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INFECTION, COMMUNITY AND CANCER

A novel zebrafish xenotransplantation model for investigating and identifying new treatments for metastatic colorectal cancer.

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Background

Colorectal cancer (CRC) causes over 900,000 deaths each year, primarily due to our inability to prevent and treat metastatic forms of the disease. While CRCs that are detected early can be successfully treated, outcomes for patients with metastatic disease remain extremely poor. Such advanced disease is present in approximately 25% of patients at initial diagnosis and develops in about half of all CRC patients. To metastasize, CRC cells that leave the primary site must overcome several physiological barriers to successfully colonize distant organs. The mechanisms by which some CRC cells successfully complete these complex processes are not well understood. Therefore, elucidating mechanisms facilitating metastatic colonisation has the potential to uncover new avenues to treat metastatic CRC.

The current gold-standard for studying metastasis are models where human cancer cells or patient-derived xenografts are transplanted into immune-compromised mice. Despite the undoubted value of these models, they do not allow easy non-invasive visualization of tumour rendering them unviable for clinical practice. To overcome these challenges, researchers have generated novel zebrafish cancer models, including optically-clear lines that facilitate *in vivo* tracking of tumor cells in real time.

Methods

To establish a model to better understand CRC metastasis and identify potential targets for treating metastatic CRCs, we developed a novel optically-clear immuno-compromised zebrafish model (Casper/Il2rgc.a-/-) lacking T and NK cells. In contrast to standard zebrafish embryonic xenotransplantation assays where animals develop immunity from 7 days post-fertilisation (dpf), Casper/Il2rgc.a-/- embryos are immune-deficient until 21 dpf, thus providing a broader window to evaluate the fate of xenotransplanted cancer cells.

Results

Xenotransplantation of various human CRC cell lines into Casper/Il2rgc.a-/-embryos revealed that over time, some small metastatic lesions progressed to form larger lesions, suggesting that this model facilitates studies into the later stages of metastasis.

Conclusion

Embryos from Casper/Il2rgc.a-/- zebrafish provide a novel xenotransplantation model for studying cancer metastasis and testing anti-cancer therapies.

Development of real-time biosensors to detect airborne allergen

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Background

Hirst-type traps are the most common sampling machines implemented at aeroallergen monitoring stations worldwide[1]. While Hirst-type traps can effectively collect airborne particulates over a long time, limitations arise from these lengthy and labour-intensive collection times, and can be error prone by an inexperienced counter. Current sampling can be significantly improved by developing "real-time" automatic monitoring methods to assist researchers with detecting sudden fluctuations in aeroallergen concentrations over shorter periods and provide valuable data for ETSA forecasts as thunderstorm asthma events are unfolding. One example of "real-time" automated sampling is biological immunosensors (biosensors), which are based on reactions between antigens and antibodies, coupled with electrical or chemical components, to produce an analytical signal[2]. The formation of antibody-antigen complexes can be measured with a level of high sensitivity to determine the presence or concentration of specific molecules[2].

Methods

Mass-sensitive biosensors were developed to detect allergens *Lol p 5/Phl p 1* and *Alt a 1* found in prominent allergenic pollen and fungi, respectively. 1:3 11-MUA and 9-MNL thiols were modified via NHS/EDC activation to enable antibody attachment.

Results

Antibody-antigen complexes were successfully formed in a simulation chamber under controlled conditions. The development of these biosensors are the first of its kind, as none had yet been built to detect pollen or fungal spore allergens.

Conclusion

This research demonstrates that biosensors may become a viable alternative to conventional methods for monitoring airborne allergens in "real-time". Future studies should focus on testing biosensors in field conditions to validate their real-world usage.

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Establishing genomic surveillance for early warning of antimalarial drug resistance in Bangladesh.

