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# Quality Assessment of Plain Chest X-Rays of the Expatriates in Jordan: Multi-Centres Study

**Mohammad Ayasrah[1], Rasha Al Hrout[2] and Khaleel Rawahneh[3]**

[1]Jordan University of Science and Technology Faculty of Applied Medical Science, IRBID, IRBID GOVERNORATE, Jordan.
[2]School of Physics, Universiti Sains Malaysia, 11800 USM, Penang, Malaysia.
[3]Madaba Health Directorate, Ministry of Health, Madaba, Jordan.

This study is a retrospective cross-sectional assessment of the quality of plain chest X-rays (CXRs) of expatriates in Jordan. It examines how well the American College of Radiology (ACR) and European Commission (EC) criteria were followed at 12 health sites. The study examined 1,020 CXR images gathered in 2023, specifically assessing the degree to which these images adhered to specific radiographic quality standards. The results showed substantial discrepancies in adherence to various quality criteria. Although key criteria such as exposure and anatomical visualization showed good compliance, overall adherence to all standards was alarmingly low, with just 15% of CXRs meeting the entire set of quality requirements. The study identified specific struggles in organ superimposition and image annotation, with compliance rates of 23% and 29%, respectively. This work introduces a novel feature: using a quantitative classification system to evaluate the quality of CXR, similar to the systems used in mammography. This technique enables the more accurate and systematic examination of radiographs, with the potential to enhance the precision of evaluations. The findings emphasize the need for improved training, greater adherence to protocols, and stronger quality control systems to guarantee consistent quality in CXR procedures. The discrepancies in adherence underscore the need for systemic changes to enhance diagnostic accuracy and patient care. These results indicate that enforcing strict criteria more rigorously might greatly improve the quality of diagnostic imaging and maximize health outcomes in healthcare settings worldwide.

# The Role of Mitogen-Activated Protein Kinases and Endoplasmic Reticulum Stress Signaling Pathways in Annatto Tocotrienol-Induced Human Chondrosarcoma SW1353 Cell Paraptosis

**Kok Lun Pang[1,2], Qixiang Wong[2], Yun Qi Ng[2], Roshan Mascarenhas[2], James Woon Sy-Keen[2] and Kok Yong Chin[3,*]**

[1]Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, Jalan Lagoon Selatan, 47500 Subang Jaya, Selangor, Malaysia.
[2]Newcastle University Medicine Malaysia, No. 1, Jalan Sarjana 1, Educity, 79200 Iskandar Puteri, Johor, Malaysia.
[3]Department of Pharmacology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jalan Yaacob Latif, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur, Malaysia.

*Corresponding Author: chinkokyong@ppukm.ukm.edu.my

Chondrosarcoma is the second most common primary bone cancer and involves cartilage. Surgical removal is the main treatment because chondrosarcoma cells are naturally resistant to chemotherapy- or radiotherapy-induced cell death, regardless of stage. However, paraptosis is a distinct type of programmed cell death that differs from typical apoptosis. Cytoplasmic vacuolation, caspase independence, and dilation of the endoplasmic reticulum and/or mitochondria are its key distinguishing features. Paraptosis induction has significant potential as an alternative strategy for eliminating apoptosis-resistant cancer cells. Tocotrienol is a natural form of vitamin E found in various vegetable oils and nuts. It has four isoforms—α-, β-, γ-, and δ-tocotrienol, based on side-chain variations at the chromanol ring. Annatto-based tocotrienol (AnTT) is unique because it contains only γ-, and δ-tocotrienols, both of which have been widely reported to possess promising anticancer properties. Furthermore, γ- and δ-tocotrienol have also been shown to induce paraptosis in several cancer cell lines, though their molecular mechanisms remain unclear. We previously demonstrated that AnTT induces SW1353 chondrosarcoma cell death with extensive cytoplasmic vacuolation. Simultaneously, transcriptomic analysis showed that endoplasmic reticulum stress (ERS) and mitogen-activated protein kinases (MAPK) signaling were involved in the effects of AnTT treatment. Therefore, in this study, we aimed to elucidate the role of ERS and MAPKs, including p38, extracellular signal-regulated kinase (ERK), and c-Jun N-terminal kinase (JNK), in AnTT-mediated SW1353 cell death. The new data showed that AnTT-induced SW1353 cell death is not dependent on caspase activation, which is another characteristic of paraptotic cell death. Chemicals that blocked ERS signaling, MAPK pathways, or both exacerbated AnTT-induced, caspase-independent cell death. In conclusion, ERS and MAPK signaling are suggested to be involved in cancer cell defense against AnTT-mediated cell death. Tocotrienols, alone or in combination with ERS and/or MAPK inhibitors could be a potential adjuvant therapy for chondrosarcoma.

# Identification of Alpha-1 Antitrypsin as a Candidate Biomarker for Post COVID-19 Syndrome by Two-Dimensional Electrophoresis Proteomic Analysis

**Norbaiyah Mohamed Bakrim[1,*], Aisyah Badrulhisham[1], Wan Nurliyana Wan Ramli[2], Hairul Aini Hamzah[1], Nor Zamzila Abdullah[3], Salmah Anim Abu Hassan[4], Dzawani Muhamad[5], Mohd Yusri Idorus[6] and Alfi Khatib[7]**

[1]Department of Basic Medical Sciences, Kulliyyah of Medicine, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang.
[2]Department of Internal Medicine, Kulliyyah of Medicine, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang.
[3]Department of Pathology and Laboratory Medicine, Kulliyyah of Medicine, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang.
[4]Department of Orthopaedics, Traumatology & Rehabilitation, Kulliyyah of Medicine, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang.
[5]Department of Medicine, Hospital Tengku Ampuan Afzan, Jalan Tanah Putih, 25100 Kuantan, Pahang.
[6]Institute of Medical Molecular Biotechnology, UiTM Sungai Buloh, 47000, Selangor.
[7]Department of Pharmaceutical Chemistry, Kulliyyah of Pharmacy, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang.

*Corresponding Author: drbaiyah@iium.edu.my

Despite a significant decrease in Coronavirus disease (COVID-19) case prevalence since the end of pandemic phase and effectiveness of the vaccination program, some patients continue to experience symptoms beyond three months of infection known as Post COVID-19 Syndromes (PSC). Common manifestations include enduring cough, shortness of breath and fatigue, which adversely affect quality of life. The primary challenge in managing PCS is its diagnosis, which relies on clinical symptoms and exclusion of other possible diagnoses. This approach is time-consuming, involves numerous tests and procedures, and leads to delayed initiation of appropriate intervention. Therefore, identifying reliable biomarkers for PCS is crucial for improving diagnosis accuracy, predicting outcomes, and developing targeted therapies. This study compared the proteomic profiles between PCS patients and healthy controls to identify disease-specific proteins that could serve as candidate biomarkers. This was a comparative cross-sectional study that recruited six PCS patients who fulfilled the inclusion criteria; persistent symptoms more than three months post infection with diagnosis was confirmed by a physician at the Post COVID-19 clinic. The control group consisted of 10 age, gender and race matched healthy subjects with no history of infection for the past one year. Protein of pooled serum from each group were extracted and separated using two-dimensional electrophoresis method. Firstly, the proteins were separated via iso-electric point, then via different molecular weight. The protein spots were analysed using PD Quest software, and proteins expression with significant difference between the two groups were recognized. Subsequently, proteins of interest were analysed using Matrix Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) Mass Spectrometry for protein identification. All subjects were Malay females with mean age of 38 ± 9.7 for PCS group and 42 ± 11.8 years for healthy control. Persistent cough and fatigue were found to be the most common presentations (83%) followed by exertional dyspnoea (67%). Proteomic profiles analysis reveals 182 proteins spots were detected in serum PCS patients in a range of pH 4 to 7. Alpha-1 antitrypsin, with molecular weight of 46.7 kDa was identified among the proteins that significantly over-expressed when compared to control ($p=0.02$). This protein is a protypical protease inhibitor that protects lung tissue from proteolytic damage by inhibiting neutrophil elastase. The elevated levels of Alpha-1 antitrypsin in the serum of PCS patients align with its known antiviral and anti-inflammatory properties, suggesting that it could serve as a potential diagnostic marker for PCS.

# Molecular Characterization of LIC10280, a Novel Putative Virulence Factor of *Leptospira interrogans* in yeast model

**Zhen Pei Wong[1], Aik-Hong Teh[2] and Eugene Boon Beng Ong[1,*]**

[1]Institute for Research in Molecular Medicine, Universiti Sains Malaysia, 11800 USM, Pulau Pinang, Malaysia.
[2]Centre for Chemical Biology, Universiti Sains Malaysia, 11900 Bayan Lepas, Pulau Pinang Malaysia.

*Corresponding Author: eugene@usm.my

Pathogenic Leptospira species are the cause of leptospirosis, a zoonotic disease. However, the molecular mechanisms of leptospiral pathogenesis are still unclear, as many genes related to virulence are still undiscovered. The budding yeast *Saccharomyces cerevisiae* is a popular eukaryotic model that has been used as an alternative model to identify bacterial VFs that target conserved eukaryotic cellular processes. Previously, our group has identified a protein with unknown function, LIC10280, as potential candidate VFs of *Leptospira interrogans* from yeast growth inhibition assay. In current study, we validated the protein LIC10280 as a leptospiral VF, studying molecular characteristics and its function in leptospiral pathogenesis. First, *in silico* analysis is carried out on protein sequence of LIC10280 to predict and analyse its molecular properties, function, and structure. Next, the targeted eukaryotic cellular processes or molecules of LIC10280 were investigated in the yeast cell model. Then, the interaction between LIC10280 and host components was investigated by *in vitro* studies. As the results showed, protein LIC10280 is predicted as a secretory protein that contains a signal peptide at the N-terminus of the protein sequence. Only heterologous expression of the full-length sequence of protein LIC10280 showed yeast growth inhibition activity, reduced the yeast cells' viability, and the possibility of causing DNA fragmentation. The mature protein part of LIC10280 showed nucleus-binding properties in the yeast microscopy study. This study is still ongoing to identify the possible target molecule(s) or cellular process of protein LIC10280 to better understand the pathogenesis of leptospirosis.

# Knockdown of OTUB1 and OTUB2 Induces Cell Cycle Arrest and Modulates Cancer Regulatory Gene Expression in Hepatocellular Carcinoma Cells

**Wu Yanming and Nurulisa Zulkifle***

Department of Biomedical Sciences, Advanced Medical and Dental Institute, Universiti Sains Malaysia, Bertam, 13200 Kepala Batas, Penang, Malaysia.

*Corresponding Author: nurulisa@usm.my

Hepatocellular carcinoma (HCC) is a leading cause of cancer mortality worldwide, ranking among the top six cancers globally. Despite advances in molecular markers, HCC remains challenging to treat, necessitating ongoing research into novel therapeutic targets. Deubiquitinating enzymes (DUBs) such as OTUB1 and OTUB2 are implicated in these processes and are found to be upregulated in HCC. This study aims to investigate the effects of OTUB1 and OTUB2 knockdown on cell cycle progression and gene expression in HCC cells to evaluate their potential as therapeutic targets. To achieve this, $HepG2^{KRAB}$ stable cells were transfected with CRISPR guide RNA plasmids targeting OTUB1 and OTUB2 using Lipofectamine. Subsequently, Cell cycle analysis was conducted using Propidium Iodide (PI) staining. Furthermore, RT-PCR was performed using primers for selected cancer-related genes, including *c-Myc, TP53, HIF-1α, VEGF, Bcl-2, NF-κB, and CDH-1.* Finally, Statistical analyses were conducted using SPSS with significance set at $p<0.05$. As a result, PI staining shows a significant increase in the proportion of $HepG2^{KRAB/OTUB1}$ and $HepG2^{KRAB/OTUB2}$ cells in the G-phase (11.74% and 12.78%, respectively) compared to control groups (6.91% for $HepG2^{WT}$ and 6.14% for $HepG2^{KRAB}$). Moreover, A similar pattern was observed in the S-phase, with increases to 30.45% and 28.80% in $HepG2^{KRAB/OTUB1}$ and $HepG2^{KRAB/OTUB2}$ respectively, compared to control groups (20.03% for $HepG2^{WT}$ and 21.02% for $HepG2^{KRAB}$). Meanwhile, RT-PCR analysis demonstrates a significant reduction in OTUB1 and OTUB2 mRNA levels following CRISPR knockdown. In $HepG2^{KRAB/OTUB1}$ cells, c-Myc expression decreased by 2.2-fold, TP53 by 2.8-fold, and HIF-1α and VEGF by 2.3-fold and 2.1-fold, respectively. Bcl-2, NF-κB, and CDH-1 levels were also significantly reduced by 3.1-fold, 2.0-fold and 2.4-fold, respectively. $HepG2^{KRAB/OTUB2}$ showed similar but less pronounced reductions in gene expression compared to $HepG2^{KRAB/OTUB1}$. Based on these findings, the increase in G-phase and S-phase percentages in PI staining indicates that knockdown of OTUB1 and OTUB2 leads to cell cycle arrest at the G2/S transition, hindering normal cell cycle progression. Consequently, this arrest results in reduced cellular proliferation, suggesting that OTUB1 and OTUB2 are critical for promoting cell cycle advancement in HCC. Moreover, the downregulation of oncogenes such as c-Myc and the tumor suppressor TP53 further supports impaired tumor growth and enhanced apoptosis. Additionally, decreased HIF-1α and VEGF levels suggest impaired angiogenesis, potentially slowing tumor progression and metastasis. In conclusion Knockdown of OTUB1 and OTUB2 disrupts key oncogenic pathways in HCC, leading to cell cycle arrest and reduced tumorigenic potential. Thus, these enzymes show promising therapeutic targets, offering new avenues for intervention in liver cancer treatment.

# *In silico* development of DNA aptamer against actin protein of *E. histolytica*

**Syahrul Amin Sa'adon[1], Nadiah Abdul Rashid[2] and Nurulhasanah Othman[1,*]**

[1]Institute for Research in Molecular Medicine (INFORMM), Main Campus, Universiti Sains Malaysia, Georgetown, 11800, Pulau Pinang, Malaysia.
[2]Department of Biotechnology, Kulliyah of Science, International Islamic University Malaysia (IIUM), Bandar Indera Mahkota, 25200 Kuantan, Pahang, Malaysia.

*Corresponding Author: nurulhasanah@usm.my

Amoebiasis imposes a substantial burden on low- and middle-income countries, including Malaysia, and necessitates timely and precise diagnosis to prevent severe outcomes such as amoebic liver abscess and mortality. However, current diagnostic methods rely primarily on microscopic and molecular tests like PCR and ELISA. In response to this challenge, we explore an innovative approach: enhancing aptamer design through *in silico* methods to meet specific binding requirements for biomolecule targets (actin protein of *E. histolytica*). Unlike antibodies, Aptamers, a single-stranded RNA or DNA with a unique tertiary structure that offers advantages such as high affinity, minimal immunogenicity, and reusability. Nevertheless, despite their potential, aptamer development remains labor-intensive and costly due to reliance on SELEX technology. To address this, the 3D structure of a single oligonucleotide strand was modeled to contain hairpin(s) and predicted to retain folding stability based on the free energy of secondary structure formation. Next, optimization of the size and number of hairpins was conducted in concert with the aptamer sequence length, employing a local docking procedure via AutoDock Vina. Subsequently, the performance and reliability of the resultant aptamer structure in targeting the actin protein of *E. histolytica* were further assessed using molecular docking and molecular dynamics (MD) simulation. The results showed that five aptamers exhibiting the lowest docking energies against the actin protein were identified using AutoDock Vina. Moreover, the clustering of molecular dynamics (MD) trajectories provided a more precise representation of the binding interactions, from which Apt1 and Apt8 emerged as the most suitable candidates for the actin protein. This study showed that the dynamic H-bond formation between the active binding residues and nucleotides stabilizes Apt1 and Apt8 binding on actin protein. In conclusion, the computational prediction of complex binding will include validations through experimental assays in future studies. This approach makes it easier and cheaper to develop better aptamers.

# A Case Report of a Novel *De Novo FBN1* c.2342del Leading to Premature Termination Codon in Marfan Syndrome Patients

**Anis Hazirah Azman[1], Ahmad Norasidi Mohd Raffie[1], Chew Hui Bein[2], Muzhirah Aisha Md Haniffa[2], Keng Wee Teik[2], Mohd Nazif Samat Darawi[1,*]**

[1]Faculty of Health Sciences, Universiti Selangor, Malaysia.
[2]Department of Genetics, Hospital Kuala Lumpur, Malaysia.

*Corresponding Author: nazifsamat@gmail.com, nazif@unisel.edu.my

Marfan syndrome (MFS) is an autosomal dominant connective tissue disorder affecting various body systems, mainly cardiovascular, ocular, and skeletal systems. This disease results from a mutation in the *FBN1* gene, which governs the production of fibrillin-1 protein vital for the integrity of the extracellular matrix across various bodily tissues. Over 7,000 *FBN1* variants have been reported, with a majority being unique to each pedigree. The presence of numerous *FBN1* variants has led to diverse phenotypic manifestations, varying severity levels, and different ages of onset posing challenges for diagnosis, treatment, and prognosis. Genomic DNA was extracted from a buccal swab specimen obtained from an 11-year-old girl who fulfilled the Ghent Nosology criteria and was subjected to exome sequencing (3Billion, Inc, Korea). A deletion of nucleotide (c.2342del) in the *FBN1* gene was detected in the sample. Subsequently, a pair of primers was designed and utilized to flank and amplify the DNA region containing the mutation for other related family members. The amplicons generated from the polymerase chain reaction were then subjected to Sanger sequencing. The cloning step followed by Sanger sequencing was necessary in the case of the heterozygous sample. Prediction of protein structure was conducted using AlphaFold2. *FBN1* c.2342del causes a shift in the reading frame at codon 781 resulting in a premature termination codon at position 22 downstream from the original mutation site (p.Cys781LeufsTer22). Sanger sequencing revealed the same mutation in the proband's twin but not in their parents and other family members. This indicates that the mutation occurred as a *de novo* event, meaning it arose spontaneously rather than being inherited. AlphaFold's protein prediction demonstrates high confidence in the local positioning of individual amino acid residues, except at positions around 0-100, 400, and 800, but exhibits low confidence in the relative domain positioning. The primers successfully amplified the correct segment of *FBN1*. Since the mutation is heterozygous in certain samples, DNA cloning followed by Sanger sequencing was employed to identify the normal and mutated allele. The identified mutation is classified as a likely pathogenic. It is predicted to result in a loss or disruption of normal protein function through nonsense-mediated decay (NMD) or protein truncation. Further validation through experimental study is needed to confirm the prediction from AlphaFold2. This research significantly enhanced the understanding of MFS. The discovery of the novel *FBN1* variant c.2342del (p.Cys781LeufsTer22) contributes to the expanding databases and mutation repositories related to MFS.

