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INSTITUT BIOLOGI MOLEKUL PERUBATAN UKM
UKM MEDICAL MOLECULAR BIOLOGY INSTITUTE
9TH REGIONAL CONFERENCE ON MOLECULAR MEDICINE
RCMM 2023
IN CONJUNCTION WITH THE
5TH NATIONAL CONFERENCE FOR CANCER RESEARCH
Precision Medicine in Healthcare
14-15 OCT 2023
08:00a.m. - 05:00p.m. MYT
Bangi Resort Hotel, Bangi, Selangor
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O1

Abstract for 9th Regional Conference on Molecular Medicine (RCMM)
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Elucidation of Epstein-Barr virus (EBV) Encoded Latent Membrane Protein-1 (LMP-1) 30 bp Deletion Mutation Effect on Immune Checkpoint Regulation in EBV-Associated Nasopharyngeal Carcinoma (NPC) and Non-NPC Cancers

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ABSTRACT

Background: Nasopharyngeal carcinoma (NPC), lymphoma, breast, and gastric cancers are Epstein-Barr virus (EBV)-associated cancers. Presently, late-stage cancer is hard to manage. Therefore, early detection and precision cancer treatment are very important for cancer management. The detection of EBV LMP1 30 bp deletion in the biopsy samples of NPC patients were previously studied and proven to have an association with the development of NPC, but many questions remain unresolved, particularly on the effects of this mutation on the regulation of immune checkpoint receptors on immune cells (particularly regulatory T cells; Tregs) in cancers other than NPC. **Methods:** The current study was conducted at the Department of Medical Microbiology and Parasitology, School of Medical Sciences, Universiti Sains Malaysia (Health campus), Malaysia. The EBV LMP1 30 bp deletion mutation variants from whole blood samples from 29 patients with NPC, 29 non-NPC and 29 Healthy controls (HC) were determined using Polymerase Chain Reaction (PCR). Furthermore, the expression levels of immune checkpoint receptors (CD3, CD4, CD25, CD127, FOXP3, LAG-3, TIM-3, and TIGIT) was determined on Tregs using flow-cytometry assay. Statistical analysis was performed using SPSS programme.

Results: The majority of NPC patients were Malay, followed by Chinese ethnicity (89.29% and 10.34%, respectively). Most of the NPC patients were diagnosed with WHO type III (n = 17, 58.62%), followed by WHO type II (n = 11, 37.93%), and WHO type I (n = 1, 3.44%). The locally advanced NPC stages were found in the vast majority of NPC patients (stage IV and stage III). LMP1 30-bp deletion mutation variant was detected in 8/29 (27.58%) of NPC patients, while none of the non-NPC and healthy control samples were found positive for LMP1 30 bp deletion mutation variant. The expression level of TIM3 on Tregs was 3.94 $\pm$ 5.79 in NPC, 17.2 $\pm$ 20.3 in non-NPC and 0.079 $\pm$ 0.25 in HC. For LAG3 the expression was 4.38 $\pm$ 9.25 in NPC, 1.83 $\pm$ 4.35 in non-NPC and 0.048 $\pm$ 0.18 in HC. For TIGIT the expression level was 29.5 $\pm$ 13.5 in NPC, 36.6 $\pm$ 22.0 in non-NPC and 1.03 $\pm$ 1.36 in HC. **Conclusion:** The results of this study contribute significantly to the understanding of EBV LMP1 30 bp deletion on the expression levels of immune checkpoint receptors in EBV-associated cancer patients which could relevant in identifying potential immune checkpoint inhibitor drugs for immunotherapy as well as prognostic biomarker in monitoring immunotherapy in EBV-associated (NPC and non-NPC) cancer patients.

O2

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High Throughput DIA-NN Proteomics Identifies Altered Extracellular Protein Network in CRISPR-Targeted AGR2 of Breast Cancer Cells.