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Abstract

Bangladesh has achieved an 80% reduction in malaria cases over the last decade, but these gains are threatened by the rapid spread of *Plasmodium falciparum* resistance to the frontline artemisinin and partner drugs in the Greater Mekong Subregion (GMS). Over 90% of malaria cases occur in the Chittagong Hill Tracts (CHT) districts, bordering Myanmar. To date, there is no evidence of artemisinin resistance in the CHT; however, large influx of refugees fleeing Myanmar into Bangladesh warrants dedicated surveillance. The National Malarial Elimination Programme (NMEP) currently receives data on antimalarial drug efficacy from clinical surveys in select locations; however, the high financial cost and logistical complexities constrain their frequency and ability to detect emerging resistance in other locations. My proposed research program aligns closely with and extend the activities of the NMEP and leading malaria genomics researchers to establish amplicon sequencing as a sustainable, high-throughput molecular surveillance platform to monitor antimalarial drug resistance and transmission dynamics. The resulting data will provide early warning signals to prevent widespread resistance to artemisinin and partner drugs in the CHT. The study will generate genomic data on antimalarial drug resistance and parasite genetic relatedness in the CHT and establish capacity for processing the data that leverages on existing informatics pipelines. The statistical outputs will inform the NMEP with clear intelligence on where to upscale antimalarial interventions, conduct clinical surveys of drug efficacy, and to change drug policy if high levels of resistance are detected.

Exploring the human tear proteome of ocular allergy sufferers in peak allergy season

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Background

Ocular allergy (OA) is a localised subset of allergy characterised by ocular surface itchiness, redness and inflammation. Inflammation and eye-rubbing, due to allergy-associated itchiness, are common in OA sufferers and may trigger changes to the ocular surface biochemistry. The primary aim of this study is to assess the differences in human tear proteome between OA sufferers and healthy controls during peak allergy season in Victoria, Australia.

Methods

31 participants (21 OA sufferers, 10 healthy controls) aged 18-45 were recruited for this study. Participants were completed symptom and quality of life questionnaires. Tear samples were collected using a non-invasive microcapillary flow technique. Extracted proteins were run on an Orbitrap Mass Spectrometer and were matched to a DIA library. Data was analysed using software MaxQuant, Perseus, FunRich and IBM SPSS.

Results

877 proteins were quantified in tear samples of OA sufferers and healthy controls, of which 23 showed a significant difference in expression between groups ($p<0.05$). 9 proteins showed increased expression in OA sufferers versus healthy controls, and 14 were decreased. Decreased proteins in OA sufferers related to cell structure, inflammatory and antimicrobial regulation. OA sufferers were shown to have increased expression of proteins relating to inflammation, immunity, and cellular development.

Conclusion

Tear protein quantification showed dysregulation of proteins involved in inflammation, immunity, and cellular structures. Proteins relating to cellular structure may suggest a possible link between OA-associated itch and the subsequent ocular surface damage via eye-rubbing, while inflammatory and immune protein changes highlight potential diagnostic and therapeutic biomarkers of OA.

Generation and characterization of kat5 mutation in zebrafish

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Abstract

DNA and proteins packaged in the cell nucleus are affected by histone acetylation, which affects chromatin organization and gene expression. The acetylation of core histones has been associated with chromatin opening and closing, gene transcription, DNA damage repair, and chromosome decondensation in mitosis and meiosis. Lysine residues are acetylated by tightly regulated histone lysine acetyltransferases (KATs) and deacetylases. There is an increasing body of research indicating that dysregulation of KATs and aberrant lysine acetylation is linked to tumorigenesis in cancer and poor prognosis, presenting an opportunity for finding new therapeutic targets in this area. Kat5 is a histone acetyltransferase family member and regulates DNA damage response by acetylating histones and remodelling of the chromatin.

My research is focussed on investigating the effects of acute deletion of kat5 using CRISPR/Cas9 mediated deletion in zebrafish and its potential role in tumorigenesis. Zebrafish have been increasingly used as an animal model to better understand the genetics and biology of vertebrate development. I found that mutation of kat5 is associated with multiple developmental issues and disease such as survival, lymphoma, oophoritis, seminoma, spinal deformity, spindle cell tumour, neurodegenerative disorders, and facial malformation. Characterization of key mechanisms causing these defects and performing further studies will pave the better understanding of role of kat5 in zebrafish development and cancer studies.

Identification of natural killer cells in zebrafish

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Background

Zebrafish has emerged as an invaluable model for studying haematopoiesis and haematopoietic diseases. There is a strong conservation of their haematopoietic cells and the key genes they express to mammals. Their genome is highly accessible to genetic modification, and they can be manipulated to examine disease pathogenesis and potential therapeutic agents. There is a growing body of literature around zebrafish immune cells, including macrophages, neutrophils, and T and B lymphocytes that have demonstrated their importance in regulating immune responses to pathogens and cancer. However, thus far less attention has been given to Natural Killer (NK) cells whose identity, ontogeny and specific roles in zebrafish remain unknown. This study seeks to address this knowledge gap by identifying and developing marker genes to investigate NK cell biology in zebrafish.