# Machine Learning-based Prostate Cancer Prediction Model: Integrating FTIR Spectra from Urinary EVs with Clinical Profiles in Modeling

**Le-Wei Wong[1], Razvan Ghiarasim[2], Janarthanan Supramaniam[3], Zhen-Hui Bu[4], Bey-Hing Goh[5,6,7], Lam-Hong Lee[4] and Wai-Leng Lee[1,*]**

[1]School of Science, Monash University Malaysia, 47500 Bandar Sunway, Selangor Darul Ehsan, Malaysia.
[2]Centre of Advanced Research in Bionanoconjugates and Biopolymers, "Petru Poni" Institute of Macromolecular Chemistry, Iasi, Romania.
[3]School of Pharmacy, Monash University Malaysia, 47500 Bandar Sunway, Selangor Darul Ehsan, Malaysia.
[4]Data Ecosystem Research and Development Centre, School of Computing, Quest International University, 30250 Ipoh, Perak Darul Ridzuan, Malaysia.
[5]Sunway Biofunctional Molecules Discovery Centre (SBMDC), School of Medical and Life Sciences, Sunway University, Sunway, Malaysia.
[6]Faculty of Health, Australian Research Centre in Complementary and Integrative Medicine, University of Technology Sydney, Ultimo, NSW, Australia.
[7]Biofunctional Molecule Exploratory Research Group (BMEX), School of Pharmacy, Monash University Malaysia, Bandar Sunway, Selangor Darul Ehsan, 47500, Malaysia.

*Corresponding Author: Lee.Wai.Leng@monash.edu

This study aims to develop an extracellular vesicle (EV)--based machine learning (ML) predictive model for prostate cancer. Screening with Prostate-Specific Antigen (PSA) has enabled early prostate cancer detection; however, its limited specificity and high false-positive rates often lead to unnecessary biopsies and over-treatment. Recently, urinary EVs have emerged as potential biomarkers for prostate cancer. Recent advancements in ML-assisted spectroscopic analysis of liquid biopsies have shown potential to develop a non-invasive predictive model for prostate cancer using FTIR spectral markers from urinary EVs. In this study, we enrolled 33 male participants, including 16 with pathologically confirmed prostate cancer and 17 non-cancerous individuals. The cohort was divided into training and testing sets in a 4:1 ratio. Datasets from 3601 FTIR features acquired from urinary EVs, and clinical profiles, including demographics, PSA values, and past medical history were integrated into the modeling. FTIR features were extracted with data weighting by the Gain Ratio algorithm, whereas the clinical features were manually selected based on their relevance to prostate cancer. ML classifiers, including k-Nearest Neighbors (k-NN), Support Vector Machine (SVM), Decision Tree (DT), and Naïve Bayes (NB), were trained using different combinations of the datasets with a five-fold cross-validation technique. The performance of the best-trained model was then evaluated on the testing set. Using FTIR features alone, the highest performance was achieved by the NB classifier when trained with the top 100 ranked FTIR features, with 85.1% area under the curve (AUC) of ROC and 84.6% accuracy. Notably, training the classifiers with PSA alone, which ranged from 1.09 to 102.56 ng/mL in this patient cohort, yielded unsatisfactory performance, with the NB classifier achieving the highest performance of 64.6% AUC and 73.1% accuracy. Despite PSA being a standard biomarker for prostate cancer, its limited classification performance indicates that relying solely on PSA for screening may be insufficient. Additional features or approaches are therefore required to improve the screening accuracy. Interestingly, combining both FTIR and clinical features offers a more holistic approach to model training, resulting in enhanced AUC and accuracy of 89.0% and 84.6%, respectively achieved by the DT model. When evaluating the DT model using the testing dataset, it demonstrated decent performance, with the AUC and accuracy reaching 91.7% and 85.7%. This study highlights the potential of ML classifiers to effectively exploit the spectral biomarkers derived from urinary EVs and clinical features that are indicative of cancer presence to address the limitations of PSA-based cancer screening.

# *KRAS* Mutation Profile of Colorectal Cancer Patients Diagnosed in Hospital Universiti Sains Malaysia

**Ng Phei Ying[1], Siti Norasikin Mohd Nafi[1,*], Nur Asyilla Che Jalil[1,4], Anani Aila Mat Zin[1,4], Lee Yeong Yeh[2,4] and Kueh Yee Cheng[3]**

[1]Department of Pathology, School of Medical Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.
[2]Department of Medicine, School of Medical Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.
[3]Biostatistics and Research Methodology Unit, School of Medical Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.
[4]Hospital Universiti Sains Malaysia, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.

*Corresponding Author: snmn@usm.my

Colorectal cancer (CRC) is the third most common cancer globally and the second most common in Malaysia. *KRAS* is a strong negative predictive biomarker for anti-*EGFR* therapy in CRC, making its detection mandatory for late-stage treatment decisions. This emphasizes the importance of gene mutation detection in precision medicine. This study aimed to determine the *KRAS* mutation profile of CRC patients in HUSM via targeted next-generation sequencing (NGS) and direct sequencing approaches. Archival CRC tissue blocks were retrieved from the Pathology laboratory, HUSM. Gene mutations, including *KRAS*, were determined using the TruSight Tumor 15 (TST15) panel through targeted NGS (n = 12). *KRAS* exon 2 profiling, which covered the detection of codon 12 and 13 variants, was then conducted using direct Sanger sequencing (n = 54). Using the TST15 panel, 66.7% of CRC cases were detected with *KRAS* mutations (G12C (25%); G12D (25%); G12S (12.5%); G12A (12.5%); A146T (12.5%); and A146V (12.5%)), whereas 33.3% of CRC cases were found with wild-type *KRAS*. Through direct sequencing, 44.4% of CRC cases were found with *KRAS* mutation, whereas 55.6% had wild-type *KRAS*. Most of the cases were detected with the *KRAS* variant G12D (87.5%), followed by G12C (4.2%), G12S (4.2%), and G12A (4.2%). Two cases with *KRAS* exon 4 variants A146T and A146V found targeted NGS were excluded from the comparison of the findings. Using both approaches, seven cases showed similar findings: two cases of G12D and one case of G12S (variant frequency in targeted NGS: 34.4%, 36.1%, and 53.5%, respectively), and four cases of wild-type *KRAS*. In contrast for another three cases: two cases of G12C and one case of G12A (variant frequency in targeted NGS: 23.8%, 34.4%, and 11.4%, respectively) were detected using targeted NGS, but all three cases were found with wild-type *KRAS* using direct sequencing. Thus, those with variant frequencies of ≤34.4% in targeted NGS were possibly detected or missed through direct sequencing. Using two molecular techniques NGS and direct sequencing, our investigation identified several variants of the *KRAS* gene in patients with CRC.

# Development of Electrochemical Aptasensor for the Detection of BipD Antigen of *Burkholderia pseudomallei* for Diagnostics Application

**Kasturi Selvam, Mohamad Ahmad Najib, Muhammad Fazli Khalid, Muhammad Hafiznur Yunus, Khairul Mohd Fadzli Mustaffa and Ismail Aziah***

Institute for Research in Molecular Medicine (INFORMM), Universiti Sains Malaysia, Kubang Kerian 16150, Kelantan, Malaysia.

*Corresponding Author: aziahismail@usm.my

Melioidosis, an infectious disease caused by *Burkholderia pseudomallei*, is prevalent in tropical regions and is associated with high mortality rates. The current gold standard for diagnosing melioidosis is culture, a method that is time-consuming and prone to cause false negative results, emphasizing the urgent need for rapid, sensitive, and specific diagnostic tests. In this study, a label-free electrochemical biosensor, named Melioidosis Aptasensor, was developed to detect *Burkholderia* invasion protein D (BipD) antigen of *B. pseudomallei*, aiming to address the aforementioned diagnostic challenges. The Melioidosis Aptasensor was fabricated using screen-printed gold electrodes (SPGE). The BipD protein-specific aptamer, AptBipD1, was thiolated at the 5' end and reduced with tris(2-carboxyethyl)phosphine (TCEP) before being immobilized onto the working electrode overnight in a water-saturated environment. The electrode surface was then passivated with 6-mercaptohexanol (MCH) to reduce non-specific binding before the incubation with BipD protein for 30 minutes. Current measurements were performed using a 5 mM potassium ferro/ferricyanide solution in 10X PBS buffer at pH 7.4. Following sensor fabrication, the aptamer concentration was optimized between 0.5 µM and 2 µM. Moreover, the specificity of Aptasensor was evaluated with lysates from *B. pseudomallei* and various Gram-negative pathogenic bacteria, including *Salmonella* Typhi, *Shigella flexneri*, *Klebsiella pneumoniae*, and *Escherichia coli*. The limit of detection (LoD) of aptasensor was determined through linear regression analysis by spiking both the buffer solution (1X PBS, pH 7.4) and healthy serum with BipD protein serially diluted from 1 µg/mL. A reduction in current was observed at each step during fabrication, indicating a decrease in the rate of electron transfer between the redox species and the electrode. The optimal aptamer concentration was 2 µM to provide sufficient binding sites for capturing the target. The aptasensor demonstrated high specificity, as confirmed by one-way ANOVA ($p < 0.001$), highlighting a significant difference between *B. pseudomallei* and other tested bacteria, thereby minimizing false-positive results. Furthermore, the aptasensor achieved LoD of 2.52 ng/mL in buffer solution and 2.29 ng/mL in serum, showcasing its ability to detect low concentrations of BipD protein. These results suggest that the developed aptasensor is a highly specific and sensitive tool for the detection of *B. pseudomallei*, offering significant potential for improving the diagnosis of melioidosis.

# Enzymatic access to the rare ΔGlcA (α1→4) Glc 3, 6, N-sulfated heparin disaccharide, implications for heparin quality control

**T. K. Kandola[1], C. J. Mycroft-West[2], M. L. Andrade De Lima[1], A. Turner[2], C. Gardini[3], E. Urso[3], G.J. Miller[1], A. Bisio[3], E. A. Yates[4,1], L. Wu[2,5] and M. Guerrini[3]and M. A. Skidmore[1,3]**

[1]Centre for Glycoscience Research and Training, Keele University, Huxley Building, Keele, Staffordshire, ST5 5BG, United Kingdom.
[2]The Rosalind Franklin Institute, Harwell Science and Innovation Campus, Oxfordshire, OX11 0QX, United Kingdom.
[3]Istituto di Ricerche Chimiche e Biochimiche 'G. Ronzoni', Via G. Colombo 81, 20133, Milan, Italy.
[4]Department of Biochemistry and Systems Biology, Institute of Structural, Molecular and Integrative Biology, University of Liverpool, Crown Street, Liverpool, L69 7ZB, United Kingdom.
[5]Division of Structural Biology, Nuffield Department of Medicine, University of Oxford, The Wellcome Centre for Human Genetics, Oxford, OX3 7BN, United Kingdom.

*Corresponding author: m.a.skidmore@keele.ac.uk

Ongoing research within the field of novel, glycosaminoglycan bacterial lyase enzymes with distinct substrate specificities continues to be an important focus of heparin / HS structure-function studies. The most widely researched heparin lyase enzymes are those from *Pedobacter heparinus*, and these are routinely used commercially in the production of low molecular weight heparins (LMWHs) and to assist in the structural characterisation of GAGs. This project highlights the latest progress and future directions regarding an alternative lyase enzyme derived from *Bacteroides eggerthii* . The recombinant expression of this *B. eggerthii* heparin lyase has highlighted distinct substrate specificities in comparison to that of the heparin lyase enzyme from *P. heparinus*.

# Evaluating the Potential of Mesenchymal Stem Cells Expressing Erythropoietin for Treating Retinal Dysfunction in a Rat Model

**Mok Pooi Ling[1,*], Avin Koh Ee-Hwan[1], Shirley Ding Suet Lee[1], Mohd Hairul Nizam Harun[2], Mae-Lynn Catherine Bastion[2], Lam Chenshen[2], Then Kong Yong[2], Munirah Binti Abd Rashid[2], Ng Min Hwei[3] and Suresh Kumar Subbiah[4]**

[1]Department of Biomedical Science, Faculty of Medicine & Health Sciences, Universiti Putra Malaysia, Selangor, Malaysia.
[2]Department of Ophthalmology, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Kuala Lumpur, Malaysia.
[3]Tissue Engineering Centre, Universiti Kebangsaan Malaysia Medical Center, Kuala Lumpur, Malaysia
[4]Saveetha Institute of Medical and Technical Sciences, Chennai, India.

*Corresponding Author: pooi_ling@upm.edu.my

Deterioration in visual function is a consequence of degeneration of retinal neuronal cells due to aging and commonly worsens when a patient has already underlying diseases, such as untreated diabetes mellitus. Stem cells have demonstrated potential clinical value in restoring the function of damaged tissues in many disease models. However, the efficacy of stem cell treatment can be affected by their reduced survivability in a pathologically harmful or inflamed tissue microenvironment. In this study, we aimed to investigate the therapeutic potential of genetically-modified Mesenchymal Stem Cells (MSCs) to express erythropoietin protein ($MSCs^{EPO}$) to reverse retinal neuronal cell degeneration and improve the visual function using a Sprague Dawley rat model. EPO is an anti-apoptotic protein, and its receptor is highly expressed on the retina to protect the eyes from intense light exposure in the early morning. The genetically-modified cells were produced by transducing a lentiviral vector encoding a human *EPO* gene into human Wharton-Jelly MSCs. The transduced cells were sorted, confirmed to secrete the EPO protein by ELISA, and culture-expanded for further downstream experiments. The $MSCs^{EPO}$ was evaluated for their ability to differentiate into neurospheres and reduce Y79 retinal neuronal cell damage induced by glutamate solution *in vitro*. The cells were then transplanted intravitreally into the rats which were treated prior with sodium iodate. Electroretinography (ERG) was performed on the rats before they were sacrificed to harvest the retina tissues for histological and gene expression analyses after one month of transplantation. The *in vitro* results indicated a relatively larger neurosphere formation from the $MSCs^{EPO}$, and a significantly enhanced survival of the Y79 cells when they were exposed to conditioned media harvested from the $MSCs^{EPO}$ group when compared with unmodified MSCs. There was also a significantly increased fold change in the b-wave amplitude, a relatively thicker retina, and expression of a unique profile of genes that likely contributed to the survival of retinas exposed to sodium iodate when transplanted with $MSC^{EPO}$ compared to unmodified MSCs or sham control rat groups. In summary, the findings of our study showed the tremendous potential of $MSCs^{EPO}$ in protecting the retina from retinal degeneration.

# Therapeutic Potential of Stingless Bee Pollen: Enhancing DNA Methylation and Genomic Stability

**Nurdianah Harif Fadzilah[1,*] and Wan Adnan Wan Omar[2]**

[1]Advanced Medical and Dental Institute, Universiti Sains Malaysia, Bertam, 13200 Kepala Batas, Penang, Malaysia.
[2]Sri Nusa Traditional Rehabilitation Centre, No 6, Lorong Panglima 4, Bertam Perdana, 13200 Kepala Batas, Penang, Malaysia.

*Corresponding Author: nurdianaharif@usm.my

Bee pollen is considered a complete food, packed with essential nutrients and therapeutic properties that offer protective effects against epigenetic alterations, which in turn help prevent genomic instability. Stingless bee pollens play a crucial role in enhancing DNA methylation patterns, specifically in Alu and LINE-1 repetitive elements. These repetitive elements are key indicators of genomic stability, and their proper methylation is essential for maintaining cellular health and preventing various diseases, including cancer. This study investigated the protective effects of stingless bee pollen against global DNA hypomethylation. Bee pollen extract (BPE) was prepared from *Geniotrigona thoracica* stingless bee species native to Malaysia. Spectrophotometric testing was used for chemical and antioxidant activity, HPLC and GC-MS analysis for phenolic compound identification, trypan blue exclusion assay for antiproliferative effects, and PCR-Pyrosequencing for global methylation quantification. In antioxidant activity, *G. thoracica* BPE exhibited an $EC_{50}$ of 0.98 ± 0.18 mg/mL. HPLC and GC-MS analysis revealed distinct chemical profiles of phenolic compounds in *G. thoracica* BPE. In the antiproliferation assay, *G. thoracica* BPE showed a therapeutic index of 3.12 and an $EC_{50}$ of 0.5 mg/mL. In global DNA methylation analysis, *G. thoracica* BPE significantly increased Alu methylation at 24 hours (26.00% ± 0.58 vs. 21.50% ± 0.96, p = 0.007) and 48 hours (31.75% ± 1.89 vs. 20.25% ± 0.95, p = 0.004). LINE-1 methylation levels were also significantly elevated at 24 hours (79.92% ± 1.26 vs. 72.83% ± 2.21, p = 0.050) and at 72 hours (95.25% ± 2.46 vs. 74.83% ± 0.44, p = 0.001) compared to untreated cells. The increased DNA methylation observed in Alu and LINE-1 elements suggests that stingless BPE could influence epigenetic regulation. The therapeutic properties of Malaysian stingless bee pollen extend beyond its antioxidant capabilities, promoting genomic stability through enhanced DNA methylation.

# The role of ROS generation in the anticancer activity of novel Schiff base compound in triple-negative breast cancer

**Reyhaneh Farghadani*, Han Yin Lim, Mahmood Ameen Abdulla, Jayakumar Rajarajeswaran**

Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, Jalan Lagoon Selatan, Bandar Sunway, Selangor Darul Ehsan 47500, Malaysia.

*Corresponding Author: reyhaneh.farghadani@monash.edu

Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer characterized by high recurrence rates and poor prognosis, often leading to distant metastases in the brain, lung, bone, and liver. Current chemotherapeutic regimens for TNBC are limited by drug resistance and adverse side effects, underscoring the urgent need for novel and more effective treatments. Reactive oxygen species (ROS) play a dual role in cancer, acting as a double-edged sword. While modest ROS levels promote tumour progression, excessive ROS levels can suppress tumours. Cancer cells with elevated ROS are highly dependent on antioxidant defences, and further increases in ROS levels beyond a tolerable threshold can induce cell death. Therefore, strategies that elevate ROS levels are being explored to preferentially induce cell death in cancer cells, contributing to the efficacy of many anticancer agents. Schiff bases, biologically active molecules with enhanced pharmacological activities, have shown significant promise in drug discovery. Recent efforts have focused on developing Schiff base derivatives with potent anticancer properties and reduced toxicity. In this study, we investigated the anticancer potential of a novel β-diiminato Schiff base compound in TNBC, focusing on its ability to induce ROS generation. Our findings demonstrate that the Schiff base derivative significantly elevates intracellular ROS levels in TNBC cells in a dose-dependent manner, as measured by DCFDA assay. This increase in ROS disrupts mitochondrial membrane integrity, activating the intrinsic apoptotic pathway. In vivo studies also confirmed the safety profile of the compound. These results suggest that ROS generation is a critical mechanism underlying the anticancer activity of Schiff base derivatives, offering a promising avenue for developing new therapeutic strategies against TNBC.

# Application of *In-Silico* Approach in Determining the Suitability of Rho GTPase and Rho GTPase Activating Protein (RhoGAP) as Drug Targets of *Giardia lamblia*

**Nurhana Jasni and Nurulhasanah Othman***

Institute for Research in Molecular Medicine (INFORMM), Universiti Sains Malaysia, Penang, Malaysia.

