stem cells into pancreatic beta cells. Frontiers in Cell and Developmental Biology 9 (2021).
20. L. Alvarez-Erviti, Y. Seow, H. Yin, C. Betts, S. Lakhal, M. J. A. Wood, Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. Nature Biotechnology29, 341–345 (2011); https://www.nature.com/articles/nbt.1807.
21. R. Rajappa, D. Sireesh, M. B. Salai, K. M. Ramkumar, S. Sarvajayakesavulu, S. V. Madhunapantula, Culturing of MIN6 cells. Frontiers in Pharmacology (2019); https://bio-protocol.org/exchange/minidetail?id=7991235&type=30.
22. "ELISA Technique" (Cleveland Clinic, 2023); https://my.clevelandclinic.org/health/articles/24990-elisa.
23. National Research Council, "Effects of Housing Density and Cage Type on Young Adult C57BL/6J Mice" (National Academies Press, 2024); https://www.ncbi.nlm.nih.gov/books/NBK25400/.
24. S. E. J. Kamli-Salino, P. A. J. Brown, T. N. Haschler, L. Liang, D. Feliers, H. M. Wilson, et al., Induction of experimental diabetes and diabetic nephropathy using anomer-equilibrated streptozotocin in male C57Bl/6J mice. Biochemical and Biophysical Research Communications 650, 109–116 (2023); https://pubmed.ncbi.nlm.nih.gov/36774688/.
25. J. H. Juang, C. L. Chen, C. W. Kao, S. T. Wu, C. R. Shen, In vivo imaging of immune rejection of MIN6 cells transplanted in C3H mice. Cells 13, 1044 (2024); https://www.mdpi.com/2073-4409/13/12/1044.

26. "Subcutaneous Injection in the Mouse" (Research Animal Training); https://researchanimaltraining.com/articles/subcutaneous-injection-in-the-mouse/.
27. K. Dinger, J. Mohr, C. Vohlen, D. Hirani, E. Hucklenbruch-Rother, R. Ensenauer, et al., Intraperitoneal glucose tolerance test, measurement of lung function, and fixation of the lung to study the impact of obesity and impaired metabolism on pulmonary outcomes. Journal of Visualized Experiments 133 (2018); https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5931777/.
28. K. Bittenglova, D. Habart, F. Saudek, T. Koblas, The potential of pancreatic organoids for diabetes research and therapy. Islets 13, 85–105 (2021).
29. M. Dahlhoff, E. Wolf, M. R. Schneider, The ABC of BTC: Structural properties and biological roles of betacellulin. Seminars in Cell & Developmental Biology 28, 42–48 (2014).
30. D. D. Wang, M. Yang, Y. Zhu, C. Mao, Reiterated targeting peptides on the nanoparticle surface significantly promote targeted vascular endothelial growth factor gene delivery to stem cells. Biomacromolecules16, 3897–3903 (2015); https://pmc.ncbi.nlm.nih.gov/articles/PMC4922499/.
31. "Electroporation" (Thermo Fisher Scientific); https://www.thermofisher.com/ca/en/home/references/gibco-cell-culture-basics/transfection-basics/methods/electroporation.html.
32. M. Alhajj, A. Farhana, M. Zubair, "Enzyme Linked Immunosorbent Assay (ELISA)" (StatPearls Publishing, 2023); https://www.ncbi.nlm.nih.gov/books/NBK555922/.
33. L. Collins, R. A. Costello, "Glucagon-like peptide-1 receptor agonists" (StatPearls Publishing, 2024); https://www.ncbi.nlm.nih.gov/books/NBK551568/.
34. Y. Bao, J. Zhu, X. Mao, M. Zhang, Q. Ao, H. Zhu, et al., Harmine inhibits ovarian cancer migration and invasion and epithelial-mesenchymal transition (EMT) by inhibiting HDAC7 to restore RECK expression. Biochemical Pharmacology 242, 117391 (2025); https://www.sciencedirect.com/science/article/pii/S0006295225006562."
35. Hyperglycemia (High Blood Glucose)" (American Diabetes Association, 2023); https://diabetes.org/living-with-diabetes/treatment-care/hyperglycemia.
36. M. A. Nauck, J. J. Meier, Incretin hormones: Their role in health and disease. Diabetes, Obesity & Metabolism 20 (suppl. 1), 5–21 (2018); https://www.ncbi.nlm.nih.gov/pubmed/29364588.
37. A. Bartolomé, The pancreatic beta cell: Editorial. Biomolecules 13, 495 (2023); https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10046343/.
38. M. de Almeida Monteiro Melo Ferraz, M. Fujihara, J. B. Nagashima, M. J. Noonan, M. Inoue-Murayama, N. Songsasen, Follicular extracellular vesicles enhance meiotic resumption of domestic cat vitrified oocytes. Scientific Reports 10, 1 (2020).
39. J. S. Dymond, Explanatory chapter: Quantitative PCR. Methods in Enzymology 529, 279–289 (2013); https://pubmed.ncbi.nlm.nih.gov/24011054/.
40. "Sonication - an overview" (ScienceDirect Topics); https://www.sciencedirect.com/topics/chemistry/sonication.
41. K. Gavini, K. Parameshwaran, "Western Blot (Protein Immunoblot)" (StatPearls Publishing, 2023); https://www.ncbi.nlm.nih.gov/books/NBK542290/
42. 42.S. Bhatnagar, The discovery and mechanism of action of letrozole. Breast Cancer Research and Treatment 105 (suppl. 1), 7–17 (2007); https://pmc.ncbi.nlm.nih.gov/articles/PMC2001216/.