Methods

Based on available transcriptomic and molecular studies, this study analyses various NK cell-related genes using Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) and high-resolution whole mount in situ hybridization (WISH) to evaluate their specific expression in the zebrafish NK cell lineage.

Results

The study presents different NK cell-specific genes to be expressed throughout zebrafish embryonic development. The expressions of these genes were significantly reduced in the IL-2Rγc knockout fish which lack NK cells compared to wildtypes. Importantly, the study shows that zebrafish NK cells reside in zebrafish thymus which also accommodates T lymphocytes.

Conclusion

Together, the study provides new evidence of zebrafish NK cells and establishes cellular markers that can be used to identify NK cells in zebrafish.

Reference

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Identification of novel malaria proteins involved in parasite-host cell interactions

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Background

Malaria is caused by protozoan parasites of the *Plasmodium* genus, of which *P. falciparum* is the most lethal. *Plasmodium* parasites invade and remodel erythrocytes to grow and obtain essential nutrients. One organelle implicated in invasion, parasite establishment within erythrocytes, and remodelling is the rhoptry. To date, only 30 *Plasmodium* rhoptry proteins have been identified using empirical approaches. Elucidating the rhoptry proteome will be crucial to understanding the roles of rhoptry proteins in mediating host-parasite interactions.

Methods

Novel techniques in proteomics, like proximity labelling, can identify proteins localising to a particular cellular region. In proximity labelling, a labelling enzyme is fused to a gene to label nearby proteins. One such enzyme, TurboID, biotinylates proximal proteins by catalysing their covalent attachment to biotin-AMP. Proximity labelling studies in *P. falciparum* are limited.

Results

The aim of the study was to identify the rhoptry proteome by fusing TurboID to proteins localising to different regions in the rhoptry. This body of work demonstrates the development of TurboID-fused *Pf*CERLI, which localises to the rhoptry cytoplasmic face, and TurboID-fused *Pb*RON3, a rhoptry bulb-localising protein. Mass spectrometry revealed 129 proximal proteins significantly enriched in CERLI-TurboID-expressing parasites, 14 of which are known rhoptry proteins. The most significantly enriched proteins localise to apical

cellular compartments or to membranous structures, and include proteins involved in vesicle-mediated transport.

Conclusion

Identification of rhoptry proteins provides the basis for understanding their trafficking through the secretory pathway and evaluation of their potential as novel therapeutic targets, which are desperately required due to rising drug resistance of parasites.

Interviewing health practitioners on their attitudes, perspectives, experiences, and influences in ocular allergy

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Background

Current research has revealed a lack of consensus in health practitioner diagnostic, treatment, and collaborative care approaches to Ocular Allergy (OA). However, the exact gaps have not been determined. Thus, the 'Survey on Ocular Allergy for Health Practitioners (SOAHP)' study was conducted to better understand the current gaps in the knowledge and practices of relevant health practitioners in OA. However, as SOAHP was a closed-ended survey study, this did not capture all aspects of health practitioner involvement in OA. Thus, interviews were conducted to gain a greater understanding of health practitioner attitudes, perspectives, experiences and influences surrounding OA. The secondary aim was to create a collaborative care model on OA.

Methods

Semi-structured in-depth interviews were conducted on 35 health practitioners which included 5 Allergists/Immunologists, 3 General Practitioners, 2 Ophthalmologists, 21 Optometrists and 4 Pharmacists. A qualitative descriptive approach was taken. Data was analysed using the Reflexive Thematic Analysis by Braun and Clarke.

Results

This qualitative study derived five themes from the data which included Attitudes towards OA, Current Practice Patterns in OA, Interdisciplinary Collaborations in OA, Barriers to Diagnosis and Management of OA, and Enablers to Diagnosis and Management of OA patients.