*Corresponding Author: nurulhasanah@usm.my

*Giardia lamblia* is a globally distributed protozoan parasite that causes an intestinal disease named Giardiasis. The primary treatment relies on nitroimidazole drugs such as metronidazole (MTZ), tinidazole, and albendazole. However, the incidence of refractory cases had increased which led to therapeutic non-compliance. Although numerous research studies have been conducted to address these concerns, they remain unresolved. Rho family GTPase and its positive regulator, Rho GTPase activating protein (RhoGAP) were found to be involved in various biological and cellular processes. They were found to be involved in regulating encystation, membrane trafficking, and the metabolic processes of the protozoan parasite. Thus, it is hypothesized that inhibiting these proteins would lead to the killing of the parasite. Target sequence-based search using DrugBank, molecular docking, and molecular dynamic simulation are the current methods that are employed in drug target discovery. Thus, these methods were applied to evaluate both proteins as drug targets. Through the target sequence analysis, two repurposed drugs were matched with Rho GTPase which are Dextromethorphan and Azathioprine. On the other hand, three drugs were matched with Rho GTPase-activating protein (RhoGAP) which were Imatinib, Dasatinib, and Ponatinib. Molecular docking analysis using Auto Dock Vina showed the binding energies of the five drugs to their respective proteins were -8.5 kJ/mol, -8.0 kJ/mol, -7.0 kJ/mol, -5.3 kJ/mol, and -6.8 kJ/mol represented by Imatinib, Ponatinib, Dasatinib, Azathioprine, and Dextromethorphan, respectively. Molecular dynamic simulation analysis performed at 100 ns using GROMACS showed all the complexes exhibit various compactness, uniqueness, and satisfactory stability. Thus, we concluded that among all five drugs, two drugs which were Dasatinib and Imatinib potentially inhibit the Rho GTPase-activating protein (RhoGAP). In contrast, one drug potentially inhibits the Rho GTPase protein.

# Gene Expression Analysis of *Giardia lamblia* Small GTPase, Rho subfamily protein treated with Dasatinib inhibitor

**Haneen Imad Abdulsattar, Nurhana Jasni and Nurulhasanah Othman***

Institute for Research in Molecular Medicine (INFORMM), Universiti Sains Malaysia, Penang.

*Corresponding Author: nurulhasanah@usm.my

Giardiasis, caused by the protozoan parasite *Giardia lamblia*, is commonly treated with Metronidazole as the primary therapeutic option. However, the emergence of adverse effects and resistance to Metronidazole has inspired research into new treatments. This study focused on investigating the potential of RhoGTPase family proteins as targets for drug intervention in *G. lamblia*. Based on its involvement in metabolic processes, as indicated by the UniProtKB database, and previous findings showing up-regulation of Rho GTPase family protein in the virulent variant of *E. histolytica*, another protozoan parasite, it was hypothesized that by inhibiting RhoGTPase family proteins, the *G. lamblia* metabolic process could be interrupted, leading to the parasite's death. Previous analyses utilised target sequence analysis in the DrugBank database, molecular docking, molecular dynamic simulations, and minimal inhibitory concentration (MIC) test, demonstrating a strong binding affinity between the putative Rho GTPase-activating protein and Dasatinib. This binding suggested that Dasatinib could be effective in killing this parasite at minimal inhibitory concentrations. Therefore, this study was designed to explore the effects of inhibiting the putative Rho GTPase-activating protein with Dasatinib on the small GTPase, Rho subfamily protein, through gene expression analysis. *G. lamblia* trophozoites were cultured and treated with MIC 12.5 µM Dasatinib at three different time points, before parasite death, which occurred within 48 hours. The RNA was extracted using the TRIzol reagent, quantified, purified, and reverse-transcribed into complementary DNA (cDNA). Quantitative PCR (qPCR) was performed using specifically designed primers targeting the small GTPase, Rho subfamily protein, with Aldolase serving as the reference gene. The qPCR parameters were optimized, resulting in high PCR efficiency (reaction efficiency = 1.18 and $R^2$ = 0.97502). Microscopic analysis revealed distinct morphological changes in the treated *G. lamblia* cells, including shrinkage, loss of motility, and compromised cellular integrity, which further supported the gene expression findings. Treatment with Dasatinib led to significant alterations in small GTPase, Rho subfamily protein gene expression. After 30 hours of treatment, the gene was upregulated with a fold change (Fc) of 3.22. However, at 36 hours, downregulation was observed with Fc = 5.12, and by 42 hours, the gene expression had further reduced with an Fc = 3.16, indicating a substantial reduction. This study validated that inhibiting the putative RhoGTPase-activating protein with Dasatinib resulted in differential regulation of the small GTPase, Rho subfamily protein gene expression, which may contribute to the killing of *G. lamblia*.

# TGFBR2 c.1220G>A Mutation and Thoracic Aortic Aneurysm and Dissection Disease: Bioinformatics Insights

**Barizah Pulli[1], Aiman Afif Roslan[1], Chew Hui Bein[2], Muzhirah Aisha Md Haniffa[2], Keng Wee Teik[2] and Mohd Nazif Samat Darawi[1,*]**

[1]Faculty of Health Sciences, Universiti Selangor, Malaysia.
[2]Department of Genetics, Hospital Kuala Lumpur, Malaysia.

*Corresponding Author: nazifsamat@gmail.com, nazif@unisel.edu.my

Thoracic aortic aneurysms and dissection (TAAD) is a genetically heterogeneous disorder characterized by abnormal dilatation of the thoracic aorta, often leading to a life-threatening condition such as aortic dissection or ruptures of the aorta. Many genes contribute to the development of TAAD, with the majority encoding proteins involved in the extracellular matrix, smooth muscle cell contraction or metabolism, or the transforming growth factor-β signalling pathway. Early identification of at-risk individuals through genetic testing is crucial for timely clinical management and potentially life-saving interventions. A heterozygous substitution of a nucleotide (c.1220G>A) in the *TGFBR2* gene was detected in the proband, a Chinese male using next-generation sequencing TAAD panel (Centrum Medische Genetica, Belgium). Bioinformatics analyses were performed using SWISS-MODEL, BIOVIA Discovery Studio Visualizer, Polyphen-2 and SIFT to predict the impact of the variant on protein structure and evaluate their effects on protein function. Subsequently, we investigated the presence of the variant in other family members for early detection. A set of primers was designed and employed to flank and amplify the DNA segment harbouring the variant. The PCR-generated amplicons were analysed using Sanger sequencing. The primers successfully amplified the correct segment of the *TGFBR2* gene with 832 bp in size. Sanger sequencing confirmed the presence of the same variant in the proband's son. A double peak in the electropherogram revealed a heterozygous genotype. This heterozygous missense substitution variant entails a guanine-to-adenine transition at position 1220 in the coding sequence of the *TGFBR2* gene, leading to a serine-to-asparagine alteration at amino acid codon 407. The high degree of conservation of the serine residue at this position across diverse species suggests a crucial role in the function of the transforming growth factor-β receptor 2 protein. This receptor plays a crucial role in signal transduction and regulates cellular processes such as proliferation and differentiation. Bioinformatics analyses effectively assessed the potential functional consequences of the genetic mutation by predicting their impact on protein stability and function. Molecular modelling studies further elucidated the structural consequences of the mutation, highlighting potential disruptions to hydrogen bonding networks critical for protein stability and ligand binding. Our findings underscore the importance of genetic testing for TAAD, particularly for family members of identified carriers, to facilitate early diagnosis and timely intervention to prevent potential complications.

# Graphene Oxide and Reduced Graphene Oxide as Novel Therapeutic Agents for Inhibiting Foam Cell Formation and Atherosclerosis Risk Reduction

**FarizahHanimLat[1], RafeezulMohamed[1,*], Ahmad NaqibShuid[2], MohdYusmaidieAziz[3] and Muhammad MahyiddinRamli[4]**

[1]Department of Biomedical Science, Advanced Medical and Dental Institute, Universiti Sains Malaysia, Bertam, 13200 Kepala Batas, Pulau Pinang, Malaysia.
[2]Department of Health Community, Advanced Medical and Dental Institute, Universiti Sains Malaysia, Bertam, 13200 Kepala Batas, Pulau Pinang, Malaysia.
[3]Department of Toxicology, Advanced Medical and Dental Institute, Universiti Sains Malaysia, Bertam,13200 Kepala Batas, Pulau Pinang, Malaysia.
[4]Institute of Nano Electronic Engineering, Universiti Malaysia Perlis, 01000 Kangar, Perlis, Malaysia.

*Corresponding Author: rafeezul@usm.my

Foam cells, which are macrophages overloaded with cholesterol, play a critical role in the development of atherosclerosis, a major cause of heart attacks and strokes. Carbon nanomaterials (CNMs), particularly graphene oxide (GO) and reduced graphene oxide (rGO), have emerged as promising candidates for cardiovascular therapies. This study investigates the impact of GO and rGO derived from oil palm trunks on foam cell formation. GO was synthesized using a modified Hummers' method and subsequently reduced thermally to produce rGO. Both materials were characterized through Raman spectroscopy, FTIR, XRD, and AFM to assess their structural properties. RAW 264.7 macrophages were treated with varying concentrations of GO and rGO (0.1 to 50 μg/mL) for 24, 48, and 72 hours to determine their effects on cell viability. Foam cell formation was evaluated by Oil Red O staining and total cholesterol quantification following treatment with oxidised low-density lipoprotein (oxLDL), GO and rGO. Pro-atherogenic cytokines secretion (IFNγ, TNFα, IL-6, IL-1β) were measured using ELISA, and gene expression of IL-1β, TNFα, ABCA1, CD36, ACAT1, SRA1, and ANXA were analysed via RT-qPCR. Raman spectroscopy indicated higher disorder in GO (ID/IG ≈ 1.23) compared to rGO (ID/IG ≈ 0.94). FTIR analysis showed that GO retained more oxygen-containing groups, and XRD revealed larger d-spacings for GO, highlighting its structural differences from rGO. AFM images confirmed that GO had greater surface roughness. Cell viability assays demonstrated that GO was more cytotoxic than rGO, particularly at higher concentrations and longer exposure times, leading to the selection of 1.0 μg/mL for further experiments. Both GO and rGO reduced lipid droplet accumulation and total cholesterol in oxLDL-treated macrophages, as evidenced by Oil Red O staining and total cholesterol quantification. Additionally, both materials decreased the secretion of pro-atherogenic cytokines (IFNγ, TNFα, IL-6, IL-1β). GO notably suppressed the expression of IL-1β, TNFα, CD36, ACAT1, and SRA1 genes, while enhancing ABCA1 expression, which facilitates lipid efflux. rGO exhibited similar effects on gene expression, though it showed increased expression of IL-1β and SRA1 genes in oxLDL-treated macrophages. These results suggest that GO and rGO hold potential as therapeutic agents for reducing atherosclerosis risk by mitigating inflammation and preventing foam cell formation in macrophages.

# Anti-Pigmentation and Anti-Ageing Effects of Mycosporines Produced by Wild-Type and Mutant Strains of *Xanthophllomyces dendrorhous*

**Neshalini Sathiabalan and Ai Lan Chew***

Institute for Research in Molecular Medicine (INFORMM), Universiti Sains Malaysia, Penang, Malaysia.

*Corresponding Author: chew@usm.my; ai_lan@hotmail.com

*Xanthophyllomyces dendrorhous* is a well-known species for carotenoid pigment production which has been exploited biotechnologically. Research has also proven that this yeast species contains UV-absorbing molecules known as mycosporines that can thrive in high oxidative and UV-exposure habitats where it is often associated with its potential as an ideal sunscreen. Hence, here we investigated the potential of mycosporines produced by *X. dendrorhous* wild-type (WD) and mutant strains red (R), yellow (Y) and white (WH) on their anti-pigmentation and anti-ageing ability using the 96-well plate assay method. Mycosporines extracted from *X. dendrorhous* strains that were cultured in an optimized condition were tested for their melanin regulation ability through tyrosinase inhibition assay. The tyrosinase inhibition assay confirmed that mycosporines of R have the highest inhibition of 75.46% with the lowest IC50 value of 20.10 µg/ml. The inhibition percentage descended with the WD, Y and WH strains (64.97%, 56.17%, 53.08%) respectively portraying a moderate tyrosinase inhibition effect. Wrinkles, the obvious indicator of ageing are due to damaged collagen and elastin in the skin epidermis. Mycosporines produced by the *X. dendrorhous* were also hypothesized to have anti-ageing effects which led to the analysis on the collagenase inhibitory assay and elastase inhibitory assay. The results showed that the R strains gave the highest collagenase inhibition activity of 89.73% which was followed by the WD, Y, and WH strains (85.34%, 81.98%, and 80.36%) respectively. Whereas the elastase inhibitory assay exhibited a strong elastase inhibition with the highest percentage (84.35%) by the R strain and accompanied by the WD, Y and WH strains (78.62%, 60.40%, and 55.95%) respectively. In short, our findings demonstrated that mycosporines produced by the *X. dendrorhous* wild-type and mutant strains have great potential as natural molecular sunscreen with their strong anti-pigmentation and anti-ageing effects.

# Single-Cell Multiomics Reveals Monocyte-Specific Dysregulation of IL1R2 and THBS1 in Colorectal Cancer

**Sabrina George[1], Nor Haslinda Abdul Aziz[2] and Nor Adzimah Johdi[1,*]**

[1]UKM Medical Molecular Biology Institute (UMBI), National University of Malaysia. [2]Department of Obstetrics and Gynecology, Faculty of Medicine, National University of Malaysia.

*Corresponding Author: adzimah@ppukm.ukm.edu.my

Colorectal cancer (CRC) remains a leading cause of cancer-related morbidity and mortality worldwide. The circulating immune cells play a pivotal role in CRC progression, with significant dysregulation often observed in CRC patients. This study aims to understand CRC's molecular and cellular immune landscape that may serve as potential biomarkers or therapeutic targets. We employed a multi-omics approach, combining single-cell RNA sequencing (scRNA-seq) with antibody-based sequencing (AbSeq), to dissect the immune profile of peripheral blood mononuclear cells (PBMCs) at a single-cell resolution. PBMCs were analysed from six CRC patients and six healthy controls using a targeted panel of 399 genes and 30 surface proteins, providing an in-depth view of these populations' immune heterogeneity and functional states. From this dataset, 12,226 high-quality PBMCs were obtained and six major immune subsets were identified: monocytes (5,131, 41.97%), CD4+ T cells (2,481, 20.29%), CD8+ T cells (1,796, 14.69%), natural killer (NK) cells (1,705, 13.94%), B cells (769, 6.29%), and dendritic cells (DCs) (193, 1.58%). Further analysis using the Seurat algorithm revealed 11 distinct clusters. Differential gene expression analysis reveals a significant upregulation of Interleukin 1 receptor, type II (IL1R2) and Thrombospondin 1 (THBS1) in monocytes derived from CRC patients compared to healthy controls. IL1R2, an anti-inflammatory receptor, and THBS1, an ECM protein implicated in various cancer-related processes, were both found to be overexpressed. This upregulation suggests potential alterations in monocyte functionality and their role in CRC pathogenesis. Specifically, the elevated IL1R2 levels may contribute to an altered inflammatory response, while increased THBS1 expression correlated with a poor prognosis. These findings suggest the potential of IL1R2 and THBS1 as prognostic biomarkers and therapeutic targets in CRC. Further investigation into the mechanistic roles of these molecules in CRC is warranted to explore their potential as biomarkers and therapeutic targets.

# Cytotoxic and Mutagenic Effects of Dewatered Sludge Treated with Microbial Fuel Cell (MFC) using *In Vitro* Systems

**Muhammad Adib Fadhlullah Muhammad Lukman[1], Nur Azzalia Kamaruzaman[1,*], Ana Masara Ahmad Mokhtar[2] and Amira Suriaty Yaakop[3]**

[1]National Poison Centre, Universiti Sains Malaysia, 11800 Minden, Penang. [2]School of Industrial Technology, Universiti Sains Malaysia, 11800 Minden, Penang. [3]School of Biological Sciences, Universiti Sains Malaysia, 11800 Minden, Penang.

*Corresponding Author: azzalia@usm.my

Significant increase in the production of activated sludge from wastewater treatment plants is causing severe environmental pollution and this has been a top-priority concern for environmental protection. Thus, there is an urgent need for sustainable waste treatment methods with innovative technological solutions. Microbial Fuel Cells (MFC) is a bio-electrochemical system which has been identified as a potential approach for sludge treatment and renewable energy production. However, utilization of dewatered sludge in conjunction with MFC technology has gained concerns regarding the environmental and health impacts of such practices especially in relation to DNA damage and cancer-inducing factors. Thus, this study aims to evaluate cytotoxic and mutagenic effects of dewatered sludge treated with MFC technology. MFC samples consisted of *Bacillus subtilis* (BS), *Bacillus cereus* (BC), *Bacillus tropicus* (BT), BS+BC, BS+BT and BS+BC+BT, and each sample was evaluated in six concentrations (1mg/ml, 0.5mg/ml, 0.25mg/ml, 0.125mg/ml, 0.0625mg/ml, 0.0313mg/ml). Cytotoxicity study was conducted using MTT assay, a cellular metabolic test, whereby human foreskin fibroblast Hs27 cells were treated with MFC samples. For mutagenicity, a Bacterial Reverse Mutation (AMES) test was performed using the MFC samples. *Salmonella typhimurium* strains TA98 and TA100 were utilized to detect frameshift and base-pair mutations respectively. Analysis of cell viability (%) from the treatment of MFC found that in comparison to the controls (xT and T0), all samples (BS, BC, BT, BS+BC, BS+BT and BS+BC+BT) showed slightly increased cell viability. Controls did not contain any bacteria in the samples where xT represents untreated non-autoclaved sludge and T0 represents untreated autoclaved sludge. Furthermore, mutagenicity test using *Salmonella typhimurium* bacteria found that the number of colonies grown for MFC samples for both strains were elevated for BT sample when compared to the negative control. Moreover, combination of bacteria BS+BC, BS+BT and BS+BC+BT showed more elevated number of colonies in comparison to control, which are higher than the single bacteria treatment. Additionally, the number of colonies for BS and BC samples was lower than the negative control. Based on cytotoxicity and mutagenicity tests performed on the MFC samples, it could be deduced that the MFC samples did not induce cytotoxicity as cell viability was increased, and mutagenic effect could be observed for certain samples especially for MFC samples treated with combination of bacteria. Thus, MFC technology when applied to dewatered sludge show some safety potential in terms of cell viability but should be used with single bacteria treatment to minimize mutagenic effect.

# Determining the Quantity and Quality of DNA from the FFPE Tissues NGS Library Preparation: A Sharing Experience

**Siti Noresah Mohd Zaim[1], Siti Norasikin Mohd Nafi[1,*] Norzaliana Zawawi[2,4] and Wan Fadhlina Wan Adnan[3,4]**

[1]Department of Pathology, School of Medical Sciences, Health Campus, Universiti Sains Malaysia, 16150, Kubang Kerian, Kelantan.
[2]School of Dental Sciences, Health Campus, Universiti Sains Malaysia, 16150, Kubang Kerian, Kelantan.
[3]Department of Obstetrics & Gynecology, School of Medical Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan.
[4]Hospital Universiti Sains Malaysia, 16150, Kubang Kerian, Kelantan.