Quantification of tracer mass in cardiac positron emission tomography using high-performance liquid chromatography

Quantification de la masse tracante en tomographie par émission de positrons cardiaques à l'aide de la chromatographie en phase liquide à haute performance

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Abstract | Résumé

Cardiac sympathetic nervous system dysfunction is associated with multiple cardiovascular diseases and may be assessed non-invasively using positron emission tomography (PET) imaging. Meta-fluorobenzylguanidine labeled with fluorine-18 ([18F]mFBG) is a radiotracer that targets the norepinephrine transporter (NET) and enables quantitative evaluation of sympathetic nerve function. However, tracer formulations contain both radioactive and non-radioactive forms, and excess non-radioactive mass may compete for transporter binding and affect imaging accuracy. This proposal aims to develop a high-performance liquid chromatography (HPLC)-based method to quantify total tracer mass and determine molar activity of [18F]mFBG. The protocol uses calibration standards of non-radioactive mFBG under formulation-matched solvent conditions and provides analysis using UV-detected HPLC to generate a calibration curve. The system analyzes formulated tracer samples to determine total mFBG concentration and allows for the calculation of molar activity as the ratio of radioactivity to total tracer mass. This approach is expected to provide a reproducible method for ensuring biologically negligible tracer mass in preclinical PET studies and may support future clinical translation.

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Le dysfonctionnement du système nerveux sympathique cardiaque est associé à de multiples maladies cardiovasculaires et peut être évalué de manière non invasive à l'aide d'imagerie par tomographie par émission de positrons (TEP). La méta-fluorobenzylguanidine marquée au fluor-18 ([18F]mFBG) est un radiotraceur qui cible le transporteur de noradrénaline (NET) et permet une évaluation quantitative de la fonction du nerf sympathique. Cependant, les formulations tracer contiennent à la fois des formes radioactives et non radioactives, et une masse non radioactive excessive peut rivaliser pour la liaison du transporteur et affecter la précision de l'imagerie. Cette proposition vise à développer une méthode basée sur la chromatographie en phase liquide à haute performance (CLHP) pour quantifier la masse traceuse totale et déterminer l'activité molaire de [18F]mFBG. Le protocole utilise des standards d'étalonnage pour mFBG non radioactif dans des conditions de solvant adaptées à la formulation et fournit une analyse utilisant du CLHP détecté par UV pour générer une courbe d'étalonnage. Le système analyse des échantillons traceurs formulés pour déterminer la concentration totale de mFBG et permet de calculer l'activité molaire comme le rapport radioactivité à la masse totale du traceur. Cette approche devrait fournir une méthode reproductible pour garantir une masse traceuse biologiquement négligeable dans les études précliniques TEP et pourrait soutenir la traduction clinique future.