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ABSTRACT

Anterior Protein Gradient-2 (AGR2), a member of the protein disulphide isomerase (PDI) family located in the endoplasmic reticulum (ER), plays a pivotal role in catalyzing protein folding and thiol-disulphide interchange reactions. Its significance emerges from its overexpression in various epithelial-origin cancer types, accompanied by extracellular secretion, yet the functions of extracellular AGR2 (eAGR2) remain to be elucidated. This study dives into the intricate of eAGR2 functions, using breast cancer cells as a model. Employing CRISPR/Cas9 genome editing tool, AGR2 was targeted in three breast cancer cell lines: MCF-7, T47D, and 1833-BoM. Cell-based in-vitro assays of the AGR2-targeted cells confirmed AGR2's oncogenic nature in breast cancer. AGR2-repressed cells demonstrated reduced proliferative capacity, a hallmark of tumorigenesis. Furthermore, the cells displayed heightened sensitivity to conventional breast cancer drugs like doxorubicin, suggesting AGR2's involvement in drug resistance mechanisms. We also constructed four different structural AGR2 variants to modulate AGR2 secretion and found that the ER-retention motif might has a role in AGR2 secretion. To gain quantitative insights into eAGR2's role, state-of-the-art mass spectrometry Data Independent Acquisition-Neural Network (DIA-NN) methodology, were utilized. After subjecting the AGR2-null cells to serum starvation and secretome enrichment, DIA-NN mass spectrometry identified over 900 differentially expressed secretome proteins, with more than 300 consistently downregulated in all AGR2-targeted cell lines. Functional enrichment highlighted the involvement of these genes in critical cellular processes such as receptor signaling, secretion, extracellular vesicle biogenesis, cell adhesion, and

inflammatory response. Protein-protein interaction network analysis uncovered a compelling interconnected hub genes within the downregulated proteins, shedding light on potential key players in the AGR2 signaling axis. This study unveiled bona-fide AGR2-modulated secretome that may influence the tumor microenvironment and breast cancer progression, thereby expanding the biology of eAGR2 function in the context of breast cancer.

O3

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Lipidome Analysis and Potential Lipid Biomarkers in Glioblastoma Tissue using Untargeted Lipidomic Analysis

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ABSTRACT

Background: Glioblastoma (GBM) is the lethal subtype of glioma, with a poor prognosis and patient survival rate. Dysregulation of the metabolism, particularly lipid homeostasis is linked to the GBM's aggressiveness and treatment resistance. This study aimed to characterise the lipidome composition and further investigate the potential lipid biomarkers in tissue samples of GBM patients. **Methods:** Human tumoural tissue lipidomes from 8 controls and 22 patients with glioblastoma were analysed using triple quadrupole liquid chromatography electrospray ionisation mass spectrometry. Differences in lipid profiles and differential lipid expression between GBM and controls were compared and investigated using univariate and multivariate statistical analysis. Receiver operating characteristic (ROC) analysis was performed, where the area under the curve (AUC) values larger than 0.75 were identified as potential lipid biomarkers. **Results:** Lipidome analysis revealed increased lipid composition with 7888 lipid compounds detected in GBM tissue. Several lipid subclasses including phosphatidylcholine, phosphatidylethanolamine, triacylglycerol, and fatty acyl were reduced, whereas ceramide was elevated in GBM tissue. Partial least squares-discriminant analysis (PLSDA) and hierarchical clustering analysis (HCA) demonstrated that GBM patient and control populations were visually separated by identified lipid biomarkers. Random forest analyses further predict the accuracy of 25 significant lipid species in GBM. Lipid metabolites, Cer 13:0;2O/26:5 (AUC=1.00), DG 43:11 (AUC=0.94) and PC O-9:0_24:5 (AUC=0.89) were among the best lipids biomarker in GBM tissue. **Conclusion**: This study demonstrates lipid metabolic rewiring in glioblastoma, which may provide insight into its underlying molecular plasticity and progression for clinical benefits.