Conclusion

It was found that health practitioners viewed OA as a routine, and non-sight threatening condition regardless of the known effects. However, although viewed in a simple light, there were multiple barriers identified including a lack of a clear diagnostic method, no framework for treatments, and no collaborative care model. Thus, most participants requested multiple enablers to alleviate such barriers. Finally, a collaborative care model on OA was created through the results from SOAHP and this interview-based study.

Investigating the role of dysregulated metabolism in paediatric glioblastoma

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Background

Paediatric glioblastoma (pGBM) is an aggressive brain tumor with limited survival, and traditional treatments for adult GBM are often ineffective in children. The distinctive biology of pGBM demands specialized therapies. While the shift towards increased glycolysis and lactate production (Warburg effect) is well-studied in adult GBM, this metabolic dysregulation in pGBM is largely unexplored.

Methods

We aimed to compare glucose metabolism in paediatric GBM (SF188), adult GBM (T98G), and non-cancerous cells (CT003 and HEK293) in both monolayer and 3D spheroids, hypothesizing that the cancers demonstrate dysregulated metabolism. The metabolic phenotype, including oxidative and glycolytic flux, was assessed using the Seahorse XF analyzer, with all data normalized to protein concentration (BCA).

Results

The pGBM and T98G cells showed significantly higher oxygen consumption (OCR) rates of 2.7 ± 0.21 and 4.5 ± 0.24 pmol/min/µg protein, respectively, and increased extracellular acidification rates (ECAR) of 0.98 ± 0.04 and 1.4 ± 0.04 mpH/min/µg protein, respectively. These values were considerably higher compared to the non-cancerous CT003 and HEK cell lines, with OCR of 0.91 ± 0.11 and 0.91 ± 0.13 pmol/min/µg protein, and ECAR of 0.30 ± 0.02 and 0.1 ± 0.02 mpH/min/µg protein, respectively (n=4 ± SEM).

Conclusion

Our study identifies that adult and pGBM cells have heightened glycolytic and aerobic metabolism, reflecting increased energy capacity, especially in 3D models and monolayers. pGBM metabolism aligns with the Warburg effect, differing from adult GBM, requiring unique research strategies. Future work will explore elevated glycolysis in pGBM, its role in proliferation and survival, and potential therapeutic targeting.

Investigating the role of TRX2 in trafficking malaria virulence proteins

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Background

Malaria is caused by infection with *Plasmodium* parasites, with ~400,000 deaths and >200 million cases each year. There is no efficacious licensed vaccine and thus anti-malaria drugs are critical for treating malaria. Due to major problems with resistance to current anti-malarials, new strategies to combat *Plasmodium* are desperately needed. This project aims to identify the role of thioredoxin (TRX2) in *P. falciparum*, a component of the parasite's protein export machinery (PTEX), in trafficking cysteine rich proteins such as *Pf*EMP1 (the major virulence factor) to the host cell surface and its contribution to parasite survival.

Methods

To determine the role of TRX2 in parasite survival, a transgenic parasite line *Pf*TRX2-HA*glmS* was created using CRISPR/Cas9 technique, enabling knockdown of HA-tagged TRX2 expression with glucosamine. The effect of TRX2 knockdown on parasites growth was tested by growing them with and without glucosamine. The comparative growth analysis was performed using student's t-test. to identify the role TRX2 in trafficking *Pf*EMP1 to the host cell surface, immunofluorescence assay (IFA) was performed by probing parasites smears with ATS antibody.

Results

Diagnostic PCR results indicated successful creation of *Pf*TRX2-HA*glmS* transgenic parasites line. Western blot analysis showed that glucosamine treatment reduced more than 85% TRX2 protein expression. Knockdown of TRX2 protein resulted in significant reduction of parasites growth (50%). In addition, IFA results revealed significant defects in *Pf*EMP1 protein export to the erythrocytes membrane when TRX2 protein expression was depleted.

Conclusion

Using molecular and biochemical techniques, it has been shown that TRX2 is important for optimal growth of *P. falciparum* and export of *Pf*EMP1 protein to the host cell surface.

Physical activity and circulating inflammatory markers and cytokines during pregnancy: a population-based cohort study

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Background

Physical activity (PA) during pregnancy has numerous benefits, partly mediated via its effect on the immune system. However, this evidence is inconsistent. We estimated the effect of PA during pregnancy on systemic inflammatory markers in mothers recruited in the Barwon Infant Study.