Keywords: Cardiac PET imaging, fluorine-18 meta-fluorobenzylguanidine ([18F]mFBG), norepinephrine transporter, high-performance liquid chromatography, molar activity, radiotracer quantification, sympathetic nervous system imaging, radiopharmaceutical quality control.

Introduction

The cardiac sympathetic nervous system plays a critical role in regulating cardiac function, and its dysfunction is associated with conditions such as heart failure and arrhythmias. Positron emission tomography (PET) imaging enables non-invasive assessment of sympathetic innervation using radiotracers that target the norepinephrine transporter (NET).

Meta-fluorobenzylguanidine labeled with fluorine-18 ([18F]mFBG) is a PET radiotracer that enters sympathetic nerve terminals via NET and exhibits measurable myocardial uptake and washout kinetics (1, 2). These characteristics provide a quantitative assessment of cardiac sympathetic activity. However, each tracer formulation contains both radioactive and non-radioactive ("cold") mFBG, and excessive non-radioactive mass may compete with the radiotracer for NET binding, potentially confounding imaging results (1).

Molar activity, defined as the ratio of radioactivity to total tracer mass, is therefore a critical parameter. Ensuring high molar activity minimizes biological perturbation and improves the validity of PET measurements. Despite the growing use of [18F]mFBG for cardiac sympathetic nervous system imaging,

studies rarely standardize quantification of total tracer mass and molar activity in formulated samples. Existing studies primarily focus on radiochemical synthesis, biodistribution, and imaging performance, while they inconsistently describe accurate measurements of non-radioactive ("cold") mFBG content (1, 2). This represents an important limitation because excess non-radioactive mass may compete with radiotracer uptake through the NET, potentially altering tracer kinetics and reducing quantitative imaging accuracy. In addition, low analyte concentrations and solvent incompatibilities may complicate reproducible HPLC-based quantification. Therefore, the development of a formulation-matched and reproducible analytical workflow may improve quality control practices and support standardized assessments of biologically negligible tracer mass in preclinical PET imaging.

This project proposes the development of a high-performance liquid chromatography (HPLC)-based method to quantify total mFBG concentration and determine molar activity in [18F]mFBG formulations used for cardiac PET imaging.

No published literature outlines a standardized HPLC-based workflow specifically for the reproducible quantification of total mFBG mass in formulated [18F]mFBG PET tracer preparations under formulation-matched solvent conditions. The proposed study therefore aims not only to quantify tracer mass, but also to establish a reproducible analytical strategy that may improve standardization of molar activity measurements in NET-targeted PET imaging studies.

Methods

Preparation of calibration standards
A series of non-radioactive mFBG calibration standards was prepared over a biologically relevant concentration range using a solvent system matching the tracer formulation. To improve solubility and analytical reproducibility, calibration standards were prepared using a formulation-matched solvent system containing 10% dimethyl sulfoxide in water. This approach was selected to minimize variability associated with solvent incompatibilities and improve consistency between calibration standards and formulated tracer samples. Serial dilutions were performed to generate standards spanning the expected concentration range of formulated samples.

HPLC analysis
All samples were analyzed using high-performance liquid chromatography equipped with UV detection. A fixed injection volume was used for all standards and samples to ensure consistency. Chromatographic separation was performed under standardized conditions, and the retention time corresponding to mFBG was identified based on reference standards. Peak areas were integrated using chromatography software.

Calibration curve generation
Calibration curves were generated by plotting integrated peak area as a function of known mFBG concentration. Linear regression analysis was performed to determine the relationship between concentration and detector response. Only calibration ranges that demonstrated linearity were used for quantification.