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TCR Profiling of Cytotoxic T Cells Targeting Neoantigens from Colorectal Cancer Patients

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ABSTRACT

Background: Neoantigens, derived from non-synonymous mutations in a patient's genome could potentially become an ideal target for robust activation of anti-cancer response. Neoantigens are only expressed in tumor cells and are completely absent in normal cells. As such, neoantigens can become a precise target for cytotoxic T cells, especially in the application of cancer vaccines and adoptive T cell therapy (ACT). **Method:** PCR sequence-specific primer was done to check for the HLA status of ten high TMB CRC samples. Prediction of neoantigen was done using MuPeXi. Gene expression and immunogenicity analysis were done using the Class I immunogenicity tool. The affinity binding of neoantigen candidates was determined using NetMHC4.0. Primary cell culture work was done by isolating PBMCs from whole blood. Monocytes were isolated using the plastic adherence method and were cultured with maturation cytokines to form matured dendritic cells (mDCs). Validation of cellular markers i.e CD14, C83, CD86, CD3 and CD8 was done using FACS Verse. Ten M of the peptide was fed into mDCs before being co-cultured with CD8 T cells. Finally, neoantigen-reactive T cells were isolated using peptide dextramer and FACS Aria III. Total RNA was obtained and immunosequencing was performed. Briefly, 5' RACE was performed for reverse transcription and the products were amplified by PCR with corresponding specific primers. The libraries were sequenced using Illumina Novaseq instrument. **Result:** 5 samples were identified as HLA-A*2402 positive from 10 CRC samples. Data from combined analysis of MuPeXi, gene expression microarray, immunogenicity tool and

NetMHC4.0 revealed one candidate that strongly binds to HLA-A*2402. Cytometric analysis revealed more than 90% of mDCs. Isolation of T cells using negative magnetic beads separation showed 93% of the T cell population. The sorting of T cells pulled down around 6% of neoantigen-specific T cells. Immunosequencing analysis of TCR revealed several dominant CDR3 clonotypes of alpha and beta chains in both CD8 and dextramer samples. Interestingly, the most dominant CDR3 sequence was also found in the tumor microenvironment, suggesting the presence of neoantigen-specific T cells from tumor-infiltrating lymphocytes. **Conclusion:** TCR sequence targeting specific neoantigen can be identified effectively using n immunosequencing of sorted T cells by peptide dextramer. We provided a fast and effective method to culture and isolate neoantigen-specific T cells. In the future, the sequence of TCR can be cloned into healthy T cells for further functional validations against the peptide neoantigen.

O5

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Lutein Inhibits the Triple Negative Breast Cancer Cell Progression by Suppressing the Protumour Effects of Tumour-Associated Macrophages

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ABSTRACT

Tumour-associated macrophages (TAMs) play an important role in tumour development by modulating the M2-type macrophage (M2) polarisation in tumour microenvironment. Several phytochemicals have been reported to have inhibitory effects on M2 macrophage polarisation. Lutein (non-vitamin A carotenoids) is a natural compound with well-known for its medicinal benefits and has been shown to have promising anticancer properties. In the current study, we used *in vitro* assays to determine whether lutein has inhibitory effects on the M2 polarisation and its protumour functions towards MDA-MB-231 (triple negative breast cancer) cells. CD14+ isolation from the peripheral blood mononuclear cells (PBMCs) of healthy donors was performed using immunomagnetic beads to isolate monocytes and the monocytes were cultured with GM-CSF/LPS/IFN-gamma or M-CSF/IL-4 to differentiate the monocytes into macrophages. MDA-MB-231 cell lines were co-cultured with human monocyte-derived macrophages (HMDMs) and subjected to the cell proliferation and transwell migration assays as well as the determination of cell surface markers. Our results showed that M2 macrophages accelerated the proliferation and migration of MDA-MB-231 cells and these protumour effects were inhibited by lutein. Additionally, lutein also significantly reduced the expression of M2 surface markers (CD206), and thus reversed the earlier elevation of CD206 induced by IL-4. Our data collectively imply that lutein may be an effective anticancer therapy or adjuvant for patients with TNBC.