*Corresponding Author: snmn@usm.my

Targeted Next-Generation Sequencing (NGS) is an approach that gains attention among clinicians and researchers to explore the specific mutation profiles of cancer. In pathology, formalin-fixed tissue (FFPE) tissues are suitable for long-term storage and preserving histological aspects, giving them key importance in retrospective research and molecular diagnostics. However, the procedure of formalin fixation and paraffin embedding leads to cross-linking and degradation of DNA, creating challenges for molecular research. Here, we share our experience in determining the quantity and quality of DNA from the FFPE tissues. as an initial step for NGS library preparation. This study aimed to evaluate the quality and quantity of DNA samples extracted from the FFPE endometrial cancer tissues and assess their suitability for NGS library preparation. A total of 28 FFPE tissue samples from endometrial cancer patients were collected from the pathology laboratory of Hospital Universiti Sains Malaysia (HUSM). The key tasks of this research include (1) extracting DNA from FFPE tissues, from which DNA was extracted using the QIAamp DNA FFPE Advanced UNG kit (QIAGEN, Germany) (2) quantifying and assessing the quality of the DNA, the quantification was measured through fluorometric assays, and the quality was assessed using spectrophotometry by measuring purity ratios (A260/A280) and the integrity of the DNA using agarose gel electrophoresis, and (3) preparing NGS libraries and evaluating their quality for sequencing, NGS library preparation was performed using a targeted gene panel, and the libraries were analysed for quality using Bioanalyzer, followed by sequencing on an Illumina platform. Library quality was evaluated based on yield, fragment size distribution, and sequencing metrics. DNA extracted from the FFPE tissues showed average yields ranging from 20 to 100 ng/μL per sample. The purity ratios (A260/280) were within acceptable limits, ranging between 1.8 and 2.0, indicating minimal protein contamination. Despite some fragmentation, most extracted DNA samples were suitable for targeted NGS library preparation. Bioanalyzer results will demonstrate library fragment sizes for sequencing. This study highlights that, although DNA extracted from FFPE tissues can be degraded, it is still suitable for NGS library preparation with proper extraction techniques and quality control measures. Ensuring high DNA quality and quantity from FFPE samples is critical for generating reliable sequencing data and improving molecular diagnostics for cancer. These findings emphasize the importance of precise quality control in the NGS workflow, particularly when working with archived tissue samples.

# Development of Carboxymethylcellulose Hydrogel from Oil Palm Empty Fruit Bunch for Efficient Curcumin Delivery

**Nurfarrahhin Rosle[1], Nurrulhidayah Salamun[2], Khairunadwa Jemon[3], and Wan Fatin Amira Wan Mohd Zawawi[1,3,*]**

[1]Department of Biosciences, Faculty of Science, Universiti Teknologi Malaysia, 81310, Johor, Malaysia.
[2]Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, 81310, UTM Johor Bahru, Johor, Malaysia.
[3]Cancer and Infectious Diseases Group, Health and Wellness Research Alliance, Universiti Teknologi Malaysia, 81310, Johor, Malaysia.

*Corresponding Author: wanfatinamira@utm.my

Malaysia stands as one of the leading global producer of oil palm, generating an enormous quantity of biomass waste, particularly oil palm empty fruit bunches (OPEFB). Due to the challenges associated with OPEFB disposal, there is growing interest in repurposing this biomass by extracting its high-purity cellulose to produce valuable biomaterials such as natural hydrogels. On the other hand, curcumin, a bioactive compound with demonstrated therapeutic efficacy in biomedical applications like wound healing and drug delivery, presents a challenge due to its hydrophobic nature, thus restricting its clinical application. To overcome this, using a hydrogel as a hydrophilic carrier is an effective approach to facilitate curcumin delivery. Therefore, this study aims to incorporate curcumin into carboxymethylcellulose hydrogel (CMC) to enhance its delivery efficiency. In this study, CMC hydrogel was synthesized from OPEFB through carboxymethylation process using sodium monochloroacetate (SMCA) and it was crosslinked at room temperature by using $CaCl_2$ as a cross-linking agent to evaluate its effectiveness as a hydrophilic system for curcumin delivery. All samples were characterized using FT-IR, XRD, EDX and FESEM to analyse chemical changes and modifications. FT-IR analysis confirmed the successful conversion of cellulose to CMC, with spectra showing only cellulose-related peaks. The XRD analysis demonstrated a significant transition from the crystalline cellulose structure of cellulose to an amorphous phase, as evidenced by the absence of OPEFB cellulose peaks in the CMC hydrogel. Furthermore, the elemental composition of the samples was verified through EDX analysis, while FESEM revealed significant morphological changes during both the extraction and cross-linking processes. To incorporate curcumin into the CMC hydrogel, physical adsorption methods was employed. The optimal concentrations of both CMC and curcumin were determined through swelling and release studies, which assessed the hydrogel capacity to load and release curcumin effectively. The results suggest that the 20% CMC hydrogel exhibit substantial potential to serve as an efficient hydrophilic system for curcumin delivery. The loading capacity analysis indicated that 0.2 mg/ml was the optimal concentration of curcumin. Moreover, release kinetics studies further confirmed that 0.2 mg/ml curcumin concentration achieved the most sustained release compared to other tested concentrations. Hence, this study offers a sustainable approach to biomass utilization while providing a promising framework for enhancing curcumin's therapeutic potential in biomedical applications.

# Cytotoxic Effects of *K. alvarezii* Crude Extract on Human Leukemia Cell Line K562

**Nur Fatin Najihah Mat Husin[1], Anisa Natasha Jefri[1], Khairunadwa Jemon[1], Nurzila Ab Latif[1], Nor Rizan Kamaluddin[2], Shaik Ahmad Kamal Shaik Mohd Fakiruddin[2], Nurriza Ab Latif[1,*]**

[1]Department of Biosciences, Faculty of Science, Universiti Teknologi Malaysia, 81310, Skudai, Johor.
[2]Cancer Research Centre (CaRC), Institute for Medical Research (IMR), National Institutes of Health (NIH), Block C, No.1, Jalan Setia Murni U13/52 Seksyen U13 Setia Alam, 40170 Shah Alam, Selangor, Malaysia.

*Corresponding Author: nurriza@utm.my

*Kappaphycus alvarezii* is a red seaweed that grows abundantly in East Malaysia and is rich in bioactive constituents. The crude extract of *K. alvarezii* has been reported to exhibit promising pharmacological effects such as anticancer, antiinflammatory, antiviral, antioxidant, antidiabetic, and cholesterol-lowering agents, as well as hemagglutination activities. Therefore, this study aimed to quantify the total phenolic and flavonoid contents, and cytotoxic properties of the *K. alvarezii* crude extract isolated from Sabah coastal area. Bioactive compounds from the seaweed powder were extracted using methanol and ethanol with different concentrations (50%, 70%, and 100%). 70% methanolic extract recorded the highest total phenolic content, which is 11.88 ± 0.56 mg GAE/g. The total flavonoid concentration of all the extracts, both methanolic and ethanolic, was found to be very low. The presence of potential bioactive compounds was profiled using liquid chromatography mass spectrometry quadrupole time-of-flight (LC/MS-QTOF). Phytochemical screening of the extract revealed a total of 225 compounds were identified including phenolics, flavonoids, coumarins, terpenes, quassinoids, thiophene, and others. The in vitro anticancer activity of 70% methanolic crude extract was further investigated against human leukemia cells, K562, and normal human fibroblast cells, HSF 1184. The cytotoxic effect of the extract was assessed using the [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] (MTT) assay. The result showed to significantly inhibit K562 cell viability in a concentration- and time-dependent manner, with minimal cytotoxic effects observed on treated-HSF 1184 cell line. Flow cytometry analysis indicates the crude extract has been shown to promote apoptosis among the K562 cell population. Collectively, results from this study suggest the presence of active compounds could contribute to the anticancer effect of *K. alvarezii* crude extract.

# Andrographolide-induced Apoptosis and Cell Cycle Arrest in MDA-MB-231 and MCF-7 Breast Cancer Cells

**Deeza Syafiqah Mohd Sidek and Daruliza Kernain Mohd Azman***

Institute for Research in Molecular Medicine (INFORMM), Universiti Sains Malaysia, 11800 USM, Penang.

*Corresponding Author: daruliza@usm.my

Breast cancer continues to pose a major health threat among women globally with high mortality rates. Andrographolide is a major diterpenoid lactone that can be found in *Andrographis Paniculata*, a medicinal herb that is popular in Southeast Asia. Andrographolide previously has been potentially identified to have anti-cancer properties. This study is aimed to evaluate the effects of andrographolide on breast cancer cells by using two different breast cancer cell lines, MDA-MB-231, a triple negative cell line, and MCF-7, an ER-positive cell line. MDA MB 231, MCF 7, and MCF 10A were treated with andrographolide at a different concentration ranging 0.781 µM to 200 µM for 24, 48 and 72 hours. The cell viability was observed using the WST-1 reagent. The $LC_{50}$ value calculated were used in the subsequent experiments. Both cells were seeded accordingly for each experiment and treated with $LC_{50}$ and ½ $LC_{50}$ concentration of andrographolide for 48 hours. Flow cytometry was used to determine the apoptosis and cell cycle distribution. The cells were stained using annexin V/propidium iodide for apoptosis and propidium iodide (PI) for cell cycle. Andrographolide was found to reduce cell viability for both MDA-MB-231 and MCF-7 cell lines in a concentration-time-dependent manner. The $LC_{50}$ values of both cells after being treated with andrographolide are 23.06 µM for MDA-MB-231 and 12.21 µM for MCF-7 cells. $LC_{50}$ value for the non-cancerous MCF-10A cell line could not be determined, as cell viability remained around ~80% even at the highest concentration of 200 µM after 24 hours. Andrographolide induced apoptosis in both cancer cell lines. The percentage of apoptotic cells in untreated controls were 3.91% for MDA MB 231 and 2.77% for MCF-7. After 48 hours of treatment with $LC_{50}$ and ½ $LC_{50}$ concentration of andrographolide, the percentage of apoptotic cells for MDA MB 231 cells increase by 7.93% and 5.21%, respectively. A similar pattern can be observed for MCF 7 which also showed an increasing number of apoptotic cells, 6.58% and 4.22%, respectively. Andrographolide treatment of $LC_{50}$ and ½ $LC_{50}$ concentration were observed to lead to cell cycle arrest in the G2/M phase in both cells which demonstrates its cytotoxic effects on these cancer cells. This study showed that andrographolide effectively reduced cell viability and induced apoptosis in MCF-7 and MDA-MB-231 breast cancer cells. It also caused cell cycle arrest in the G2/M phase in these cancer cells which suggests that andrographolide may have selective effects on cancer cells.

# The Protective Effect of THICAPA on Familial Alzheimer's Disease Pathogenesis Using Skin-derived Fibroblast Cell Lines

**Danesh Thangeswaran[1], Shaharum Shamsuddin[2] and Venugopal Balakrishnan[1,*]**

[1]Institute for Research in Molecular Medicine (INFORMM), Universiti Sains Malaysia, 11800 Minden, Penang.
[2]School of Health Sciences, Universiti Sains Malaysia, Kubang Kerian 16150, Malaysia.

*Corresponding Author: venugopal@usm.my

Familial Alzheimer's disease (fAD) is hereditary and developed at an unusually early age. The accumulation of toxic amyloid-beta (Aβ) protein in the brain induces inflammation that leads to neurodegeneration, a hallmark of AD. The prevalence of fAD symptoms is alarming, and there are no drugs available to cure this neurological disorder. The search for novel fAD treatments has shifted towards formulations with the potential to mitigate toxic protein aggregations by modulating the amyloid precursor protein (APP) pathway. THICAPA is a novel compound belonging to the tetrahydroisoquinoline group of amines. These amines are naturally found in the brain and foods with broad medicinal properties, including anti-inflammatory properties and affine ligands for central nervous system receptors. Therefore, this study investigated the modulatory effect of THICAPA on the APP processing pathway using the fAD patient skin-derived fibroblast cell line. In this study, we have shown that 50 μM THICAPA has exerted a potent scavenging effect on aged Aβ42 oligomers in the healthy skin-derived fibroblast cell line. Following that, the impact of THICAPA on the gene and protein expressions linked to both amyloidogenic processing of the APP pathway was assessed through the qRT-PCR and Western blot techniques. THICAPA reduced the gene expression of APP, BACE1 and PSEN1 in the amyloidogenic pathway, indicating the downregulation of the β- and γ-secretase activities, respectively. Furthermore, the ELISA quantification of Aβ proteins displayed a significant reduction of Aβ40, and Aβ42 isoforms produced from the amyloidogenic pathway resulting from downregulated amyloidogenesis. Hereby, our study identifies THICAPA as a potential anti-AD candidate with significant therapeutic activity against toxic Aβ productions.

# Comparative Genomic Analysis of a *Mycobacterium tuberculosis* STB-T6A Strain Isolated from Spinal Epidural Mass in Sabah, Malaysia

**Kai Ling Chin[1,2,*], Eraniyah Jastan Suing[1], Ruhini Andong[1], Choong Hoon Foo[3], Sook Kwan Chan[3], Jaeyres Jani[1,2], Kamruddin Ahmed[1,2] and Zainal Arifin Mustapha[1]**

[1]Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia.
[2]Borneo Medical and Health Research Centre, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia.
[3]Department of Orthopaedics, Queen Elizabeth Hospital, Kota Kinabalu, Sabah, Malaysia.

*Corresponding Author: chinkl@ums.edu.my

Spinal tuberculosis, also known as Pott's spine, is an extrapulmonary form of tuberculosis caused by *Mycobacterium tuberculosis* (Mtb). This condition can result in bone destruction, deformity, and paraplegia. The high burden of tuberculosis among undocumented immigrants in Sabah suggests the potential spread of Mtb strains from other countries into Sabah. This study aims to determine the origin of a Mtb strain from a patient diagnosed with spinal tuberculosis in Sabah. A 45-year-old male presented with clinical symptoms including chronic back pain, spinal tenderness, neurological deficits, paradiscal lesions, loss of anterior vertebra, and narrowing of the disc space, during his visit to Queen Elizabeth Hospital in Kota Kinabalu, Sabah. A biopsy was conducted to collect the epidural mass in the spine. The specimen was tested positive for tuberculosis using Xpert® MTB/RIF Ultra. To isolate the bacteria, the specimen was cultured in BACTEC™ Mycobacterium Growth Indicator Tube system. DNA was extracted and subjected to whole genome sequencing with the Illumina NovaSeq™ 6000 sequencing system. The sequencing data of Mycobacterium tuberculosis STB-T6A strain has been submitted to the National Center for Biotechnology Information (NCBI) and can be accessed under the following accession numbers, i.e., BioProject: PRJNA1091826, BioSample: SAMN40864168, Sequence Read Archive: SRR28580507, and GenBank: JBBWUO000000000. The sequencing output was used for k-mer size estimation with KmerGenie version 1.7051, followed by de novo assembly using SPAdes version 3.15.4. Variant calling was performed with the Genome Analysis Toolkit (GATK), and the variants were annotated with SnpEff version 5.0. Comparative genomic analysis was then conducted through single nucleotide polymorphism (SNP)-based phylogenetic analysis, utilizing KSNP3 and MEGA 11 software. Additionally, drug resistance analysis was performed using Mykrobe Predictor. The de novo assembly generated 208 contigs with an N50 of 162,410 base pairs. The whole genome size of the Mtb STB-T6A strain is 4,395,691 base pairs with a GC content of 65.58%, comprising 4,233 protein-coding sequences, 45 tRNAs, 3 rRNAs (5S, 16S, and 23S rRNAs), and 3 ncRNAs. The variant calling analysis showed that 99.21% of the generated reads were successfully mapped to the M. tuberculosis H37Rv reference genome. The comparative genomic analysis showed that the Mtb STB-T6A strain belongs to the Indo-Oceanic lineage (Lineage 1) and is closely associated with the Mtb T46 strain from the Philippines. Moreover, the drug susceptibility analysis predicted the strain to be drug-susceptible. In summary, these findings offer significant insights into the genetic diversity and evolutionary connections of the Mtb STB-T6A strain.

# Biomarkers in Endothelial Dysfunction-Associated Type 2 Diabetes Mellitus

**Nur Fatin Zalikha Zailan, Nazanin Talebabadi, Eusni Rahayu Mohd. Tohit, Hasni Mahayidin and Masriana Hassan***

Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

*Corresponding author: masriana@upm.edu.my

Diabetes mellitus (DM) is a chronic metabolic disorder by which the severe complications of the disease could lead to an increased risk of cardiovascular disease (CVD) mediated by vascular endothelial dysfunction. Chronic inflammation and oxidative stress, characterized by the imbalance of reactive oxygen species (ROS) production, are among the factors that influence endothelial damage. Evaluating biomarkers in an in vitro model representative of endothelial dysfunction could aid in the pathophysiological studies, clinical evaluation, and therapeutic potential for CVD-related diseases. Serum and plasma of both males and females from normal (healthy) individuals (n=41) as control population and Type 2 DM (T2DM) patients (n=41) were collected from the Faculty of Medicine and Health Sciences, UPM, and the Family Medicine Clinic, Hospital Sultan Abdul Aziz Shah, respectively. The production of nitric oxide (NO) (by Griess assay) and cytokines (by flow cytometry-based LEGENDplex immunoassays) was determined in plasma from both normal and T2DM samples. The production of ROS (by dichlorodihydrofluorescein diacetate [DCFDA]) and endothelial damage were evaluated by serum induction in human umbilical vein endothelial cells (HUVEC). In the trans-endothelial permeability assay, the cells were cultured in a 0.4μm transwell insert and the endothelial injury was evaluated through fluorescein isothiocyanate (FITC)-Dextran analysis. The level of plasma NO was comparatively lower but not significant in T2DM patients than in the control group. Similarly, non-significant lower plasma TNF-α, IL1-β, and IL-6 in T2DM patients than the normal group were observed. Conversely, serum-induced ROS production in T2DM (2213.67 ± 393.17 RFU) was significantly higher ($p = 0.0176$) than the control group (1303.00 ± 129.27 RFU). A significantly higher ($p = 0.0035$) trans-endothelial permeability was also detected in HUVEC stimulated with T2DM serum (1227.67 ± 122.99 RFU) than with the control serum (568.67 ± 31.32 RFU). In addition, exogenous IL-1β and/or H2O2 promote a significant increase in ROS production and/or trans-endothelial permeability. The study shows that the reduction of NO and an increase in ROS production in T2DM could potentially contribute to the damage of the endothelial cells, thus suggesting the pivotal role of oxidative stress in endothelial dysfunction in DM. This in vitro model will contribute to the assessment of potential biomarkers for early clinical evaluation and physiological studies of endothelial cell dysfunction in DM.

# Alterations of Tissue Inhibitor of Metalloproteinase Levels Among Smokers with Coronary Artery Disease

**Nazirah Samah[1], Shawal Faizal Mohamad[2], Beh Boon Cong[2], Nik Azuan Nik Ismail[3], Faridah Mohd Nor[4], Wan Mohd Hafiz Wan Razali[4,5], Faizal Amri Hamzah[6], Adila A. Hamid[1], Azizah Ugusman[1,*] and Amilia Aminuddin[1,*]**

[1]Department of Physiology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jalan Yaacob Latif, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur, Malaysia.
[2]Department of Cardiology, Universiti Kebangsaan Malaysia, Jalan Yaacob Latif, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur, Malaysia.
[3]Department of Radiology, Universiti Kebangsaan Malaysia, Jalan Yaacob Latif, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur, Malaysia.
[4]Forensic Unit, Department of Pathology, Universiti Kebangsaan Malaysia, Jalan Yaacob Latif, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur, Malaysia.
[5]Department of Forensic Pathology, Faculty of Medicine, Sungai Buloh Campus, Universiti Teknologi MARA, 47000 Sungai Buloh, Selangor, Malaysia.
[6]Department of Emergency Medicine, Universiti Kebangsaan Malaysia, Jalan Yaacob Latif, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur, Malaysia.