Quantification of tracer samples
Aliquots of formulated [18F]mFBG batches were analyzed using the same HPLC method. The integrated peak area corresponding to mFBG was used to determine total mFBG concentration by interpolation from the calibration curve. Total mFBG amount was calculated based on formulation volume.

Molar activity calculation
Total radioactivity of each tracer batch was measured independently using a dose calibrator. Molar activity was calculated as the ratio of radioactivity to total mFBG amount. All variables were defined prior to calculation to ensure consistency across samples.

Expected Outcomes
It is anticipated that calibration standards will produce a linear relationship between mFBG concentration and integrated HPLC peak area within a defined concentration range. The retention time for mFBG is expected to remain consistent across runs, allowing reliable identification of the compound.

Analysis of formulated [18F]mFBG samples is expected to yield measurable peaks corresponding to mFBG, enabling quantification of total tracer mass. Interpolation from the calibration curve should allow accurate estimation of mFBG concentration.

Molar activity values are expected to fall within a range consistent with biologically negligible tracer mass, supporting the validity of PET imaging measurements. The proposed method is anticipated to provide reproducible and consistent results across multiple samples.

Discussion

The proposed study aims to establish a practical and reproducible HPLC-based method for quantifying total mFBG concentration and determining molar activity in [18F]mFBG tracer formulations. Accurate molar activity measurement is essential to ensure that injected tracer mass does not interfere with biological processes, particularly in transporter-mediated imaging (1). A major strength of the proposed method is the incorporation of formulation-matched calibration standards designed to improve analytical reproducibility under conditions closely resembling formulated tracer samples. This may reduce variability associated with analyte solubility and solvent mismatch, which can affect chromatographic peak shape and detector response at low concentrations. By improving consistency of molar activity measurements, the proposed workflow enhances confidence that injected tracer masses remain biologically negligible during NET imaging studies. This is particularly important for transporter-mediated PET tracers, where competition between radioactive and non-

radioactive ligand may influence quantitative interpretation of uptake kinetics.

The use of a formulation-matched solvent system is expected to improve consistency and reduce variability in calibration. However, potential limitations include variability in injection volume, detector sensitivity, and chromatographic conditions. These factors introduce error and will require careful standardization.

This work contributes toward the development of more standardized quality control protocols for PET radiotracers where tracer mass effects are a concern. Beyond [18F]mFBG, the proposed workflow applies to other transporter-targeted PET tracers requiring accurate molar activity determination. Improved standardization of tracer mass quantification may ultimately support greater reproducibility across preclinical imaging studies and facilitate future clinical translation.

Conclusion

This proposal outlines the development of an HPLC-based method to quantify total mFBG concentration and calculate molar activity in [18F]mFBG tracer formulations. The expected outcomes include a reproducible calibration method and reliable estimation of tracer mass. This approach has the potential to improve the accuracy and biological validity of cardiac PET imaging studies and may be extended to other radiotracers in both preclinical and clinical settings.

References

1. U. S. Ismailani, A. Buchler, J. d. C. Tso, S. M. Pulente, F. Abdirahman, E. L. Hoddinott, M. Fortier, E. E. Mulvihill, R. A. deKemp, B. H. Rotstein, PET imaging of cardiac sympathetic nervous system dysfunction using meta-[18F]fluorobenzylguanidine. Eur. J. Nucl. Med. Mol. Imaging 53, 3325-3339 (2025).
2. M. Grkovski, P. B. Zanzonico, S. Modak, J. L. Humm, J. Narula, N. Pandit-Taskar, F-18 meta-fluorobenzylguanidine PET imaging of myocardial sympathetic innervation. J. Nucl. Cardiology 29, 3179–3188 (2022).