O6

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Super Enhancer Upstream Regulators Drive KLF6-PDGFB Expression and mTORC1 Pathway Activation in Renal Carcinoma

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ABSTRACT

Kruppel-like factor 6 (KLF6), a super enhancer-driven transcription factor, supports clear cell renal cell carcinoma (ccRCC) growth by controlling cellular loop that links several ccRCC hallmark features including mTORC1 pathway hyperactivation. We have previously demonstrated that KLF6 transcriptionally regulates *PDGFB* expression, which will subsequently activate mTORC1 pathway in ccRCC via autocrine and paracrine manners. Nonetheless, whether the KLF6-PDGFB axis-mediated mTORC1 activation in ccRCC is also dependent on the function of super enhancer regulators, namely BRD4 and P300, remains elusive. Hence, to address this, we chemically perturbed BRD4 and P300 activities in a panel of ccRCC in-vitro models using inhibitor JQ1 and A485, respectively. This was followed by assessing (i) H3K27ac level, marker for active enhancer, via ChIP-qPCR, (ii) *KLF6* and *PDGFB* expression level via qPCR and (iii) mTORC1 activity via Western blot. We found that BRD4 and P300 inhibitions reduced H3K27ac signals at several *KLF6* locus constituent enhancers, in line with the critical roles of these factors in maintaining the super enhancer landscape. Moreover, the inhibition of BRD4 and p300 significantly reduced the expression of *KLF6* and *PDGFB* in ccRCC cells, which consequently impaired the mTORC1 activity in the JQ1 and A485-treated cells. Our findings unravel the functional link between super enhancer upstream regulators, BRD4 and P300, and mTORC1 activation in ccRCC, which is via the transcriptional regulation of KLF6-PDGFB axis. Collectively, this study provides another important jigsaw for

the construction of transcriptional dependency landscape in ccRCC, contributing to the holistic understanding on ccRCC pathogenesis for the development of novel and efficient clinical strategies.

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Predictive Modelling of Cardiovascular Risk in Type 2 Diabetes Mellitus Patients: A K Nearest Neighbours Approach

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ABSTRACT

Background: Type 2 diabetes mellitus patients have a relatively higher risk of developing cardiovascular disease compared to non-diabetic individuals due to other microvascular complications. A prediction model is necessary to reduce the risk of cardiovascular disease development and the economic burden due to the treatment costs. **Methods:** This study has developed a cardiovascular disease risk prediction model with 220 participants recruited from Endocrine and Cardiology Clinic in Hospital Canselor Tuanku Muhriz (HCTM). After ranking the 15 features using ANOVA, several classifiers were trained with 80:20 data split and 10-fold cross validation using the organized data. **Results:** A K Nearest Neighbours model with 88.6% accuracy, 88.1% F1 score, and AUC of 0.9383 was selected. A graphical user interface was also developed to provide a user-friendly platform to

run the prediction model. The prediction model developed in this study has a high accuracy and precision even though it is trained with a relatively small data size. **Conclusion:** Prediction models using machine learning should be introduced to the medical settings to assist in the development of cardiovascular complications among diabetes patients.

O8

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Alteration of Gut Microbiota in Systemic Lupus Erythematosus (SLE)

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ABSTRACT

Alteration of gut microbiota could potentially be the initial step inducing dysregulation of the immune system in the gut-kidney axis that led to systemic inflammation. It is increasingly recognized in many diseases but studies in systemic lupus erythematosus (SLE) are still limited. This research was to determine the gut microbiota profile in SLE and its association with disease activity. A prospective study of 15 active renal SLE (aSLE) with age and sex matched control consisted of 15 renal SLE in remission (rSLE) and 15 non-renal SLE (nSLE) from PPUKM. Demographic data, stool samples, routine bloods and urine tests were collected and analysed. Stool DNA was extracted and 16S rRNA metagenomic sequencing was performed on an Illumina MiSeq Platform. The aSLE group had significant high SLEDAI-2K ($p<0.001$), low serum albumin ($p<0.001$) and total protein ($p<0.001$), lower serum haemoglobin ($p=0.031$) and high urea ($p=0.008$). No significant difference was observed in the richness and diversity (α-diversity), and the compositional difference (β-diversity) of bacterial species among the groups. However, the α-diversity metrics Simpson was significantly higher in rSLE than aSLE ($p=0.036$) which represent lower evenness of gut microbiome. The Firmicutes/Bacteroidetes ratio showed a decreasing trend between groups with aSLE (27.37 ± 80.31), rSLE(7.535 ± 12.23) and nSLE (5.314 ± 6.028), despite not significant. The most dominant phyla observed in aSLE was the proteobacteria, whereas actinobacteriota in the nSLE. Interestingly, LEfSe analysis revealed that bacteria genus *Veillonella* was enriched in the aSLE as compared to rSLE and nSLE, whilst *Akkermansia* was over-represented in the rSLE as compared to the nSLE. The genus *Veillonella* was positively correlated with disease activity