*Corresponding Author: amilia@ppukm.ukm.edu.my, dr.azizah@ppukm.ukm.edu.my

Coronary artery disease (CAD) is characterized by narrowing or blockage of coronary arteries due to atherosclerotic plaque. CAD remains the leading cause of morbidity and mortality worldwide. Smoking is recognized as a significant risk factor for the development of CAD. Among these mechanisms, emerging evidence suggests the involvement of various molecular players such as tissue inhibitors of metalloproteinase (TIMPs). TIMPs are matrix metalloproteinase (MMP) inhibitors involved in tissue remodeling and vascular integrity. Alterations in TIMP levels have been implicated in the pathogenesis of CAD, yet the specific impact of smoking on TIMP protein levels among individuals with CAD remains inadequately explored. Therefore, this study aimed to determine the protein levels of TIMPs among smokers with CAD. Serum isolated from whole blood samples of patients with three main groups (acute coronary syndrome (ACS), chronic coronary syndrome (CCS), and control) was collected for ELISA analysis. The patients were then grouped into ACS and smoker (n=17), ACS and non-smoker (n=14), CCS and smoker (n=11), CCS and non-smoker (n=10), control and smoker (n=20), and control and non-smoker (n=20). The percentage of stenosis was significantly higher in the ACS and CCS groups than in the control group. In non-smokers, TIMP 3 protein level was significantly increased in ACS (18.89 ± 6.07 ng/mL) and CCS (21.84 ± 8.63 ng/mL) groups compared to the control group (12.53 ± 2.62 ng/mL). Meanwhile, among smokers, TIMP 3 levels were higher in the CCS group compared to the control group (CCS=22.4 ± 6.80 vs. Control=15.24 ± 3.48 ng/mL; $p<0.05$). However, there was no significant difference in the TIMP 3 levels between smokers and non-smokers within the three main groups. The finding supports the role of TIMP in the development of atherosclerotic plaque. However, smoking itself did not affect the levels of TIMP 3 in subjects with and without CAD.

# The Influence of Receptor Modulation in Neurogenic Niche of LPC-Induced Demyelinated Postnatal Spinal Cord

**Sharmili Sivanathan, Che Aishah Nazariah Ismail, Rahimah Zakaria, Shazana Hilda Shamsuddin and Nazlahshaniza Shafin***

Department of Physiology, School of Medical Sciences, Universiti Sains Malaysia, 16150, Kubang Kerian, Kelantan, Malaysia.

*Corresponding Author: drshaniza@usm.my

A chronic autoimmune, neurodegenerative, inflammatory non-traumatic demyelinating disease can impair the proper functioning of axons. The resulting demyelination leads to destructive damage to the nervous tissues and causes chronic disability, and relapsing symptoms and can progress into a chronic phase. Understanding the mechanism of remyelination is essential to stop the disease progression. Additionally, stimulation of endogenous remyelination could have a prodigious therapeutic impact. This study aims to elucidate the mechanism of GABA(B) modulation on remyelination of neural stem cells of the spinal cord. Notably this research represents the exploration of GABAB in potential neural stem cells of the spinal cord. Postnatal Sprague Dawley rats D10-D15 were sacrificed, and their spinal cord were collected for proliferation studies. Spinal cord samples were assigned to different groups; Lysophosphatidylcholine (LPC), LPC+GABA(B) Agonist, LPC + GABA(B) Antagonist, GABA(B) Antagonist, GABA(B) Agonist and the control group. The thymidine analogue 5-Ethynyl-2'-deoxyuridine (EdU) was added to the slices in the presence or absence of modulators. The expression level of cell proliferation, differentiation and inflammatory response is measured by immunofluorescence, immunohistochemistry(IHC) and Apt-immunostaining. The numbers of EdU-positive cells were significantly lower in GABA(B) agonist-treated slices ($p < 0.05$) compared with GABA(B) Antagonist-treated slices specific to the central canal area in LPC-induced demyelination postnatal spinal cord. Our findings suggest that slices treated with baclofen had considerably fewer proliferative cells and GABAB receptor antagonists might be able to initiate remyelination. By using aptamer for targeting the immunomodulatory markers TNF-α and IL-1, the digital aptamers were created to find the aptamer that has the highest affinity to the target molecules. Findings from this study will provide novel insight into the potential role of GABAB receptor modulation on neural stem cell proliferation of the spinal cord. Furthermore, understanding the underlying mechanisms may guide future researchers in designing potential interventions to combat spinal events that lead to the remyelination of neurons.

# Polymeric Nanowires as 3D Scaffold for Cellular Cultivation Intended for Downstream Biomarker or Molecular Diagnostic Applications

**Salman H. Alrokayan[1,*], Haseeb A. Khan[1], Tajamul Hussain[1], Fouzi Mouffouk[2], Salman Alamery[1] and Khalid Abu-Salah[3]**

[1]Department of Biochemistry, College of Science, King Saud University, Riyadh 11451, Saudi Arabia.
[2]Department of Chemistry, Faculty of Science, Kuwait University, Safat 13060, Kuwait.
[3]Department of Nanomedicine, King Abdullah International Medical Research Center, Riyadh, Saudi Arabia.

*Corresponding Author: salrokayan@ksu.edu.sa

The advent of 3D cell culture not only revolutionized the areas of drug discovery and tissue engineering but also influenced molecular diagnostic approaches, particularly in cell-based assays. This technique can also be used to screen for small molecule drugs for personalized medicine or genetically manipulated to understand disease pathways. Polymeric nanofibers represent an exciting new class of materials that has drawn substantial attention due to the ability of these materials to mimic the arrangement of fibrils of the extracellular matrix, making them suitable for a wide range of medical applications. We have developed a novel polymeric 3D scaffold based on the self-assembly of a star-like amphiphilic co-polymer of poly (caprolactone)-poly(ethylene oxide) base unit into nanowires. This nanomaterial has a scale similar to the cellular extracellular matrix and is capable of mimicking the extracellular microenvironment and has been tested for the growth and proliferation of stem cells. This nanomaterial may have direct implications in 3D cell culture for downstream biomarkers or genetic analyses for diagnostic purposes. (This project was funded by the National Plan for Science, Technology and Innovation (MAARIFAH), King Abdulaziz City for Science and Technology, Kingdom of Saudi Arabia, Award Number: 14-BIO-1066-02).

# Identification of A Novel Variant in A Proximal Symphalangism Family by Whole-Exome Sequencing

**Xin Chen[1,2], Qian Zhang[1], Litao Qin[1], Yihui Wang[1], Ke Yang[1], Congmin Li[1] and Shixiu Liao[1,*]**

[1]Medical Genetics Institute of Henan Province, Henan Key Laboratory of Genetic Diseases and Functional Genomics, Henan Provincial People's Hospital, People's Hospital of Zhengzhou University, Zhengzhou University, Zhengzhou, Henan Province, PR China.
[2]Medical Genetics Institute of Henan Province, Henan Key Laboratory of Genetic Diseases and Functional Genomics, Henan Provincial People's Hospital, School of Clinical Medicine, Henan University, Zhengzhou, Henan Province, PR China.

*Corresponding Author: ychslshx@zzu.edu.cn

Proximal symphalangism (SYM1) is a rare congenital deformity of limbs. Because of the high heterogeneity, the genotype-phenotype analysis disturbed the clinical practice. We recruited an SYM1 family at Henan Provincial People's Hospital to elucidate the genetic etiology and examine its correlation with the clinical phenotype. The proband underwent whole-exome sequencing (WES). Candidate variants were validated in the family members through Sanger sequencing. The pathogenicity of the variants was assessed using bioinformatics software. Limited flexion of the ring and little fingers of both hands was observed in the proband. His father, sister and niece had similar symptoms. X-ray film showed fusion of the proximal and middle phalanx of the bilateral ring and little fingers with interphalangeal joint space disappeared, and short bilateral first toes for proband. A heterozygous variant c.613T>G (p.Trp205Gly) of *NOG* gene was identified by WES. This variant has not been reported in the literature before and was co-separated from the phenotype in the pedigree. The 205th tryptophan of Noggin protein encoded by *NOG* was highly conserved among species. The protein modeling demonstrated that hydrophobic tryptophan was mutated into hydrophilic glycine, which expanded the cavity (74.304Å^3) and reduced the ΔΔG (-3.282kcal/mol, $\Delta\Delta G_{pred}$=4.371), leading to instability. Most of the reported *NOG* variants were located in the conserved region and cause diseases by affecting the secretion or dimerization of Noggin. Variant c.613T>G destroyed the hydrophobic interaction among Pro56, Trp205 and Trp217. In accordance with the recommendations of the American College of Medical Genetics and Genomics, this variant was classified as a likely pathogenic variant. It is worth noting variant p.Trp205Cys at the same amino acid site was reported in a family with facio-audio-symphalangism syndrome. Another patient carrying p.Trp205Cys displayed multi-system anomalies including hearing loss, glaucoma, asthma and vertebral deformities. These results reflected the complex phenotypic heterogeneity of NOG-related disorders. We confirmed a novel variant c.613T>G of the NOG gene in a SYM1 family by WES. Our findings enriched the variation spectrum of the *NOG* gene and provided a basis for genetic counseling and prenatal diagnosis.

# Multiplex PCR Assay for Simultaneous Detection of *Vibrio parahaemolyticus* & *Vibrio alginolyticus*

**Nurlina Rosli[1], Noorizan Miswan[2], Nur Ain M. Hussin[3], Muhammad Syafiq Izzuddin Abd. Hadi[4], Azila Abdullah[4] and Rashidah Abdul Rahim[1,2] and Lau Nyok Sean[2,*]**

[1]School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia.
[2]Centre for Chemical Biology, Universiti Sains Malaysia, 11900 Penang, Malaysia.
[3]Analytical Biochemistry Research Centre, 11900 Penang, Malaysia.
[4]National Fish Health Research Center (NaFisH), Department of Fisheries Malaysia, 11960 Penang, Malaysia.

*Corresponding Author: nyoksean@usm.my

The *Vibrio* genus plays major roles in aquaculture and foodborne pathogens*. V. parahaemolyticus* (VP) is a well-known species in this genus for causing human infections through uncooked or contaminated seafood. Another significant species is *V. alginolyticus* (VA), which is also associated with aquaculture infections and antibiotic resistance in human infections. Identifying Vibrio species by phenotype and biochemical characteristics is a challenge, thus necessitating nucleic acid-based methods. Nevertheless, certain popular toxin genes, such as *toxR*, are overlappingly present within different Vibrio species, rendering them unsuitable for species identification. Additionally, horizontal gene transfers between *Vibrio* species can occur within the same environment, further complicating species identification. A previous study has utilized comparative genomics techniques to identify sequences that showed the potential as biomarker regions for *V. parahaemolyticus* and *V. alginolyticus* detections. They were revealed via genome clustering of multiple *Vibrio* species genomes based on open reading frames (ORFs). Based on these findings, our study aimed to develop new primer sets for the species-specific identification of *V. parahaemolyticus* and *V. alginolyticus*. These primer sets detect a hypothetical and heme-binding proteins in the two species, respectively. To further improve efficiency, the primers were designed for a multiplex PCR assay, allowing simultaneous detection of both species in one reaction. Multiplexing is advantageous, considering both species are typically present in the same environments. Our study utilized the PrimerPremier and PrimerBlast software to generate several primer set candidates based on the target sequences. PrimerPremier software was utilized to screen the hypothetical and heme-binding protein gene sequences for potential primer sequences. The resulting primers were then checked for specificity using PrimerBlast. A pair of primers was then selected for each species for multiplex PCR assay assessment using pure culture DNA samples of *V. parahaemolyticus* and *V. alginolyticus*. Our results showed that the primer sets were able to detect *V. parahaemolyticus* and *V. alginolyticus* in both singleplex and multiplex assays at the DNA template concentration of 50 ng/µl with good specificity. The amplicons were 299 and 217 bp in size, corresponding to the hypothetical protein of *V. parahaemolyticus* and the heme-binding protein of *V. alginolyticus*. No undesired amplifications were detected when the primer sets were tested against several other common clinical bacterial species. These findings underscore the efficacy of the developed primers in distinguishing between these two significant *Vibrio* species, offering a reliable tool for rapid and accurate detection in clinical and environmental samples.

# Enhancing Molecular Diagnostic Testing for Leptospirosis: Role of External Quality Assurance (EQA) Program in Referral Laboratories

**Mohd Nadzuan Yahaya*, Muhammad Ridhuan Mohd Ali, Meruwan Amin Shoib, Muslihah Mohamad, Nor Hazrin Abd Hazis, Rohaidah Hashim and Murnihayati Hassan**

Bacteriology Unit, Infectious Disease Research Centre (IDRC), Institute for Medical Research (IMR), National Institutes of Health (NIH), Setia Alam, Selangor, Malaysia.

*Corresponding Author: nadzuan.yahaya@moh.gov.my

Leptospirosis, caused by the spirochete bacteria Leptospira, is a zoonotic disease with significant public health implications worldwide and poses a considerable health burden due to its prevalence and potential severity in Malaysia. Leptospira polymerase chain reaction (PCR) plays a crucial role in identifying the presence of the causative bacteria and confirming the diagnosis. The Institute of Medical Research (IMR) is a referral laboratory offering leptospirosis confirmatory tests including *Leptospira* microscopic agglutination test (MAT), culture and PCR. This study aimed to identify the role of an External Quality Assurance (EQA) programme for Leptospira PCR diagnostic testing from a referral laboratory perspective. We examined the performance data from the EQA report for the first year of enrolment (2023). The data were tabulated for descriptive statistics. A literature review was conducted to obtain evidence compared to our laboratory experience in similar scenarios. In 2023, the EQA module was conducted in three dispatches between March and November. For each dispatch, five samples were examined in the same manner as the patient sample, giving a total of 15 EQA samples tested for one calendar year. Only one discordant result was recorded in March 2023 due to a transcription error (80% concordance) during the first dispatch. Otherwise, the subsequent dispatches in June and October 2023 yielded 100% results in concordance, with the overall concordance rate being 93.3%. Result concordance indicates the consistency of our test results which is essential for ensuring the accuracy and reliability of the test currently offered at our centre. EQA programs are generally a requirement for laboratory accreditation and molecular modules in infectious disease and serve as an external platform for laboratories to assess their ability to generate accurate, reliable, and consistent reports. Laboratory performance in the EQA program is one of the quality performance indicators (QPI) for our institute with a minimum concordance result of 85%. Our laboratory was able to achieve the QPI, which also translates to accurate and replicable results. EQA participation allows laboratories to detect errors or discrepancies by comparing their findings with those of other laboratories and reference standards. EQA plays a crucial role in instilling confidence in healthcare providers, patients, and public health authorities regarding the efficacy of molecular testing for disease diagnosis and management. EQA significantly enhances the quality, reliability, and accuracy of molecular diagnostic testing, crucially contributing to high-quality diagnostic service delivery in referral laboratories.

# Application of Median Nerve Electrical Stimulation to Regulate miR-1-3p in the BDNF/TrkB-MAPK Pathway for Promoting Myelin Regeneration in A Rat Stroke Model

**Rui Li[1,2], Jingyi Lu[1], Chengcai Zhang[3], Pengyue Zhang[3] and Kai Ling Chin[2,*]**

[1]Department of Rehabilitation Medicine, The Second People's Hospital of Kunming, Rehabilitation Hospital affiliated to Kunming University, Kunming, China.
[2]Department of Biomedical Sciences, Faculty of Medicine & Health Sciences, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia.
[3]Institute of Acupuncture, Tuina, and Rehabilitation, The Second Clinical Medical School, Yunnan University of Traditional Chinese Medicine, Kunming, China.

*Corresponding Author: chinkl@ums.edu.my

Restoration of the upper limb motor dysfunction following ischemic stroke remains a major challenge for rehabilitation medicine. Clinically, we had demonstrated that median nerve electrical stimulation (MNES) could significantly improve the upper limb motor function in stroke patients, but the underlying mechanism is still unclear. Myelin regeneration is crucial for restoring nerve function after a stroke. Within this therapeutic process, the microRNA miR-1-3p, which targets brain-derived neurotrophic factor (BDNF), may have a significant impact. In this study, we aimed to investigate the potential involvement of MNES in regulating miR-1-3p in the BDNF/TrkB-MAPK pathway to promote myelin regeneration in a rat stroke model. Adult male Sprague-Dawley rats (n=18) were divided into three groups, i.e., control (sham group), left middle cerebral artery occlusion stroke model (MCAO group), and MCAO group receiving MNES treatment (MNES group). The target genes for miR-1-3p were predicted using the TargetScan database. The miR-1-3p and BDNF gene expression were detected with quantitative reverse transcription polymerase chain reaction (RT-qPCR). The expression of BDNF, tropomyosin-related kinase B (Trk-B), and mitogen-activated protein kinases (MAPK) were detected by Western blotting. Luxol Fast Blue (LFB) staining was employed to assess myelin regeneration. Using bioinformatics analysis, BDNF was identified as the target gene for miR-1-3p. RT-qPCR analysis demonstrated that MNES treatment significantly decreased miR-1-3p expression ($p<0.05$) and increased BDNF expression ($p<0.01$) following ischemic stroke in rats. Moreover, the expression levels of BDNF ($p<0.05$), Trk-B ($p<0.01$), and MAPK ($p<0.01$) proteins were elevated. The LFB staining in the MCAO group is lighter and patchier, indicating a reduction in myelin; however, it becomes noticeably darker and more uniform after MNES treatment, suggesting enhanced myelin regeneration. In conclusion, the MNES treatment could inhibit miR-1-3p and activate the BDNF/TrkB-MAPK pathway to promote myelin regeneration after a stroke.

# Autoantibodies Profile in Paraneoplastic Neurological Syndromes Associated Disorders in Malaysia

**Ching Yee Ming[1,*], Siti Fatimah Abdullah[1], Ainur Yusniza Yusof[1], Siti Asma Bakar[1], Bavithra Venu[1], Syuriatika Dionysius[1], Nur Syahirah Alias[1] and Masita Arip[2]**

[1]Autoimmune Unit, Allergy and Immunology Research Centre, Institute for Medical Research, National Institutes of Health, Ministry of Health Malaysia.
[2]Allergy and Immunology Research Centre, Institute for Medical Research, National Institutes of Health, Ministry of Health Malaysia.