Understanding Immune Tolerance and Escape in Gestational Choriocarcinoma Using Single-Cell Transcriptomics and Organoid Co-Cultures

Comprendre la tolérance immunitaire et l'évasion dans le choriocarcinome gestationnel à l'aide de transcriptomique unicellulaire et de co-cultures organoïdes

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Abstract | Résumé

Gestational choriocarcinoma (GChC) is an aggressive trophoblastic malignancy that displays an unusual degree of immune evasion. Emerging data suggest that GChC hijacks these same tolerance pathways to suppress cytotoxic immune responses. These mechanisms include programmed death receptor 1 (PD-1) and its ligand, programmed death ligand 1 (PD-L1) signaling pathway and human leukocyte antigen G (HLA-G)-mediated Natural killer (NK) cell inhibition. However, the precise cellular interactions and checkpoint circuits responsible for tumour persistence remain poorly defined.

This research proposal aims to integrate a single-cell and organoid-based strategy to identify and functionally validate the immune-tolerance mechanisms that GChC appropriates from the healthy placenta. Single-cell RNA sequencing of archived GChC tumours and term placental tissues will resolve tissue subpopulations and quantify the expression of immune evasive genes. Ligand–receptor inference models will map suppressive communication networks between tumour trophoblasts and maternal immune cells. Spatial multiplex immunofluorescence will confirm the anatomical localization of tolerance markers and identify immune-exclusion zones within the tumour microenvironment. Finally, trophoblast organoid–immune co-culture assays will directly test whether blockade of PD-L1, HLA-G, or other identified pathways restores T-cell and NK-cell activation.

By combining single-cell profiling with functional disruption, this framework aims to define the associated immune-tolerance circuits that enable GChC immune escape. These insights could inform biomarker development and guide selective immunotherapeutic strategies that target tumour- specific tolerance without disrupting normal pregnancy physiology.

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Le choriocarcinome gestationnel (GChC) est une malignité trophoblastique agressive qui présente un degré inhabituel d'évasion immunitaire. Les données émergentes suggèrent que le GChC détourne ces mêmes voies de tolérance pour supprimer les réponses immunitaires cytotoxiques. Ces mécanismes incluent le récepteur de mort programmé 1 (-1) et son ligand, la voie de signalisation du ligand de mort programmé 1 (-L1) ainsi que l'inhibition des cellules tueuses naturelles (NK) médiée par l'antigène leucocytaire humain G (HLA-G). Cependant, les interactions cellulaires précises et les circuits de points de contrôle responsables de la persistance des tumeurs restent mal définis.

Cette proposition de recherche vise à intégrer une stratégie basée sur une seule cellule et un organoïde afin d'identifier et de valider fonctionnellement les mécanismes de tolérance immunitaire que le GChC s'approprie du placenta sain. Le séquençage à ARN unicellulaire des tumeurs GChC archivées et des tissus placentaires à terme résoudra les sous-populations tissulaires et quantifiera l'expression des gènes immunoévasifs. Les modèles d'inférence ligand-récepteur cartographient les réseaux de communication suppressifs entre les trophoblastes tumoraux et les cellules immunitaires maternelles. L'immunofluorescence multiplex spatiale confirmera la localisation anatomique des marqueurs de tolérance et identifiera les zones d'exclusion immunitaire dans le microenvironnement tumoral.

Enfin, les tests de co-culture organoïde–immunité du trophoblaste testeront directement si le blocage de-L1, HLA-G ou d'autres voies identifiées restaure l'activation des lymphocytes T et des cellules NK. En combinant le profilage unicellulaire avec la perturbation fonctionnelle, ce cadre vise à définir les circuits de tolérance immunitaire associés qui permettent l'évasion immunitaire du GChC. Ces connaissances pourraient orienter le développement des biomarqueurs et guider des stratégies immunothérapeutiques sélectives ciblant la tolérance spécifique aux tumeurs sans perturber la physiologie normale de la grossesse.