(r=0.195, p=0.2) whereas *Ruminococcus torques* were negatively correlated with disease activity (r=-0.166, p=0.276). The genus *Veillonella*(AUC=0.721, 95%CI 0.568-0.874) and R*uminococcus torques*(AUC=0.694, 95%CI 0.536-0.853) were identified as a potential predictor for disease activity via ROC analysis.

O9

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Gene Expression Profiling of the Extracellular Vesicles from the Insulin-Resistant Adipocytes

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ABSTRACT

Background: Metabolic dysfunction-associated fatty liver disease (MAFLD) is the most common liver disease, often associated with obesity, insulin resistance/ diabetes, or metabolic syndrome. Extracellular vesicles (EVs) are nano-sized particles secreted from the cells, with their cargos containing nucleic acids and proteins to mediate the communication between adipocytes and hepatocytes during MAFLD disease development. This study aimed to characterise the EVs isolated from insulin-resistant adipocytes and their cargos, focusing on gene expression profiles. **Methods:** Insulin-resistant (IR) and insulin-sensitive (IS) mature adipocytes were established *in vitro*. Their EVs were characterised via surface marker flow cytometry and morphology analysis using the transmission electron microscope (TEM) and dynamic light scattering (DLS). Total RNAs were isolated from the EVs and analyzed for differential gene expression using the human gene expression microarray (Agilent SurePrint v3). Significant genes were filtered based on the adjusted p-value (p.adj)<0.05 and -2<fold change>2. Biological pathway and gene network analyses were performed using the g:profiler, STRING, and miRNet database. **Results:** EVs from IR and IS-adipocytes have similar surface markers (CD63, CD81, C9, and ALIX). Morphologically, both EVs were cup-shaped, though the EVs from IS-adipocytes were bigger in diameter (363.1 nm) than IR adipocytes (102.9 nm). The gene expression analysis identified that one gene was up-regulated, and 200 genes were down-regulated in EVs from IR-adipocytes compared to IS-adipocytes. Seventy-three genes were associated with EVs (p.adj=$1.1e^{-23}$), and 75

genes were associated with adipocyte cell types (p.adj=1.48e^{-2}). The top three biological pathways enriched are oxidative phosphorylation (p.adj=3.88e^{-8}), reactive oxygen species (ROS) (p.adj=6.09e^{-6}), and MAFLD (p.adj=8.76e^{-4}). Network analysis identified one critical molecular network comprising the miR-146a-5p and its target genes (*PTPN11*, *SLC25A6*, and *NDUFB2*) involved in ROS regulation. **Conclusion:** Identifying the molecules inside the EVs from the adipocytes may provide a better understanding of the molecular communication between the adipocytes and hepatocytes, possibly leading to MAFLD development.

Keywords: Adipocytes; cell-cell communication; extracellular vesicles; insulin resistance; MAFLD

Acknowledgement: This study is sponsored by the Fundamental Research Grant Scheme (FRGS) from the Ministry of Higher Education (FRGS/1/2019/SKK08/UKM/03/8).