*Corresponding Author: chingym@moh.gov.my

Paraneoplastic neurological syndromes (PNS) are a heterogeneous group of neurological disorders associated with cancer which is not directly caused by metastatic invasion, nutritional deficiencies, infections or side effects of the treatment. The presence of well-characterised paraneoplastic antibodies facilitates the diagnosis of PNS and to identify of the possible underlying tumour. In this study, we have evaluated the positivity rate of paraneoplastic antibodies and the clinical symptoms of patients with suspected PNS. Paraneoplastic antibodies test results from 793 samples sent to the Autoimmune Lab of the Institute for Medical Research (IMR), Setia Alam, from January 2023 to December 2023 were retrospectively reviewed. The paraneoplastic antibodies test detects the presence of antibodies against 12 antigens, namely amphiphysin, CV2, PNMA2 (Ma2/Ta), Ri, Yo, Hu, recoverin, SOX1, titin, zic4, GAD65 and Tr (DNER) by immunoblot. The age of this study cohort ranged from 9 to 89 years old, with a median age of 58 years, predominantly male (53.2%). Of the total 793 samples reviewed, 780 were serum samples, while 13 were cerebrospinal fluid (CSF) samples. The positivity rate was 10.8%, including 3 samples which were positive for more than 1 antigen. Among the positive samples, the most common high-risk paraneoplastic antibodies (>70% associated with cancer) were anti-Yo (18.4%). The most common clinical symptom reported in this cohort was encephalitis-related features (46.2%), with a positivity rate of 5.7%. In this study, the positivity rate of paraneoplastic antibodies was 10.8%, with anti-Yo antibodies as the most reported high-risk antibodies.

# Genome-Wide DNA Methylation Analysis in Cytogenetically Normal Acute Myeloid Leukemia (CN-AML) Patients from Malaysia

**Nor Rizan Kamaluddin[1,2,*], Norfarhana Mazlan[2], Zahidah Abu Seman[2], Noor Atiqah Fakharuzi[2], Azian Naila Md Nor[2], Elsa Haniffah Mejia Mohamed[3], Muhammad Redha Abdullah Zawawi[4], Mohd Khairul Nizam Mohd Khalid[5], Saiful Effendi Syafruddin[4], Ezalia Esa[1,2] and Yuslina Mat Yusoff[2]**

[1]Virology Unit, Infectious Disease Research Centre, Institute for Medical Research, National Institutes of Health, Ministry of Health Malaysia.
[2]Hematology Unit, Cancer Research Centre, Institute for Medical Research, National Institutes of Health, Ministry of Health Malaysia.
[3]Department of Pharmacology, Faculty of Medicine, Universiti Malaya, Kuala Lumpur.
[4]UKM Medical Molecular Biology Institute, Universiti Kebangsaan Malaysia, Kuala Lumpur.
[5]Inborn Errors Metabolism and Genetic Unit, Nutrition, Metabolic and Cardiovascular Research Centre, Institute for Medical Research, National Institutes of Health, Ministry of Health Malaysia.

*Corresponding Author: norizan.k@moh.gov.my

Cytogenetically normal acute myeloid leukaemia (CN-AML) is characterised by the absence of chromosomal abnormalities, and they constitute between 40 to 50% of all AML cases. In the cases of CN-AML, epigenetic alterations could be an important factor driving the pathogenesis. Thus, this study aimed to explore the epigenetic landscape in CN-AML utilising Whole Genome Bisulfite Sequencing (WGBS) technology. A cohort of 70 CN-AML patient samples and 10 healthy controls were subjected to WGBS. Differential methylation analysis was performed to identify genome-wide hypermethylated and hypomethylated regions. A total of 18,998 hypermethylated and 7,979 hypomethylated gene regions were found. The extensive hypermethylated regions found in our cohort were consistent with the literature on AML. Among the hypermethylated regions include the promoter region of key tumour suppressor genes, including *CDKN2A*, *TP53I3*, and *WT1*. Pathway enrichment analysis indicated a significant enrichment of the Ras-associated protein 1 (Rap1) signalling pathway. This finding was supported by a recent AML transcriptome study which identified the Rap1 signalling pathway as one of the top pathways that are dysregulated in AML. In conclusion, the results showed that CN-AML patients exhibited distinct epigenetic changes and this study confirmed that aberrant DNA methylation is an important hallmark of CN-AML.

# CML-miRNA Relapse Detector, a Ready-To-Use Test for Guiding Treatment-Free Remission in Chronic Myeloid Leukaemia Adult Patients at the Molecular Response

**Aliza Mohd Yacob[1,*], Latifah Ibrahim[2], Nor Asiah Muhamad[3], Hamidah Akmal Hisham[1], Shuhaila Mat Sharani[1,4], Ernie Zuraida Ali[5], and Yuslina Mat Yusoff[1]**

[1]Cancer Research Centre, Institute for Medical Research, National Institutes of Health, Malaysia.
[2]Special Research Centre, Institute for Medical Research, National Institutes of Health, Malaysia.
[3]Sector for Evidence-Based Healthcare, National Institutes of Health, Malaysia.
[4]Biomedicine Program, School of Health Science, Universiti Sains Malaysia, Kelantan.
[5]Nutrition, Metabolic and Cardiovascular Research Centre, Institute for Medical Research, National Institutes of Health, Malaysia.

*Corresponding Author: aliza.yacob@moh.gov.my

No treatment-free remission (TFR) test is yet known. The decision for TFR in chronic myeloid leukemia (CML) patients is based on a test to monitor disease response to tyrosine kinase inhibitors (TKIs) treatments. Thus 50% of patients relapse within 2 years and 80% within the first 6 months after treatment cessation. The gold standard, Real-time quantitative PCR is currently used and the new digital PCR with improved sensitivity is under study to be the next reference test. However, a test dedicated to TFR is much needed to overcome the limitation of detecting disease at levels beyond the current lower limit of detection or undetectable. In this study, we developed a test with an indication table for guiding TFR in CML adult patients at the molecular response: The test, CML-miRNA Relapse Detector involves a quantitative analysis followed by a qualitative analysis. The quantitative analysis uses a custom microRNA (miRNA) quantitative PCR array followed by a qualitative analysis of cancer genes. miRNAs of the array were selected from next generation sequencing (NGS) analysis. Targets of three validated miRNAs were predicted using molecular prediction tools and databases. Predicted genes were compared to mRNA NGS data. This is followed by gene analysis on the Proteomic and Genomic Data Commons (PDC/GDC), a repository for tumors and cancer. We developed a TFR-CML Indication Table using results from the 3 validated miRNAs, NGS mRNA analysis, miRNA target analysis, and Proteomic and Genomic Data Commons analysis. This table will guide the right time to stop TKI treatment in adult Chronic Myeloid Leukemia (CML) patients who have consistently reached the deep molecular response stage. It can also be used to recognize the signs of early relapse in patients. This test uses existing laboratory setups and thus is ready to use to guide TFR in CML Adult Patients at the Molecular Response. Further analysis using a bigger sample size is recommended and will be conducted.

# Unravelling the Impact of Covid-19 Vaccination on Ribonucleic Acid (RNA) Quantities: A Comparative Analysis

**Norfarhana Khairul-Fahmy*, Tengku Nurainna Fatihah Tengku Abdullah, Jamiila Ismail, Masita Arip and Norhazlin Mustafa**

Allergy & Immunology Research Centre, Institute for Medical Research (IMR), National Institutes of Health (NIH), Ministry of Health Malaysia.

*Corresponding Author: norfarhana.kf@moh.gov.my

Covid-19 vaccination has shown remarkable effects in transitioning the global pandemic to the endemic phase. Although this is an important milestone, vaccine effectiveness varies from one person to another. Inter-individual variations in vaccine responses highly affect the magnitude of vaccine immunity and it is predicted that genetic factors contributed significantly to it. Prior to carrying out any genetic studies, nucleic acid isolation is always the starting point for all downstream experiments. Thus, this study aims to assess the change in RNA quantity before and after COVID-19 vaccination. The types of vaccines included in the study were BNT162b2 (Pfizer), CoronaVac (Sinovac) and ChAdOx1 nCoV-19 (AstraZeneca). Subjects were divided into two groups: those with a history of Covid-19 infection (convalescent) and no history of Covid-19 infection (healthy). Blood was collected in PAXgene tubes at two time-points: before the administration of the first dose Covid-19 vaccine (pre-) and before the administration of the second dose (post-). RNA was extracted using the QIAsymphony SP instrument. RNA concentration was assessed using Qubit fluorometric quantification and concentration is translated as the quantity of RNA present in each sample. Results showed that RNA concentrations were significantly decreased from pre- (mean = 63.35 µg/ml, s.d. = 23.07) to post-vaccination (mean = 59.24 µg/ml, s.d. = 26.17) in all samples ($t(133) = -2.22$, $p<0.05$). Changes in RNA concentration pre- and post-vaccination were also significantly different across all types of studied vaccines. Further assessments revealed that convalescent subjects (median = 57.97 µg/ml, IQR = 29.95) contained significantly higher RNA concentration than healthy individuals (median = 52.34 µg/ml, IQR = 23.56), $Z = -2.12$, $p<0.05$. Stratification of subjects according to the types of vaccines showed no significant difference between the convalescent and healthy groups. RNA quantity reflects how vigorously a gene has been transcribed and those genes are expressed according to the needs of the moment. Results from this study may indicate the immune response dynamics towards Covid-19 vaccination. The body may initially ramp up gene expression prior to vaccination and downregulate certain responses after vaccination, resulting in a net decrease in RNA concentration. Additionally, the vaccine acts as a booster for convalescent subjects by triggering a strong reactivation of the immune memory established during the initial infection. This reactivation leads to a more sustained transcriptional activity, as reflected by higher RNA levels. In conclusion, this study provides foundational insights into the differential immune responses to Covid-19 vaccination.

# Whole-Genome Sequencing Analysis of Multi-Drug Resistant Salmonella enterica Serovar Typhi from Clinical Isolates in Malaysia

**Nor Hazrin Abd Hazis*, Hana Farizah Zamri, Mohammad Ridhuan Mohd Ali, Jama'ayah Mohamed Zahidi and Rohaidah Hashim**

Bacteriology Unit, Infectious Disease Research Centre, Institute for Medical Research, NIH Setia Alam, Ministry of Health Malaysia.

*Corresponding Author: norhazrin@moh.gov.my

Typhoid fever, caused by *Salmonella enterica* serovar Typhi (*S*. Typhi), poses a significant public health challenge in low- and middle-income countries. The rise of multidrug-resistant (MDR) *S.* Typhi, which shows resistance to both first-line and second-line antibiotics, has compromised the effectiveness of antimicrobial treatments. This study aimed to examine the antimicrobial resistance genes and the molecular epidemiology of MDR *S*. Typhi over 10 years (2014–2023) in Malaysia. Twenty-four MDR *S*. Typhi isolates, confirmed through antimicrobial susceptibility testing were subjected to whole-genome sequencing (WGS) using the Illumina NextSeq platform. The WGS data were processed with TheiaProk Illumina PE Version 1.3.0, and a single-nucleotide polymorphism (SNP) phylogenetic tree was generated using kSNP3_PHB Version 2.01 within Terra.bio (https://app.terra.bio). The resulting phylogenetic tree was visualized using Microreact (http://microreact.org). WGS analysis identified all isolates as genotype 4.3.1.1 (H58 haplotype lineage 1), a lineage strongly associated with multidrug resistance. The analysis identified five antimicrobial resistance genes linked to first-line antibiotics: *catA1* for chloramphenicol resistance; *dfrA7*, *sul1*, and *sul2* for trimethoprim-sulfamethoxazole resistance; and *blaTEM-1D* for ampicillin resistance. Additionally, WGS detected single-point mutations in the *gyrA* gene (*gyrA*_S83Y and *gyrA*_S83F), which are associated with reduced susceptibility to second-line fluoroquinolone antibiotics, such as ciprofloxacin. A single-nucleotide polymorphism (SNP)-based phylogenetic tree, constructed using selected genomes from public databases, clustered the Malaysian MDR *S*. Typhi isolates with internationally reported strains from Bangladesh and Pakistan, where genotype 4.3.1.1 is known to be endemic. WGS of the MDR *S*. Typhi genotype 4.3.1.1 offers valuable insights into the genetic mechanisms driving its resistance to multiple antibiotics and its global dissemination. As a sub-lineage of the H58 haplotype, this genotype is especially alarming because of its strong association with widespread multidrug resistance, particularly in areas where typhoid fever is endemic.

# Demographic Characteristics and Antifungal Susceptibility of *Kodamaea ohmeri* Isolates from Hospitals in Malaysia

**Nur Fadhilah Abu Bakar*, Mohammad Ridhuan Mohd Ali, Salina Mohamed Sukur, Murnihayati Hassan and Rohaidah Hashim**

Bacteriology Unit, Infectious Disease Research Centre (IDRC), Institute for Medical Research (IMR), National Institutes of Health, Ministry of Health Malaysia.

*Corresponding Author: nurfadhilah.ab@moh.gov.my

*Kodamaea ohmeri* is an emerging yeast pathogen that has gained attention in various parts of the world, including Malaysia, due to its association with invasive infections, particularly in immunocompromised individuals. Despite its growing clinical importance in invasive fungal diseases (IFD), there is a lack of comprehensive global data on the occurrence and distribution of *K. ohmeri*. The purpose of the study is to outline the demographics of patients infected with *K. ohmeri* and to assess the antifungal susceptibility in Malaysia. All infection cases of *K. ohmeri* were extracted from the National Surveillance of Antimicrobial Resistance (NSAR) database from the year 2019 to 2023. Subsequently, the data were analysed to determine the distribution and antifungal susceptibility patterns. Out of the 188,193 fungi cases reported in the NSAR database, 0.064% were identified as *K. ohmeri* (n=120) and there was a rising pattern from 2019 to 2023. The majority of cases (16%) were among individuals aged 25-44 years, followed by those aged 65 and above, who accounted for 11%. In addition, *K. ohmeri* was predominantly found in sterile blood specimens (62%), while non-sterile sources like fluid and urine made up 13% and 11% of cases, respectively. Out of the 8 isolates tested, 3 were resistant to fluconazole, but all 8 were sensitive to the other 5 antifungal agents. The rising trend, with most cases coming from blood samples, shows that K. ohmeri infections are becoming more common, particularly in healthcare settings. To address this, healthcare providers and public health officials must enhance treatment strategies and improve infection control measures to reduce the risk of nosocomial *K. ohmeri* infections. Given the significant resistance to fluconazole, its use may be unsuitable for treating infections caused by *K. ohmeri*. Further research on *K.ohmeri* is essential to better understand its prevalence and optimize patient care.

# MEF2A-Mediated Transcriptional Activation of NFKBIA Reduces Cisplatin Resistance in Gastric Cancer Cells

**Chenghao Chu[1,2], Yongwei Zhang[1,2], Ruiran Yu[3], Bin Liu[1,2], Bin Wang[1,2], Zhangxuan Xu[1] and Kai Ling Chin[2,*]**

[1]Department of General Surgery, Anqing First People's Hospital, 246000, Anqing, China.
[2]Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, 88400, Kota Kinabalu, Sabah, Malaysia.
[3]Department of Oncology, Anqing First People's Hospital, 246000, Anqing, China.

*Corresponding Author: chinkl@ums.edu.my

Cisplatin (DDP) resistance represents a pivotal contributing factor to chemotherapy failure and adverse patient outcomes in the context of gastric cancer (GC). The objective of the present study was to delineate roles and the underlying mechanisms through which myocyte enhancer factor 2A (MEF2A) in DDP resistance in GC. The human gastric cell lines AGS and MKN-45 were used to construct DDP-resistant cells (AGS/DDP and MKN-45/DDP). Cell Counting Kit-8 (CCK-8) assay was used to assess cell proliferation and determine the half-maximal inhibitory concentration (IC50) of cisplatin (DDP). Colony formation ability was evaluated by colony formation assay and cell apoptosis was measured by flow cytometry. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and Western blotting analysis were used to quantify the mRNA and protein levels. Chromatin immunoprecipitation (ChIP) and dual luciferase assays were used to affirm the relationship between MEF2A and its target NF-κB inhibitor alpha (NFKBIA). CCK-8 analysis showed that AGS/DDP and MKN-45/DDP cells exhibited higher DDP resistance and IC50 values than their parental cells. Moreover, both qRT-PCR and Western blot experiments uncovered that NFKBIA was significantly reduced in AGS/DDP and MKN-45/DDP cells than their parental cells. Upregulation of NFKBIA led to a decreased cell survival rate and IC50, decreased the number of cell colonies, and enhanced cell apoptosis in AGS/DDP and MKN-45/DDP cells. Furthermore, the mRNA and protein levels of MEF2A were significantly reduced in AGS/DDP and MKN-45/DDP cells than their parental cells. The binding motif of MEF2A was identified from the JASPAR online database (https://jaspar.elixir.no/), revealing two putative transcriptional sites on the NFKBIA promoter (#1, -558~-572; and #2, -59~-73). ChIP and dual luciferase assays confirmed that MEF2A interacts with the NFKBIA promoter, where MEF2A transcriptionally activates the expression of NFKBIA in AGS/DDP and MKN-45/DDP cells. In conclusion, MEF2A mitigated the DDP resistance in GC cells by activating NFKBIA, shedding light on MEF2A/NFKBIA might be a promising intervention target for improving DDP resistance in GC.

# Humoral Response and Neutrophil Degranulation Pathways Are Key Factors in Diffuse Large B-Cell Lymphoma (DLBCL) Modulation

**Nor Adzimah Johdi[1,*], Amanda Seng[2], Wei-Kang Lee[2], Hanif Zulkhairi Mohamad Said[1] and Wan Fariza Wan Jamaluddin[3]**

[1]UKM Medical Molecular Biology Institute, Universiti Kebangsaan Malaysia, Cheras, Kuala Lumpur, Malaysia.
[2]Codon Genomics Sdn Bhd, Seri Kembangan Selangor Darul Ehsan, Malaysia.
[3]Cell Therapy Center, Universiti Kebangsaan Malaysia, Cheras, Kuala Lumpur, Malaysia.

*Corresponding Author: adzimah@ppukm.ukm.edu.my

Diffuse large B-cell lymphoma (DLBCL) is globally recognized as the most prevalent and aggressive subtype of non-Hodgkin lymphoma. While conventional treatments are effective initially, the disease can become resistant or relapse over time. The study aimed to examine the differentially expressed genes at the transcriptome level and molecular pathways in DLBCL patients. This investigation utilized RNA sequencing analysis to compare differentially expressed gene samples from five diffuse large B-cell lymphoma patients with two healthy volunteers. These participants were admitted to Hospital Canselor Tuanku Muhriz UKM (HCTM)between 2019 and 2020. Using a negative binomial distribution model, the differentially expressed genes were conducted using the DESeq2 R package (version 1.10.1). The obtained P values were corrected with the Benjamin and Hochberg method and identified using a False Discovery Rate threshold of <0.05, with log2 fold change (FC) of ≥2 or ≤-2. Results showed 73 differentially expressed genes between the two groups, among which 70 genes were downregulated, and three were upregulated. The differentially expressed genes analyzed with the Reactome pathway were significantly associated with the downregulation of antimicrobial humoral response (P<0.001), neutrophil degranulation (P<0.001), chemokine receptors bind chemokines (P=0.028), defensins (P=0.028) and metabolism of angiotensinogen (P=0.040). These findings suggest that the identified pathways may contribute to cancer progression and weaken the immune response in diffuse large B-cell lymphoma patients. This study offers insights into previously undiscovered downstream targets and pathways modulated by diffuse large B-cell lymphoma.