Keywords: Gestational choriocarcinoma, Placental immune tolerance, PD-L1 signaling, HLA-G signaling, Trophoblast organoids, Immune evasion, Single-cell RNA sequencing, Maternal–fetal interface, tumour microenvironment

Background

Gestational choriocarcinoma (GChC) is a rare and highly aggressive trophoblastic cancer that employs many of the same immune-evasion strategies that the healthy placenta uses to protect the fetus from the maternal immune system. This cancer forms because trophoblasts, specialized placental cells that establish the maternal-fetal interface, naturally regulate immune tolerance. This physiological process suppresses or restrains immune response to prevent tissue damage during pregnancy. The formation of the trophoblasts gives the tumour an unusual ability to evade immune detection, which allows for aggressive metastasis. GChC has good prognosis when detected early (5-year survival >80%). However, mortality persists when diagnosed late or in high-risk groups (1). Understanding the immune escape mechanisms exploited by GChC can allow for more targeted therapies to be developed that preserve maternal fertility and reduce chemotherapy exposure. The proposed study aims to map the specific trophoblast–immune interactions that drive immune escape.

The Medawar paradox describes the unique immunological state of pregnancy, since a fetus, which is semi-allogeneic due to paternal genetic contributions, can survive without being rejected by the maternal immune system (2). Reproductive immunology research has highlighted the importance of extravillous trophoblasts in immune tolerance. Extravillous trophoblasts are a subtype of trophoblasts, specialized placental cells that embed the placenta into the uterine wall, which express non-classical MHC molecules such as Human leukocyte antigen G (HLA-G). HLA-G is specialized for Natural killer (NK) cell modulation and interacts with maternal immune receptors which suppresses immune cell proliferation, cytotoxicity, and inflammatory cytokine secretion. HLA-G functions in parallel with immune system pathways such as programmed death receptor 1 (PD-1) and its ligand programmed death ligand 1 (PD-L1), responsible for inhibition of T cell cytotoxic effect, creating a suppressive immune microenvironment (3-4).

Several pathways responsible for placental immune regulation are also associated with GChC progression and tumour immune evasion. In both placental development and cancer progression, PD-L1 and HLA-G are implicated, as they inhibit T cell cytotoxicity and NK activation (5). GChC arises from trophoblasts of a preceding pregnancy, almost always in the uterus. Choriocarcinoma are semi-allogeneic, as they contain paternal DNA and by extension paternal antigens (6). NLRP7, a gene involved in immune regulation, has been identified as a key driver of GChC immune evasion, enhancing PD-L1, HLA-G, and macrophage/T-cell suppression, while silencing it restores immune visibility and reduces tumour burden. This positions NLRP7 as a central tolerance regulator whose downstream circuits warrant targeted mapping (7). Clinically, PD-1 blockades induce durable remissions in drug-resistant gestational trophoblastic neoplasia, emphasizing the centrality of the PD-1/PD-L1 axis in GChC immune escape (8).

Current literature highlights that GChC relies on a coordinated tolerance network rather than any single pathway (9). Previous studies have suggested GChC parallels placental biology. However, these findings derive primarily from bulk or immunohistochemical analyses and do not clarify the cellular circuitry or causal dynamics of immune evasion. These approaches cannot resolve which trophoblastic or immune subpopulations drive tolerance, nor can they reconstruct the ligand–receptor interactions or involved immune-suppressive circuits that sustain tumour persistence. The project addresses the lack of cell-level mechanistic understanding through single-cell resolution mapping and functional co-culture assays. This mechanistic clarity is essential for developing safer fertility-preserving immunotherapies and improving outcomes in advanced or drug-resistant disease.

Research Hypothesis

It is hypothesized that gestational choriocarcinomas take advantage of maternal immune-tolerance pathways, particularly the PD-1/PD-L1 axis and HLA-G-mediated inhibition to suppress cytotoxic and NK cell responses. This creates a microenvironment analogous to the healthy placenta's mechanisms of immune evasion. By mapping immune signaling pathways at a single-cell resolution and functionally testing the blockade of these immune axes in organoid co-cultures, the aim is to identify tolerance circuits that drive choriocarcinoma invasion, proliferation, and persistence.