O10

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Functional Impact of Selected DNA Repair Gene Polymorphisms: Insights from *In Silico* Analyses

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ABSTRACT

Background: DNA repair genes play a central role in maintaining genomic integrity. While single nucleotide polymorphisms (SNPs) in DNA repair genes are frequently associated with the risk of various diseases, the precise functional basis of these associations remains elusive. In this study, we investigated the functional implications of selected DNA repair gene polymorphisms using bioinformatics tools. **Methods:** Seven SNPs were selected for the *in silico* studies: *ERCC1* rs3212986, *ERCC2* rs1052555, *ERCC2* rs1799793, *XPC* rs77907221, *XRCC1* rs25487, *XRCC1* rs1799782, and *XRCC3* rs861539. A total of 32 different tools were used to predict the structure, domain, missense pathogenicity, splicing alteration, protein interactions, transcriptional and post-translational modification regulation, and the clinical effects of the SNPs. **Results:** *ERCC2* rs1799793, *XRCC1* rs25487, *XRCC1* rs1799782, and *XRCC3* rs861539 are missense SNPs, while *ERCC1* rs3212986, *ERCC2* rs1052555, and *XPC* rs77907221 are 3'-UTR, synonymous, and indel mutations, respectively. *XRCC1* rs1799782 is predicted to have the highest probability of damaging effects, while *ERCC2* rs1799793 is likely to be deleterious. Structural changes in *XRCC1* rs1799782 and *XRCC3* rs861539 may affect protein-protein interactions and downstream biological processes. *XRCC3* rs861539 results in a loss of a phosphorylation site, impacting transcriptional regulation. *ERCC1* rs3212986, *ERCC2* rs1052555, and *XPC* rs77907221 were predicted to have altered splicing effects, with *ERCC2* rs1799793 and *XRCC1* rs25487 showing the most significant effects. **Conclusion:** Bioinformatics predictions of the functional effects, interactions and altered splicing effects of SNPs provide early insights into the underlying mechanisms of these SNPs in diseases, providing a basis for future experimental validation and better personalised treatment approaches.

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Characterisation of Extracellular Vesicle Released from Hepatocytes with Excessive Lipid Accumulation

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ABSTRACT

Background: In metabolic dysfunction-associated fatty liver disease (MAFLD), fibrosis occurs as a result of uncontrolled wound-healing processes involving various interactions between several cell types, including hepatocytes, hepatic stellate cells (HSCs), Kupffer cells, as well as infiltrating immune cells. Studies suggested that extracellular vesicle (EV) released from hepatocytes following excessive lipid accumulation (lipotoxic) can contribute to cell interactions via their bioactive cargos during this event. One such is the miR-21, which has been reported to increase during fibrosis. This study aimed to characterise the EV from lipotoxic hepatocytes and its miR-21 expression. **Methods:** Human hepatocyte cell line (HepG2) treated with 0.5mM palmitate (PA) conjugated with 5% bovine serum albumin (BSA) (lipotoxic), and HepG2 cells treated with 5% BSA (CON) grown in serum-free media for 48 hours. Lipid accumulation was confirmed using the Oil Red O (ORO) staining and lipid marker expression. EVs were isolated and characterised using Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS), and flow cytometry to assess their morphology, size, and surface markers. Total RNA was isolated from the EVs, and expression of miR-21 was measured using the Real-Time quantitative PCR method. **Results:** Lipotoxic hepatocytes have higher lipid accumulations ($P<0.001$) with significant upregulation of Fatty acid synthase (FAS) gene expression ($P<0.05$) compared to CON. EVs from lipotoxic and CON hepatocytes have similar surface markers, such as CD63, CD81, and CD9. Morphologically, both EVs were cup-shaped with similar sizes. Within the EV from lipotoxic hepatocytes, there was an enrichment of miR-21 expression (59.4-fold, P-value <0.0001). **Conclusion:**

EV miR-21 enrichment in lipotoxic hepatocytes may suggest that miR-21 could mediate fibrosis when the surrounding liver cells, such as HSC and Kupffer cells, take in these EVs.

Keywords: Cell-cell communication; extracellular vesicle; hepatocytes; lipid accumulation; MAFLD; miR-21

Acknowledgement: This study is sponsored by the UKM University Grant (GUP-2021-067).