# Mechanisms of Chemoresistance in Colorectal Cancer: Insights from Gene Expression and Protein Profiling in Cisplatin-Resistant Models

**Zhijack Fong[1], Zainal Abidin Syafiq Asnawi[2], Bey-Hing Goh[3,4], Wai-Leng Lee[1,*]**

[1]School of Science, Monash University Malaysia, Jalan Lagoon Selatan, Bandar Sunway, 47500 Subang Jaya, Selangor, Malaysia.
[2]Jeffery Cheah School of Medicine, Monash University Malaysia, Jalan Lagoon Selatan, Bandar Sunway, 47500 Subang Jaya, Selangor, Malaysia.
[3]Department of Biological Sciences, School of Medicine and Life Sciences, Sunway University, 47500 Subang Jaya, Selangor Malaysia.
[4]College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, China.

*Corresponding Author: lee.wai.leng@monash.edu

Colorectal cancer (CRC) is one of the most commonly diagnosed cancers and a leading cause of cancer-related deaths. The ineffectiveness of chemotherapy, including combination drug regimens, complicates the survival rates of advanced CRC. The molecular mechanisms underlying chemoresistance in CRC remain largely elusive. This study aimed to investigate these mechanisms from both clinical and in vitro perspectives. First, bioinformatics analysis of transcriptomic data from colon and rectum adenocarcinoma patients in The Cancer Genome Atlas revealed a substantial number of differentially expressed genes (DEGs) between chemosensitive and chemoresistant CRC patients. Further, clustering of these genes using Gene Ontology and KEGG databases, coupled with literature mining, implicated apoptosis inhibition as a pivotal factor in CRC chemoresistance. In our laboratory, we established *in vitro* CRC cell lines with acquired drug resistance. Three HCT116 sublines with acquired cisplatin resistance were developed through prolonged drug exposure with stepwise dosage increases: HCT116/I24781, HCT116/I248, and HCT116/I555, exhibiting 24.5-fold, 19.2-fold, and 17.2-fold increases in cisplatin resistance, respectively. Proteomics analysis and cell-based assays validated that these cisplatin-resistant HCT116 sublines developed insensitivity to cisplatin-induced apoptosis. Intriguingly, both clinical transcriptomic *in silico* analysis and proteomics studies of *in vitro* resistant CRC lines in this study suggested that hypoxia-related pathways may play a role in regulating CRC chemoresistance. Unlike the reactive oxygen species (ROS)-dependent cell death observed in cisplatin-sensitive HCT116 parental cells, ROS failed to sensitize the resistant lines to cisplatin treatment, resembling the behavior of hypoxic cancer cells. *In vitro* cells were supplemented with oxygen for their growth, leading us to speculate that cisplatin-triggered pseudohypoxia may occur in the resistant lines. Together, these findings suggest that both apoptosis evasion and therapy-induced pseudohypoxia may contribute to chemoresistance in CRC cells.

# Spinal Muscular Atrophy (SMA) Carrier Screening in Malaysia

**Nor Azimah Abdul Azize[1,*], Vengadeshwaran A/L Suberamaniam[1], Lua Seok Hian[1], Ch'ng Gaik Siew[2], Moey Lip Hen[2], Muzhira Aisha Md Haniffa[3], Winnie Ong Peitee[3], Chew Hui Bin[3], Leong Huey Yin[3], Kavitha A/P Rethanavelu[3], Ngu Lock Hock[3] and Yusnita Yakob[1]**

[1]Institute for Medical Research, National Institutes of Health, Ministry of Health Malaysia, Kuala Lumpur.
[2]Genetics Department, Pulau Pinang Hospital, Ministry of Health Malaysia, Pulau Pinang.
[3]Genetics Department, Kuala Lumpur Hospital, Ministry of Health Malaysia, Kuala Lumpur.

*Corresponding Author: azimahazize@moh.gov.my

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder presented with progressive proximal muscle weakness and paralysis, affecting approximately 1:10,000 live births, with reported carrier frequency of 1:41 in Europe and 1:51 worldwide. Majority of the SMA cases due to the homozygous deletion of exons 7 and 8 of the survival motor neuron (SMN) 1 gene. The severity of the disease is determined by the SMN2 gene. In Malaysia, carrier frequency of SMA is currently not available. The aim of this study is to analyse copy number of SMN1 and SMN2 gene using two different methods in identifying SMA carriers among volunteered parental and family members at risk. A retrospective data from 2009-2023 (n=2018) were collected. The laboratory methods involved DNA extraction from blood-EDTA samples, followed by PCR-RFLP method for samples received in 2009-2015 (n=1024) and by multiplex ligation-dependent amplification (MLPA) method for samples 2016-2023 (n=994). Copy number were analysed using GeneMarker software. About 132 parental and 32 family members were analysed for carrier status. Of 1024 samples analysed by PCR-RFLP, 168 (16%) were diagnosed as SMA and remaining 84% could not be determined whether normal or carrier. By MLPA, 168/994 (17%) patients were SMA positive. Whereas 92% (122/132) of parental samples carry 1 copy of SMN1 gene, 7% (9/132) carry 2 copies and 1% (1/132) carry 1 copy with intragenic mutation. Analysis of 32 family members revealed 81% (26/32) carry 1 copy, 16% (5/32) and 3% (1/32) carry 2 copies and 3 copies, respectively. PCR-RFLP was unable to identify SMA carrier because it cannot detect heterozygous deletion. In contrast, MLPA can provide the copy number of both SMN1 and SMN2 gene hence is able to genotype the carrier and severity of SMA as well. In carrier screening, 92% of parents carry 1 copy with genotype [1+0]. About 7% of parental SMA carry 2 copy and may have genotype of silent carrier [2+0]. For analysis of family members, 81% are SMA carrier with genotype [1+0]. Family members with 2 SMN1 copy number have genotype either normal [1+1] or silent carrier [2+0], therefore segregation analysis in the family is recommended. A higher frequency rate in our study (92% from parental and 81% from family members) suggested the importance on carrier screening as it can reduce the risk of having affected offspring. MLPA is a gold standard method not only for SMA diagnosis and carrier screening but also prognosis.

# Expanding the Mutational Spectrum: Identification and Phenotypic Correlations of Four Putative Novel Variants in *PAFAH1B1* and *DCX* Genes Related to Lissencephaly

**Nur Hasmi Abdul Multalib[1,*], Nor Azimah Abdul Azize[1], Vengadeshwaran a/l Suberamaniam, Malini a/p Mahalingam[2], Ahmad Rithauddin Mohamed[3] and Yusnita Yakob[1]**

[1]Institute for Medical Research, National Institutes of Health, Ministry of Health Malaysia, Kuala Lumpur.
[2]Paediatric Department, Raja Permaisuri Bainun Hospital, Ministry of Health Malaysia.
[3]Perak Tunku Azizah Women & Children Hospital, Ministry of Health Malaysia, Kuala Lumpur.

*Corresponding Author: nurhasmi@moh.gov.my

Lissencephaly (LIS) refers to a group of neurodevelopmental disorders that is marked by a smooth brain surface arises from neuronal migration disruption occurring between 12 and 24 weeks of gestation. The clinical presentation manifests in varying degrees of developmental delayed, epilepsy, and intellectual disability, primarily driven by mutations in *PAFAH1B1* and *DCX* gene. The *PAFAH1B1* gene mutations are responsible for over half of LIS cases and is inherited in autosomal dominant pattern. While the *DCX* gene mutations cause about 10 percent of cases with X-linked inheritance. The aim of the present study was to identify pathogenic variants in patients clinically suspected for having LIS in these two causative genes. We conducted data analysis from 22 patients clinically diagnosed with LIS referred to our laboratory from 2015 to 2024. Blood EDTA samples were collected for DNA extraction, followed by PCR amplification and sanger sequencing of both genes. The identified variants were evaluated for pathogenicity by in-silico prediction tools and in accordance to American College of Medical Genetics and Genomics (ACMG) guidelines. Four pathogenic variants were identified in 4 out of 22 patients (18%) with two variants in *PAFAH1B1* (p.(Glu34LysfsTer4) and p.(His379Arg)) and *DCX* gene (p,(Ser74LeufsTer2) and p.(Ala204Asp)). All variants were not previously reported elsewhere; thus, novel. We believe these variants are the causative as it is consistent with imaging and phenotypes presented in the patients. Symptoms like intellectual disability and seizures occurs in most LIS patient and given the phenotypic overlap between the two genes a comprehensive molecular testing is recommended to ensure more accurate diagnosis of LIS. About 80% of patients remain unsolved in our study, mainly due to test limitation as the method used was for single gene and not able to detect large deletion. Therefore, future molecular study using exome and genome sequencing is imperative to identify other genes that responsible for LIS, thus enhance detection rates and improve genetic counselling as well as patient management. Our findings not only expanded the spectrum of genetic mutations in *PAFAH1B1* and *DCX* genes but also contributed in understanding genotype-phenotype correlations in LIS, which would be invaluable for clinical management and diagnostic strategies.

# Phenotypic and Genetic Characteristics of Mucopolysaccharidosis Type II Patients: A Single Centre Retrospective Study in Malaysia

**Affandi O[1,2], Salina AR[1], Fatimah Diana AN[1], Norashareena MS[1], Mohd Shihabuddin AN[2], HY Leong[3], LH Ngu[3] and Julaina AJ[1]**

[1]Inborn Errors of Metabolism and Genetics Unit, NMCRC, Institute for Medical Research (IMR), National Institutes of Health, Ministry of Health, Setia Alam, Selangor, Malaysia.
[2]Faculty of Pharmacy, Universiti Teknologi MARA (UiTM), Puncak Alam Campus, Puncak Alam, Selangor, Malaysia.
[3]Genetics Department, Hospital Kuala Lumpur, Ministry of Health Malaysia, Jalan Pahang, 50586 Kuala Lumpur, Malaysia.

*Corresponding Author: julaina.jalil@moh.gov.my

Mucopolysaccharidosis type II (MPS II; Hunter syndrome) is a rare, X-linked disorder caused by deficient activity of the enzyme iduronate-2-sulfatase which contributes to a multisystemic metabolic manifestation. Treatment is available as enzyme replacement therapy (ERT) with recombinant iduronate-2-sulfatase (idursulfase). The prevalence of MPS II is quite common in Asian countries including Malaysia. This report conducted a retrospective analysis to investigate the clinical characteristics, genotypes and of Malaysian patients with MPS II. Skin biopsy samples were collected from subjects at Hospital Kuala Lumpur who had consented to participate in the NMRR-20-669-54509 research and for future research projects. Descriptive analyses were performed using SPSS version 23.0. Results are presented as mean ± standard deviation and frequency. Eight patients agreed to participate in this study with the mean age (in years) of 12.88 ± 6.03. IDS activity and glycosaminoglycans (GAGs) levels were 4.75 ± 1.16 nmol/4hr/mg protein and 828.51 ± 243.32 ng/mg protein, respectively. Six patients were of Malay ethnicity, whereas two were of Chinese descent. Most patients were from the central region (5 out of 8). Most of the patients presented with claw-like hands, coarse facies and obstructive sleep apnea. Five patients were reported to have missense mutation, and one each for splice mutation and rearrangement of enzyme complex. The reduction in GAG levels ($p<0.05$) observed in patients 1, 2, 3, and 5 demonstrated a significant response to enzyme replacement therapy. This study provided additional information on the clinical and genotype in MPS II patients. Based on these data, characterisation of this cohort group could be used as a baseline for pharmacological chaperone testing in cell studies in the future.

# Revolutionizing Aptamer Development: Electrochemical-based SELEX for Rapid Target Binding

**Shankariswari Yadevan, Nur Fatihah Mohd Zaidi and Khairul Mohd Fadzli Mustaffa ***

Institute for Research in Molecular Medicine (INFORMM), Health Campus, Universiti Sains Malaysia, Kubang Kerian, Malaysia.

*Corresponding Author: khairulmf@usm.my

Aptamers are single-stranded oligonucleotides that fold into 3-dimensional shapes capable of binding non-covalently with high affinity and specificity to a target molecule. Lately, they have been gaining more attention than antibodies due to their advantages as affinity ligands. They are isolated through *in vitro* process called Systemic Evolution of Ligands by EXponential enrichment (SELEX) and offer advantages like longer shelf life, low-cost production, better stability, and low immunogenicity. However, the use of conventional SELEX becomes a barrier to the production of aptamer due to its laborious and costly nature. To overcome these challenges, this study introduces an electrochemical-based SELEX designed for rapid, real-time, and effective aptamer development. This selection platform involves immobilizing target protein by using linkers containing thiol and carboxyl group on the gold electrode, followed by DNA aptamer. To analyse the binding, the electrode surface is exposed to a ferro/ferricyanide redox couple solution which yields square wave voltammetry (SWV) signal. This electrochemical approach eliminates the need for beads and fluorescence labelling, providing easier aptamer/target incubation, washing, and elution steps. After the elution, the bound aptamers are amplified using polymerase chain reaction (PCR), and the resulting PCR products are regenerated to produce single-stranded DNA (ssDNA) pool for subsequent cycles. Negative selection is also carried out by incubating electrodes with linkers and DNA aptamer without the target protein. As a result, six SELEX cycles were successfully performed where high-affinity aptamers bind to the target analyte. To confirm the success of immobilization steps, cyclic voltammetry (CV) and SWV were employed to check for stability and peak reduction current, respectively. The SWV reduction peak current was calculated for each SELEX cycle before and after incubating with DNA aptamers using the formula [(i° − i)/i°%] to determine the most enriched cycle. In conclusion, DNA aptamers bound to target proteins were successfully isolated and later will be characterized for its binding and affinity. These potential high-affinity aptamers hold promise for use in diagnostic or therapeutic applications.

# Manufacturing and Validation of Current Good Manufacturing Practices (cGMP)-Compliant Umbilical Cord-Derived Mesenchymal Stromal Cells (UCMSC) for Clinical Application

**Noor Atiqah Fakharuzi, Kamal Shaik Fakiruddin, Rohaina Abd Razak, Govathini Sathiaselan, Muhammad Nazirul Fahmi, Nur Alia Syuhada, Mughuntha Anujana Nagen, Sujatha Suthandiram, Yuslina Mat Yusoff, Gowrisankari Navaretnam, Wan Sakeenah Wan Nazri, Adiratna Mat Ripen and Lim Moon Nian***

Good Manufacturing Practice (GMP) Laboratory, Biomedical Research Core Facility (BioRCF), Institute for Medical Research (IMR), National Institutes of Health (NIH), Ministry of Health Malaysia (MOH), Setia Alam, 40170 Shah Alam, Selangor, Malaysia.

*Corresponding Author: limmn@moh.gov.my

Umbilical cord-derived mesenchymal stromal cells (UCMSCs) hold significant promise in treating various diseases due to their regenerative and immunomodulatory properties. In Malaysia, MSCs are classified as Cell and Gene Therapy Products (CGTPs) and regulated as biologics. The regulatory framework for CGTPs is outlined in the Guidance Document and Guidelines for Registration of CGTPs, as well as in the third edition of the Drug Registration Guidance Document (DRGD). In this regulatory context, producing MSCs on a large scale using a robust manufacturing protocol is crucial to meet clinical demands. Thus, this study aimed to validate the production of UCMSCs in compliance with regulatory requirements. The product validation involves several key steps including validation of raw materials, qualifying GMP personnels and validation of manufacturing processes. In this study, we established a well-characterized two-tiered system of Master Cell Bank (MCB) and Working Cell Bank (WCB) to generate a large-scale expansion of UCMSCs. Comprehensive in-process and product release tests including immunophenotype, karyotyping, endotoxin test, mycoplasma, and sterility were evaluated to determine the identity, potency, safety, and purity of the UCMSCs in accordance with the International Society for Cell and Gene Therapy (ISCT) and Pharmaceutical Inspection Co-operation Scheme (PIC/S) GMP Guide on Manufacture of Sterile Medicinal Product (Annex 1). Altogether, our results demonstrated that UCMSCs maintain their multipotent differentiation capacity, morphology and surface marker expression. No genetic abnormalities were detected and all batches were confirmed to be free of microbial contamination. In conclusion, we successfully adapted our clinical-grade MSC production process to meet the GMP requirements. UCMSCs were efficiently derived from three donors using a GMP-compliant culture system, and three consecutive production batches were successfully validated under stringent regulatory conditions.

# Quantitative Analysis of TNF-α Levels in Plasma: Evaluating the Impact of Different Celastrol Doses in High-Fat Diet-Fed ApoE-Knockout Mice Using ELISA

**Arifah Ahmad Damahuri[1], Thuhairah Hasrah Abdul Rahman[1], B. Vimala R.M.T Balasubramaniam[2] and Nasibah Azme[1,*]**

[1]Department of Medicine, Faculty of Medicine, University Teknologi MARA.
[2]Nutrition, Metabolism & Cardiovascular Research Centre, Institute for Medical Research, Selangor.

*Corresponding Author: nasibah@uitm.edu.my

A notable trend in contemporary society is the consumption of a hypercaloric (Western) diet, which is associated with the induction of systemic inflammation. Celastrol has been explored in multiple models as a promising drug to highlight its therapeutic properties in reducing inflammation. However, the effects of different celastrol doses on inflammatory cytokine levels in ApoE-knockout mice remain to be determined. Thus, this study aims to investigate the effect of different doses of celastrol on TNF- α levels in the plasma of ApoE-knockout mice fed a high-fat diet (HFD) via ELISA. Four groups of male ApoE-knockout mice were fed with HFD for 12 weeks starting at 8 weeks of age. Meanwhile, the control normal diet group was fed with a chow diet. During the last 4 weeks, three groups that were fed a HFD received intraperitoneal injection of celastrol treatment at doses of 1.5 mg, 2 mg, and 2.5 mg/kg/day, respectively, while the control HFD and normal diet groups received 2% DMSO/day. At the end of the treatment, blood was collected through cardiac puncture before mice were sacrificed and centrifuged for 15 minutes at 1000 xg at 4°C within 30 minutes of collection. Then, plasma was assigned for double-sandwich ELISA according to the manufacturer's instructions, Finestest (Mouse TNF- α, Tumor Necrosis Factor Alpha ELISA kit; EM0183). Data were analyzed using Thermo Scientific Multiskan FC 357 Microplate Photometer and Kruskal-Wallis test in GraphPad Prism 10. The findings showed there were no significant changes between all groups, but all celastrol-treated groups displayed a decreasing pattern in TNF- α level compared to the control HFD group. Celastrol, as an anti-inflammatory therapy, warrants further studies on prolonged Western diet consumption, as it may be effective in other models, particularly those at high risk of developing inflammation. Additionally, we need to explore the local inflammatory status in tissues, which may yield different results compared to plasma inflammatory cytokine levels.