Methods

Participants reported their previous week's PA at their 28-week antenatal appointment. Women were grouped into low, moderate and high PA categories based on duration and frequency of walking, moderate- or vigorous-intensity PA. Women reporting moderate levels of PA, which is consistent with current recommendations, served as the comparison group. Markers of systemic inflammation, high sensitivity C-reactive protein (hsCRP) and glycoprotein acetyls (GlycA), and cytokines were measured at 28 weeks gestation. Regression analyses adjusted for maternal smoking, gestational diabetes mellitus, pre-pregnancy BMI and household size were performed.

Results

Compared to women in the moderate group (n = 371, 42%) women reporting low PA (n = 436, 50%) had a 10.1% higher hsCRP (95% CI (3.7%, 16.6%), p<0.01) while women in high PA (n = 76, 9%) had a 14% higher hsCRP (95% CI (3.1%, 24.8%), p=0.01). Women in high PA category had higher interleukin (IL)-4 (q=0.03) and IL-9 (q=0.03) levels compared to those in moderate category. Each vigorous MET-minute/week was associated with lower GlycA (p = 0.03).

Conclusion

Low and high PA is associated with higher systemic inflammation and high PA affects T cell-associated cytokines during pregnancy. As excessive inflammation is a risk factor for pregnancy-related complications, studies are now required to assess impact of this on clinical outcomes.

Targeting PD-L1 positive sEVs: Developing aptamer-based liquid biopsy for enhanced therapies

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Background

PD-L1 (Programmed death ligand-1) is a crucial protein that contributes to immune cell tolerance by acting as an immune checkpoint ligand of the co-inhibitory receptor PD-1. However, when PD-L1 binds to T-cells, it suppresses their growth and proliferation, promoting cancer cell growth. PD-L1 on the surface of small extracellular vesicles (sEVs) that are derived from tumour cells, thus, can facilitate drug resistance by binding to PD-L1 antibodies upon treatment, reducing their effectiveness. Therefore, a quantitative detection system is necessary to identify and capture PD-L1 positive sEVs in the liquid samples. This project aims to develop an aptamer that specifically targets PD-L1 positive sEVs, enhancing the efficacy of therapies.

Methods

The aim is to identify the most promising aptamers candidate for further engineering and development of effective liquid biopsy. In this study, potential aptamers targeting PD-L1 peptide were selected using SELEX, followed by primary screening and engineering. Also, these aptamers have been characterized. The techniques of choice were ELISA and flow cytometry for this study.

Results

Twenty-nine candidate aptamers were obtained from SELEX where PD-L1-24-apt and PD-L1-29-apt showed promising binding to the PD-L1 protein. PD-L1-24-apt and PD-L1-29-apt affinity were 310 nM and 286 nM respectively. Moreover, these aptamers have shown substantial selective binding to the native structure of PD-L1 protein anchored in the cell surface.

Conclusions

Final aptamers selected will have fast on-rate and slow off-rate and will be able to establish aptamer-based capture of PD-L1 positive sEVs through liquid biopsy. However, further verification is required to confirm aptamers efficacy.

The use of single-cell RNA sequencing to understand virus-host interactions: the extracellular matrix enzyme ADAMTS7 and its role in influenza virus pathogenesis.

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Background

Single-cell RNA sequencing (scRNA-seq) is a technique that combines next-generation sequencing (NGS) and single-cell technology to facilitate quantitative characterization of cell heterogeneity following virus infection. Our laboratory has previously identified the importance of extracellular matrix enzymes in viral immunity as the absence of one such enzyme, ADAMTS5, a member of ADisintegrin and Metalloproteinase with Thrombospondin motifs (ADAMTS) family leads to delayed virus clearance and sub-optimal virus-specific CD8 T cell migration following influenza virus infection [1]. Another enzyme if interest is ADAMT7, a protein associated with inflammatory responses in rheumatoid arthritis. Its contribution to influenza pathogenesis is not yet characterized [2].

Methods

In order to establish and compare the transcriptome baseline between ADAMT7 knock-out (KO) and WT mice at the single cell level, a BD Rhapsody™ Whole Transcriptome Analysis protocol was established. cDNA libraries from both strains of mice were constructed from 20,000 splenocytes in accordance with the BD Rhapsody™ Express methodology (a single cell capture system) and sequencing was performed using an Illumina NovaSeq 6000. Data processing was conducted using the Seven bridge platform (cloud computing) and Seurat package in R programming.