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Abstract for 9th Regional Conference on Molecular Medicine (RCMM)
in Conjunction with 5th National Conference for Cancer Research (NCCR)
14-15th October 2023, Bangi Resort Hotel, Selangor

Metabolic Signatures in Obesity: Discovery of Promising Independent Biomarkers

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ABSTRACT

Obesity is characterized by the excessive accumulation of body fat. This condition will lead to changes in metabolic profile which reflects the alteration of key metabolites and the biochemical pathways. Therefore, the discovery of signatory metabolites in obese individuals will help us to elucidate the mechanisms and pathogenesis of obesity. In this nested case-control study, a serum metabolomics profiling was performed of 60 samples from The Malaysian Cohort comprising of lean and obese participants, by utilizing BMI values of 18.5-22.9 kg/m^2 and 30 kg/m^2, respectively. A total of 85 significant metabolic features were identified between cases and controls. Due to weak correlations among metabolites detected, a metabolite model was built using a stepwise logistic regression and this model revealed 6 highly promising markers that are linked to obesity, and this showed performance of AUC = 0.950. Metabolites with elevated risk (OR > 1) were suggested to exhibit impairment in lipid metabolism. In order to assess the individual contribution of each metabolite to predicting obesity, we conducted multiple logistic regression analysis. All metabolites displayed enhanced capabilities in predicting obesity following the adjustment for confounding variables. 14-methylheptadecanoic acid (OR = 3.952; 95% CI = 1.396-11.184), 4'-apo-beta,psi-caroten-4'-al (OR = 1.180; 95% CI = 1.062-1.311) and 7,8-Dihydro-3b,6a-dihydroxy-alpha-ionol 9-[apiosyl-(1->6)-glucoside] (OR = 0.873; 95% CI = 0.765-0.995) exhibited independent

associations after being adjusted for age, gender, race, occupation, and smoking status. This metabolite prediction could provide a clearer understanding of the development of obesity and potentially facilitate personalized medical approaches.

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P1

Abstract for 9th Regional Conference on Molecular Medicine (RCMM)
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14-15th October 2023, Bangi Resort Hotel, Selangor

Expression of Receptor Activator of Nuclear Factor-kappa B (RANK) Protein in ER-Positive Pre-Therapeutic Breast Cancer Tissues

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ABSTRACT

Background: RANK-RANKL signalling is a potential therapeutic target for breast cancer, regulating breast tumour initiation by modulating the proliferative response to progesterone hormone and mammary stem cells extension. RANK expression correlates with poor survival and chemotherapy resistance in estrogen receptor (ER)-negative breast cancer. This study explores the expression pattern of RANK in ER positive-breast cancer surgical tissues in relation to other clinicopathological features. **Method:** This is a cross-sectional study conducted at Hospital University Science Malaysia (USM). The protein expression of RANK was assessed in (n=65) ER-positive breast cancer pre-therapeutic tissue samples using immunohistochemical (IHC) staining. The intensity and the percentage of stained tumour cells were used to determine the H-score. **Results:** 72.3% (47/65) of ER-positive breast cancer have higher expression of RANK protein (H-score >200). Higher expression of RANK was significantly associated with negative lymph node invasion status (22/25) P <0.045. However, the RANK protein's expression level did not exhibit significant associations with other clinicopathological parameters. **Conclusion:** Intriguingly, the findings suggests that RANK protein might play an inhibitory role in ER-positive breast cancer, in contrast to its observed implications in ER-negative breast cancer. However, further research is crucial to unravel their complex role.