# Detection of Large Deletions and Duplication in F8 Gene of Haemophilia A Patients

**Abdullah Aziz Nursaedah[1,*], Lam Kah Yuen[1], Lukman Izzatul Nabila[1], Asrapil Waitul Fifika[1], Salam Syarini[1], Mohamad Fadilah Nur Hakimah[1], Nik Mat Zin Nik Nor Imam[1], Jradeh Bilal[3], Jaslowska Ewa[3], Yee Thynn Thynn[3], Gomez Keith[3], Mohd Sahid Ermi Neiza[1], Musa Nurul Hidayah[1], Mohd Yasin Norafiza[1], Md Nor Azian Naila[1], Somasundram Suguna[1], Md Afandi Faridah[2], Mohd Fathullah Tun Maizura[2], Jamaludin Sufiza[2], Mat Ripen Adiratna[1] and Mat Yusoff Yuslina[1]**

[1]Haematology Unit, Cancer Research Centre, Institute for Medical Research, National Institutes of Health, Ministry of Health, Selangor, Malaysia.
[2]National Blood Centre, Ministry of Health, Kuala Lumpur, Malaysia.
[3]Katharine Dormandy Haemophilia Centre and Thrombosis Unit, Royal Free London NHS Foundation Trust, London, United Kingdom.

*Corresponding Author: nursaedah@moh.gov.my

Haemophilia A (HA) is an X-linked recessive bleeding disorder caused by mutations in the *F8* gene, leading to deficiency of blood coagulation factor VIII. Diverse types of mutations have been reported in HA patients including intron 22 inversion, intron 1 inversion, missense, nonsense, frameshift and splice site mutations. However, in a small subset of patients, conventional PCR fails to amplify any exons or no mutations are detected upon analysis, suggesting the possibility of structural variations including large deletions and duplications. In this study, multiplex ligation-dependent probe amplification (MLPA) was carried out to detect large deletions and duplications in the *F8* gene of HA patients. Fragment analyses were performed using genetic analyzer with GS500-ROX as the internal size standard followed by data analysis using Coffalyser software. MLPA probe ratios were determined by comparing relative probe signals from each patient sample to those from healthy male DNA controls. Probe ratios in between 0.75 and 1.25 were considered as normal whereas ratios of <0.75 and >1.25 indicated deletion, and duplication, respectively. Among the studied 129 HA patients, 16 (12.4%) patients who did not have a mutation detected by inversion testing or *F8* sequencing were subjected to MLPA. Large deletions and duplications were detected in 6.2% (n=8) and 0.8% of patients (n=1), respectively. The gross deletions spanned across various locations including exon 1-7 (n=1), exon 4-6 (n=2), exon 13-21 (n=1) and exon 26 (n=4) of *F8* gene. Meanwhile, the gross duplication spanning exon 23-25 of the *F8* gene was observed in one HA patient. These large structural variations lead to severe (n=7) and moderate (n=2) bleeding phenotypes in HA patients. MLPA is a valuable molecular diagnostic approach for identifying large deletions and duplications in the *F8* gene of HA patients, thereby facilitating precise genetic testing, carrier identification, and treatment decisions.

# Frequency of Anti-Acetylcholine Receptor (AChR) Antibody among Suspected Myasthenia Gravis Patients in Malaysia

**Nurhanani Mohamed Nor, Nur Syahirah Alias, Syuriatika Dionysius, Bavithra Venu, Ainur Yusniza Yusof, Siti Asma Bakar and Siti Fatimah Abdullah**

Autoimmune Unit, Allergy and Immunology Research Centre, Institute for Medical Research, Malaysia.

Myasthenia gravis (MG) is a chronic neuromuscular autoimmune disease, characterised by muscle weakness and is potentially life-threatening. It is caused by autoantibodies targeting components of the neuromuscular junction and this include acetylcholine receptors (AChR). The objective of this study is to evaluate the frequency and diagnostic utility of anti-AChR antibody in Malaysian MG-suspected patients. This is a retrospective study analysing the frequency of anti-AChR antibody (IgG) measured by enzyme-linked immunoassay (ELISA) selected from our diagnostic database from 2023. Results equal to and above 5 nmol/L are considered positive. A total of 905 patients' sera were analysed, of which 30% (274/905) of them had a positive anti-AChR antibody with a mean age of 48.3 years. Among the youngest seropositive patients were 8-month-old female twins that presented with bilateral ptosis since birth and were diagnosed with ocular MG. More than half of seropositive patients were Malays (54.7%), followed by Chinese (31.8%), other races (10.2%) and Indians (3.3%). A female predominance was also observed (56.9%) and there was a significant mean difference between males and females who were seropositive ($p<0.05$). Serological tests of autoantibodies are useful as non-invasive biomarkers to support the diagnosis of MG. In this study, anti-AChR antibody were detected in suspected MG patients presented with typical clinical features, thus proving its diagnostic utility. However, for further classification of MG subtypes especially among seronegative patients, detection of other autoantibodies such as those against muscle-specific tyrosine kinase (MuSK) and lipoprotein receptor-related protein 4 (LRP4), is warranted to assist clinicians in disease management.

# Advancing Biochemical Diagnosis of Aromatic-L-Amino Acid Decarboxylase Deficiency (AADC-D): HPLC-Based AADC Activity Assay Preceding Genetic Testing

**Norashareena Mohamed Shakrin[1,4,*], Norzahidah Mohd Khalid[2], Nor Azimah Azize[2], Yusnita Yaakub[2], Ch'ng Gaik Siew[3], Abdah Md Akim[4] and Julaina Abdul Jalil[1]**

[1]Inborn Error of Metabolism & Genetic Unit, NMCRC, Institute for Medical Research (IMR), National Institute of Health Malaysia, Ministry of Health Malaysia (MOH), Setia Alam, Selangor.
[2]Biochemistry Unit, SDC & Molecular Diagnostic Unit, SDC, Institute for Medical Research (IMR), National Institute of Health Malaysia, Jalan Pahang, Kuala Lumpur, Malaysia.
[3]Genetic Unit, Pulau Pinang General Hospital, Georgetown, Penang.
[4]Department of Biomedical Sciences, Faculty of Medicine & Health Sciences, Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia.

*Corresponding Author: shareena.ms@moh.gov.my

Aromatic-L-amino acid decarboxylase deficiency, AADC-D (OMIM #608643) is a rare genetic disorder caused by pathogenic homozygous or compound heterozygous variants of the dopa decarboxylase (*DDC*) gene leading to severe neurological impairments. The AADC enzyme, which requires pyridoxal 5-phosphate as a cofactor, is responsible for the decarboxylation of L-3,4 dihydroxyphenylalanine (L-dopa) and 5-hydroxytryptophan (5- HTP) and is the final step in the well-characterized synthesis of the monoamine neurotransmitters dopamine and serotonin. The exact global incidence of AADC deficiency is unknown, but it is more prevalent in Asian populations. Early and accurate diagnosis is crucial for timely intervention, yet current biochemical diagnostic methods lack of specificity. Low concentrations of biogenic amines profile in cerebrospinal fluid (CSF) are suggestive but not specific to this disorder. The discovery of reliable biomarkers is essential to improve diagnosis and patient outcomes. Our objectives were to validate a High Performance Liquid Chromatography (HPLC) based assay for determination of the AADC activity in plasma for the diagnosis of AADC-D. HPLC with electrochemical and fluorescence detection was used to quantify AADC enzyme activity in two plasma samples of AADC-D patients and healthy controls using both of its substrates, 5-hydroxytryptophan (5-HTP) and 3,4-dihydroxyphenylalanine (L-dopa). The assay of AADC enzyme activity in patients' plasma showed significantly reduced activity compared to normal reference ranges. The measured enzyme activity of patient 1 and patient 2 were 12.4±6.1 and 17.9±11.5 (pmol/min/mL) respectively; below the lower limit of the reference range, highly suggestive of AADC deficiency. The results showed clear discrimination between confirmed AADC-deficient patients and healthy controls (37.2±4.7pmol/min/mL). This low enzyme activity is consistent with the clinical features and genetic findings. The genetic analysis revealed a pathogenic variant in the *DDC* gene, which encodes the aromatic L-amino acid decarboxylase enzyme. Compound heterozygous mutations were detected at c.175G>A, p.(Asp59Asn) in exon 2 and c.714+4A>T, p.(?) in intron 6 of the *DDC* gene. This diagnosis aligns with the patient's clinical symptoms and confirms the presence of this rare genetic disorder. In conclusion, the combination analysis of markedly reduced enzyme activity and a pathogenic mutation in the *DDC* gene supports a definitive diagnosis of AADC-D. The findings suggest that plasma AADC activity assay by HPLC can serve as a reliable biomarker for biochemical diagnosis of AADC deficiency.

# Colorimetric Aptablot Assay for the Detection of Chikungunya Virus (CHIKV)

**Low Yong Chun[1,*], Magdline Sia Henry Sum[1] and Anna Andrew[2]**

[1]Institute of Health and Community Medicine, Universiti Malaysia Sarawak.
[2]Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak.

*Corresponding Author: yongchun_@hotmail.com

Chikungunya virus (CHIKV) infection is responsible for causing febrile illness in humans and posed a public health concern especially since the largest outbreak in the Indian Ocean in 2004. Patients infected with CHIKV might suffer from persistent joint pain for months to years. Serological tests detecting CHIKV antigens, such as enzyme-linked immunosorbent assays, have several limitations, including high cost, the need for well-trained personnel, batch-to-batch variation, and less stable. Unlike antibodies, aptamers (single-stranded DNA or RNA molecules) offer high affinity and specificity for target molecules, can be produced in vitro and do not have batch-to-batch variation. In this study, an Aptablot assay was developed using an aptamer, replacing antibodies commonly used in conventional dot blot assays. The aptamers were selected via Systematic Evolution of Ligands by Exponential Enrichment and are specific against CHIKV. The aptamers were modified with Thiol group at 5' end and conjugated with 20 nm maleimide activated gold nanoparticles (AuNP) using a conjugation kit. The Aptablot assay was verified by immobilizing a series of concentrated CHIKV on the nitrocellulose membrane and incubated with aptamer-conjugated AuNP after the membrane was blocked with Bovine Serum Albumin in Phosphate Buffer Saline with Tween-20. The limit of detection of the Aptablot assay was $10^4$ PFU/ml, which is within the typical CHIKV load ($10^4$ to $10^8$ PFU/ml) in patients' samples during the viremic stage of infection. The colorimetric Aptablot assay developed in this study is simple, fast, and user-friendly, and it can be done without the need for advanced equipment or skilled personnel. Our findings suggest that the Aptablot assay is a promising tool for the detection of CHIKV, especially in resource-limited settings where CHIKV is endemic.

# Discovery of Ten Novel Germline RB1 Gene Mutations in Unrelated Malaysian Patients with Retinoblastoma: A 12-Year Experience

**Ilia Nazihah Mohamad Ayob[1,*], Anis Frasha Mohamad[1], Mohd Khairul Nizam Mohd Khalid[1], Chew Hui Bin[2], Leong Huey Yin[2] and Yusnita Yakob[1]**

[1]Unit of Molecular Diagnostics, Institute for Medical Research, National Institutes of Health, Ministry of Health, Malaysia.
[2]Genetics Department, Kuala Lumpur Hospital, Malaysia.

*Corresponding Author: nazihah.ma@moh.gov.my

Retinoblastoma (Rb) is the most common malignant intraocular tumor in children. Globally, retinoblastoma affects around 1 in 17,000 live births, with about 8,000 new cases reported annually. In Malaysia, an average of 14.5 new Rb cases are diagnosed every year. Rb arises due to mutations in the RB1 gene located on chromosome 13q14.2 and is recognized as a prototypical hereditary cancer in humans. Around 30-40% of cases follow an autosomal dominant inheritance pattern, while the remaining 60-70% are sporadic and non-inherited. In this study, we present our 12-year experience in identifying RB1 gene variants among retinoblastoma patients in Malaysia. This retrospective cohort study analyzed over 350 samples sent to the Institute for Medical Research for molecular testing between 2012 and June 2024. Genetic testing involved amplifying all coding regions and splice sites of the RB1 gene using PCR, followed by Sanger sequencing to detect causative mutations. Pathogenic variants were confirmed using public databases and in silico prediction tools like VarSome and Franklin, according to ACMG guidelines. For cases with no mutation detected by PCR and sequencing, MLPA (Multiplex Ligation-Dependent Probe Amplification) analysis was performed using the SALSA MLPA Probemix P047 RB1 kit. Products were run on an Applied Biosystems 3500 Genetic Analyzer with GeneScan™ 500 LIZ® as the size standard, and results were analyzed with Coffalyser.Net software. A total of 81 distinct mutations were identified using the PCR-sequencing method and MLPA. Out of the 384 samples tested, 10 samples were found to harbour novel RB1 gene variants. Identification of mutational origin was important especially to inform the family of the affected proband regarding the risk of Rb to other siblings and relatives. Screening relatives at risk for RB1 mutations before the disease begins to manifest, was also found to be more cost-effective than conventional ophthalmological examinations. Prenatal diagnosis could also be offered to the mother in the future. In our samples, 21% of Malaysian patients with Retinoblastoma received the definitive molecularly proven diagnosis. For the remaining patients, tumor sample analysis is recommended, as this was not done in the current study. Additionally, the discovery of novel variants expands the genetic spectrum of the RB1 gene, contributing to future research on this disease. Accurate diagnosis is crucial not only for patient management but also for genetic counseling and early detection of at-risk family members.

# The Effect of Lauric Acid-Rich Diets on CD4+ And CD8+ Cell Counts: A Flow Cytometer Analysis

**Maisarah Ab Fatah[1,*], Yap Sia Yen[1], Voon Phooi Tee[1], Ng Yen Teng[1], Ammu Radhakrishnan[2] and Kanga Rani Selvaduray[1]**

[1]Malaysian Palm Oil Board (MPOB), Kajang, Selangor, Malaysia.
[2]Monash University Malaysia, Bandar Sunway, Selangor, Malaysia.

*Corresponding Author: maisarah.abfatah@mpob.gov.my

Lauric acid is a medium-chain saturated fatty acid, with a 12-carbon atom chain (C12:0). Previous in vitro studies showed that lauric acid activates the immune system through various pathways. However, studies also showed that lauric acid does not activate the immune system. Therefore, further investigation needs to be done to investigate the contradicting findings. Here, we examined the effect of lauric acid-rich diets on CD4+ and CD8+ cell counts in mice blood. Three types of diets of different kinds of fat sources were used. Diets A and B are rich in lauric acid, originating from different plant sources but containing similar amounts of lauric acid. Diet C is the basal diet. Each diet contains 50 % kcal of carbohydrate, 25 % kcal of protein and 25 % kcal of fat. These pellets were given to BALB/c female mice for six weeks to observe the effects of different diets on the CD4+ and CD8+ cell counts. The blood from each mouse was collected after sacrifice and the whole blood analysis was done based on the BioLegend® protocol, using Agilent ACEA NovoCyteTM flow cytometer. Our data analysis showed that Diet A and Diet B show an increase in CD4+ and CD8+ cell count, compared to Diet C.

# Exploring The Positional Distribution of Fatty Acids in Human Plasma Chylomicrons Using $^{13}C$ Nuclear Magnetic Resonance Spectroscopy

**Ng Yen Teng and Voon Phooi Tee***

Malaysian Palm Oil Board (MPOB), Kajang, Malaysia.

*Corresponding Author: vptee@mpob.gov.my

Analysis of the positional distribution of fatty acids in triacylglycerols (TAG) using conventional lipase-catalysed hydrolysis and gas chromatography (GC) is laborious. $^{13}C$ nuclear magnetic resonance (NMR) spectroscopy has been validated to be a rapid, direct and accurate analytical method for determining the positional distribution of fatty acids in edible oils and fats. However, the utilisation of $^{13}C$ NMR in the regiospecific distribution of fatty acids in human plasma samples has yet to be studied extensively. In this study, we aimed to determine the positional distribution of fatty acids located at sn-1, 3 and sn-2 positions of blood TAG extracted from human chylomicrons using the $^{13}C$ NMR method. The study employed two test fats, namely palm olein (PO) and lard (LD), with predominantly oleic and palmitic acids situated at the sn-2 position of the TAG backbone, respectively. A postprandial, crossover-designed feeding intervention was conducted on 14 subjects (female=7, male=7), and postprandial blood samples were collected at 3, 4, and 5 hours. Plasma chylomicrons, packed with the ingested TAG (~90%), were harvested from the postprandial blood samples using the ultracentrifugation method. The TAGs extracted from the plasma chylomicrons were then analyzed using the 600 MHz $^{13}C$ NMR spectrometer (JEOL ECZR Series 600). Both test fats were utilized to optimize the acquisition and processing parameters of $^{13}C$ NMR spectroscopy for routine regiospecific analysis of blood TAG. The Limit of Detection (LOD) was visually determined at 40mg/ml. The obtained $^{13}C$ NMR spectrum of extracted TAG was compared with the optimized spectrum profiling produced from the two test fats. Subsequently, the positional distribution of fatty acids composition (SFA, MUFA, PUFA) of the extracted TAG from plasma chylomicrons was identified. The $^{13}C$ NMR spectrum reflected the positional distribution of fatty acids from the intake of the two test fats in the human chylomicron samples. Consistent with the *sn*-2 hypothesis, the PO-diet produced more MUFA, while the LD-diet produced more SFA at the sn-2 position in the plasma chylomicrons at 3, 4, and 5 hours postprandially. In conclusion, $^{13}C$ NMR spectroscopy is able to provide a direct measurement and serve as one of the rapid methods to determine the positional distribution of fatty acids in blood TAG extracted from human plasma chylomicrons after a high-fat diet intake, which is valuable to the healthcare industry.

# In Silico Analysis of Natural Agents and Antiviral Drug Combinations Targeting Key Proteins in Monkeypox Virus

**Mutaman Hussein Abdullah***

Department of Biomedical Science, Advanced Medical and Dental Institute, Universiti Sains Malaysia, 13200 Kepala Batas, Penang Malaysia.

*Corresponding Author: mutamanhussein@usm.my

The emergence of the Monkeypox virus (Mpox), a zoonotic Orthopoxvirus, as the global decline in smallpox immunity has accelerated a significant health concern. The virus, equipped with crucial enzymes for its replication and immune evasion, such as the mRNA Cap Methyltransferase (E12) and Primase D5, presents an urgent need for targeted therapies to halt its spread. This research, of paramount importance, aims to find drugs that can effectively block the Monkeypox virus. It will use the affinity of typical antiviral drugs and existing natural compounds to the determined active sites of E12 and Primase D5. The provided list combined nine antiviral drugs and nine natural compounds that would undergo docking research. E12 and Primase D5 structures were ascertained by analysing the structural information available (PDB IDs: 8JED, 8HWB). AutoDock Vina was the software used for docking purposes to accurately cypher the individual ligands' binding affinities. LigPlus, PyMOL, and BiNANa 2 were the software program tools used to visualise and investigate the docking interactions. PrankWeb was the tool used to make binding site predictions. The ligand with the lower binding affinities was discarded, while the rest of the compounds were used together and re-docked to assess the potential synergistic effects. The binding tests demonstrated that all natural agents had substantial binding affinities towards the target proteins, with Baicalin and Epigallocatechin Gallate peaking the charts in docking scores for both proteins. On the other hand, Baicalin combined with Nirmatrelvir and Valganciclovir had much stronger binding affinities than the drugs considered on their own, which could mean that natural agents could be incorporated into antiviral treatments. This study underscores the potential of drug-natural compound combinations as potent inhibitors of the Monkeypox virus. The identified compounds and their combinations provide a foundation for further experimental validation and instil hope for developing effective therapeutic strategies against Mpox.

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