Rationale

The proposal framework is designed as an ethically robust way to safely study immune evasion in pregnancy without human experimentation. GChC provides a unique model to study the mechanisms of immune tolerance. The approach aims to identify checkpoint pathways that maintain immune tolerance and identify pathological pathways establishing a comparative immune-tolerance profile of the healthy placenta and GChC. This will demonstrate whether PD-1 and HLA-G blockages restore immune activation and inform potential targets for safe immunotherapy in pregnancy-related cancers. The design combines a discovery phase using single-cell RNA sequencing with a validation phase through immunofluorescence and organoid co-culture assays. Based on single-cell RNA sequencing data, pathways showing strong gene expression differences will be identified during the discovery phase and subsequently validated through downstream experiments.

Methods

Tissue and single-cell preparation

Individual cells from archived GChC tumours and healthy term placentas are isolated via enzymatic dissociation to compare their cellular composition and microenvironments at single-cell resolution. These will be obtained through ethically approved

biobank collaborations. Healthy term placenta will be defined as tissue collected from uncomplicated pregnancies delivered at term (≥37 weeks gestation), with no evidence of infection. Inclusion criteria will consist of well-preserved samples with sufficient cellular integrity, while degraded cells will be excluded. For frozen specimens where enzymatic dissociation may not be applicable, single-nucleus RNA sequencing may be used for high-resolution cell-specific transcriptomic analysis. Each cell's transcriptome will be characterized through single cell preparations to allow the tumour microenvironment to be dissected into its individual components.

Single-cell RNA sequencing (scRNA-seq) and data processing
Transcriptomic data will be analyzed to identify distinct cell states, differentially expressed tolerance genes, and immunomodulatory trophoblast subpopulations. Differential gene expression across cell types will be assessed. Quality control filtering will be applied to remove low-quality cells, ensuring reliable downstream analysis. As a result, cells will be clustered based on gene-expression similarity to identify cell types and states. scRNA-seq is a technique that measures gene expression at the individual cell level, enabling identification of cellular subtypes and signaling pathways indicating which trophoblast subpopulations are immunomodulatory. These bioinformatic tools will be used determine whether the tumour differentially expresses immune checkpoint molecules or cytokines. By comparing GChC with healthy placenta tissue, differences in the magnitude and regulation of shared tolerance pathways will be determined.

Ligand–receptor and cell–cell interaction inference
Immune system evasion relies on cell communication which involves the computational prediction of cell–cell communication based on matching ligands in one cell type with receptors in another. To map immunosuppressive signaling, ligand–receptor pair activities between trophoblastic and immune cells will be quantified using CellChat and NicheNet. Communication patterns will be computationally predicted based on the expression of receptor-ligand gene pairs and will be used to provide insight into the active signaling pathways as well as targets for therapeutic intervention.

Validation and imaging
Spatial localization of tolerance markers will be confirmed via immunofluorescence with fluorophore-conjugated antibodies targeting PD-L1, PD-1, and HLA-G in both tumour samples and healthy tissue. This analysis will assess co-localization and confirm whether transcriptome data translates to functional protein expression within the tissue microenvironment.

Organoid–immune co-culture assays
3D trophoblast organoids derived from patient stem or progenitor cells will be developed to model the tumour environment *in vitro* (10). These organoids will be paired with matching immune cells to recreate the tumour–immune environment *in vitro*. By selectively blocking pathways such as PD-L1 or HLA-G, immune cell reactivation will be assessed to determine the functional role of these pathways in tumour immune evasion, complementing the descriptive data from single-cell sequencing. While organoid-to-organoid variability represents a limitation, the use of matched tumour–placental pairs will be put in place to reduce this effect.

Limitations

Archived gestational choriocarcinoma samples may influence cell integrity, which can introduce variability in the data set. Inter-patient differences may also impact the consistency of pathway expression and influence downstream analyses. Furthermore, the use of lab-grown 3D organoids represents an *in vitro* model that cannot fully capture the complexity of the *in vivo* tumour environment.