Results

A total of 19 cell subsets were identified from WT and KO mice. KO mice exhibited exclusive cell subsets that included CD177+ neutrophils, germinal centre and marginal zone B cells. There were also higher frequencies of erythrocytes, monocytes, red pulp macrophages and activated B cells when compared with WT mice.

Conclusion

These results suggest that expression of ADAMTS7 may impact influenza immunopathogenesis through differential cell subsets and further investigation using in vivo mouse models is required.

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Tp53 knockout zebrafish autonomously develops anaplastic sarcoma and hemangiosarcoma which are transplantable and metastatic in nature.

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Background

Tp53 plays a very significant role in maintaining the genomic integrity of multicellular organism by preventing mutation of the genome and oncogenic transformation under any stressful condition. More than 50% of all human cancers have disrupted *tp53* function. However, to date the effect of *tp53* mutation in developing cancers, their overall progression and metastatic nature of the cancers is not well studied. Therefore, to understand *tp53* knockout effect *in vivo*, we have generated *tp53* mutant zebrafish and characterized them.

Methods

CRISPR/CAS9 technique has been used to create *tp53* mutation in zebrafish. Regular monitoring and histopathological test have been conducted to identify zebrafish cancer spectrum. Adult xenotransplantation has been performed to generate syngeneic zebrafish cancer model and observe the metastatic nature of the cancers.

Results

tp53 mutant (*tp53*$^{-/-}$) zebrafish autonomously developed cancers, mainly anaplastic sarcoma along with a good number of hemangiosarcoma and few rhabdomyosarcomas. By 4 months, the onset of tumour was observed while 66% of homozygous *tp53*$^{-/-}$ animals developed tumour by 27 weeks. Xenotransplantation of two largely developed cancer, anaplastic and hemangio-sarcomas in both immune competent and compromised zebrafish showed that both are transplantable and metastatic in nature where immune cells might play different role in metastasis and tumour engraftment.

Conclusion

tp53 knockout zebrafish allowed us to study two deadly cancers anaplastic sarcoma and hemangiosarcoma. The transplantable nature of the cancers has allowed to have the cancer model faster for further dissecting as well as to understand the role of immune cells in cancer microenvironment.

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Understanding the role of rodent malaria clag genes in new permeation pathway formation

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Abstract

Malaria is one of the leading infectious diseases in the world and is caused by protozoan parasites of the species *Plasmodium*. *Plasmodium* parasites infecting humans have developed resistance to all antimalarial drugs, and it is therefore critical for new therapeutics

to be developed. New permeation pathways (NPPs) have been validated as a crucial modification of the host erythrocyte, facilitating nutrient acquisition, and are therefore an attractive therapeutic target. Understanding the channel's structure is critical for targeted drug design. *RhopH1/clag* genes have previously been implicated in NPP formation (Nguitragool et al. 2011). Rodent malaria species *Plasmodium berghei* could be used to investigate *clag's* contribution to NPPs if it can be shown functionality remains the same across species.

However, there is currently no method available for assessing NPP functionality in a rodent model.

Firstly, this study aimed to develop an osmotic lysis assay to determine NPP functionality in infected rodent erythrocytes. A series of compounds were screened on infected erythrocytes for their ability to selectively cause lysis, guanidinium-hydrochloride was found to exclusively lyse infected erythrocytes. Subsequently, NPP inhibitors and synchronous population assays showed lysis was mediated by the NPPs.

Next, the *P. berghei clag* gene was modified such that it expressed *P. falciparum* c-terminal region to reveal if *clag* gene functionality is conserved across the two species. Transgenic parasites were generated, proposing the conservation of *clag* gene across species. This work shows that *clag* genes can be studied in rodent model *P. berghei* with a higher confidence of relatability to *P. falciparum* parasite function.

Nguitragool, W, Bokhari, AAB, Pillai, AD, Rayavara, K, Sharma, P, Turpin, B, Aravind, L & Desai, SA 2011, 'Malaria parasite *clag3* genes determine channel-mediated nutrient uptake by infected red blood cells', *Cell*, vol. 145, no. 5, pp. 665-77.