Ethical Consideration

Direct patient contact and the collection of biological samples can raise ethical concerns. To address these concerns, this study will use archived human tissues obtained through approved biobank collaborations, avoiding direct patient involvement and maintaining compliance with ethical guidelines.

Conclusions

The proposed study will establish the first cellular-resolution mechanistic mapping of immune tolerance in gestational choriocarcinoma. By integrating transcriptomic data with functional organoid assays, the immunological checkpoints and ligand-receptor circuits responsible for immune suppression will be identified. These insights may guide the development of selective immunotherapies that restore anti-tumour immunity without disrupting reproductive health. This approach is not only relevant to gestational choriocarcinoma but may also be extended to other trophoblastic cancers that share similar immune-evasion pathways.

Editorial Conflict of Interest Statement

Ishaan S. Goswami and Zoha Fatima are members of the OSURJ editorial team. Both authors were fully recused from all aspects of the editorial process for this manuscript, including reviewer selection, peer review, and final decision-making. The manuscript was handled independently by other members of the editorial board.

References

1. C. M. Tarney, C. Tian, E. R. Craig, B. A. Crothers, J. K. Chan, G. D. Gist, N. W. Bateman, T. P. Conrads, C. A. Hamilton, G. L. Maxwell, K. M. Darcy, Relative Effects of Age, Race, and Stage on Mortality in Gestational Choriocarcinoma. International Journal of Gynecological Cancer 28, 338–345 (2017).

2. V. Male, Medawar and the immunological paradox of pregnancy: in context. Oxford Open Immunology doi:https://doi.org/10.1093/oxfimm/iqaa006 (2022).
3. B. Zhuang, J. Shang, Y. Yao, HLA-G: An Important Mediator of Maternal-Fetal Immune-Tolerance. Frontiers in Immunology 12, 744324 (2021).
4. M. G. Petroff, S. L. Nguyen, S. H. Ahn, Fetal-placental antigens and the maternal immune system: Reproductive immunology comes of age. Immunological Reviews 308, 25–39 (2022).
5. H. Pang, L. D Lei, Y. Guo, Y. Yu, T. Liu, Y. Liu, T. Chen, C. Fan, Three categories of similarities between the placenta and cancer that can aid cancer treatment: Cells, the microenvironment, and metabolites. Frontiers in Oncology 12, 977618 (2022).
6. H. Cheng, L. Zong, S. Yu, J. Chen, X. Wan, Y. Xiang, J. Yang, Expression of the immune targets in tumor-infiltrating immunocytes of gestational trophoblastic neoplasia. Pathology oncology research 29, 1610918 (2023).
7. D. Reynaud, R. A. Nahed, N. Lemaitre, P. Bolze, W. Traboulsi, F. Sergent, C. Battail, O. Filhol, V. Sapin, H. Boufettal, P. Hoffmann, T. Aboussaouira, P. Murthi, R. Slim, M. Benharouga, N. Alfaidy, NLRP7 Promotes Choriocarcinoma Growth and Progression through the Establishment of an Immunosuppressive Microenvironment. Cancers 13, 2999 (2021).
8. L. Hennah, M. Seckl, E. Ghorani, Novel approaches to managing gestational trophoblastic tumors in the age of immunotherapy. International journal of gynecological cancer 33, 414–419 (2023).
9. N. Sharma, R. Kundal, V. Kaushal, Immunobiology and immunotherapy of gestational trophoblastic disease. Gynecology and Obstetrics Clinical Medicine 2, 76–81 (2022).
10. M. Y. Turco, L. Gardner, R. G. Kay, R. S. Hamilton, M. Prater, M. S. Hollinshead, A. McWhinnie, L. Esposito, R. Fernando, H. Skelton, F. Reimann, F. M. Gribble, A. Sharkey, S. G. E. Marsh, S. O'Rahilly, M. Hemberger, G. J. Burton, A. Moffett, Trophoblast organoids as a model for maternal–fetal interactions during human placentation. Nature 564, 263–267 (2018).