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Abstract for 9th Regional Conference on Molecular Medicine (RCMM)
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14-15th October 2023, Bangi Resort Hotel, Selangor

The Incidence Rate and Characteristics of Positive Individuals for COVID-19 in Perak, Malaysia: July – December 2021

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ABSTRACT

Background: As of 12th August 2023, Malaysia had more than 5.1 million COVID-19 cases and Perak reported 243,083 confirmed cases with 2,168 deaths. Between 1st July 2021 and 31st December 2021, a total of 45,329 COVID-19 samples were screened and tested by real-time RT-PCR. This study aimed to determine the incidence rate of COVID-19 infection based on districts in Perak and explore the characteristics of patients who tested positive for COVID-19. **Methods:** This is a retrospective study. Secondary data of Perak COVID-19 cases were obtained from Sistem Informasi Makmal Kesihatan Awam (SIMKA), a national reporting system by the Disease Control Division, Ministry of Health Malaysia. All 11 districts in Perak were included in this study. All COVID-19 positive cases confirmed by Real-time PCR between 1st July 2021 to 31st December 2021 were included in this study. All data were run for descriptive analysis using IBM SPSS v28. **Results:** A total of 9,432 cases were confirmed positive for COVID-19 infection. The overall incidence rate in Perak was 377 cases per 100,000 population. The majority of them were among Malay ethnicity (74.3%) and female contributed 53.8% of total cases. High cases were seen among the elderly aged >60 years and middle age group 30-39 years with the percentage of 21.1% and 20.3%, respectively. The incidence rate of COVID-19 was highest in Kuala Kangsar (605 cases per 100,000 population), followed by Hulu Perak (548 cases per 100,000 population), Larut Matang & Selama (512 cases per 100,000 population), Hilir Perak (485 cases per 100,000) and Manjung (341 cases per 100,000). Among the reasons for the COVID-19 screening were those categorized as a probable case (25.3%, n=2238), close contact (24.8%,

n=2196), symptomatic screening (15.3%, n=1463), severe acute respiratory illness, SARI (9.1%, n=988), Pre-admission (4.4%, n=622) and others (21.2%, n=1,921). **Conclusion:** Data provide useful insights of COVID-19 incidence rate in Perak and their socio-demographic characteristic and an in-depth understanding of the infection trend. These data will be helpful in evaluating further infection rates and improve future protective measures for the Perak population.

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Abstract for 9th Regional Conference on Molecular Medicine (RCMM)
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Clinical Validation of a CircANAPC7 Basescope Probe for The Detection of FOLFOX Chemoresistant Colorectal Cancer via In Situ Hybridization

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ABSTRACT

Background: FOLFOX chemotherapy is one of the standard regimens for treating colorectal cancer (CRC). However, overcoming chemoresistance is a major hurdle in achieving favorable results. Hence, it is imperative to identify biomarkers that can predict the response to FOLFOX treatment, which can aid in better patient management. Circular RNAs (circRNAs) have emerged as promising biomarkers in cancer research due to their stability and dysregulation in various diseases. Our previous results have indicated that circANAPC7 is implicated in chemoresistance in CRC. Therefore, we aim to identify circANAPC7 using a Basescope probe method. **Methods:** In this study, we aimed to clinically validate a Basescope probe for detecting circANAPC7 expression in FOLFOX chemoresistant CRC via in situ hybridization. The patients were divided into two groups, responders and non-responders, based on their clinical response to FOLFOX treatment. This study analyzed tumor tissue samples from 26 CRC patients, which consisted of 14 non-responder and 12 responder patients who underwent FOLFOX treatment. Staining scores were assigned to quantify the expression levels of circANAPC7 in the samples. **Results:** We identified that non-responder samples exhibited higher total staining scores, indicating a higher circANAPC7 expression compared to responder samples. **Conclusion:** These findings validate the clinical utility of the CircANAPC7 Basescope probe as a potential biomarker in predicting FOLFOX treatment response in colorectal cancer patients, enabling personalized treatment decisions and improved management of chemoresistant cases.

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Abstract for 9th Regional Conference on Molecular Medicine (RCMM)
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14-15th October 2023, Bangi Resort Hotel, Selangor

Assessment of WGS *In Silico* HLA Typing Tools against Molecular HLA Typing Method

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ABSTRACT

The HLA genes, which are highly variable and located in the MHC region on chromosome 6, play a crucial role in how the immune system recognizes tumor antigens. This makes accurate HLA allele typing methods essential for studying immune response variations among cancer patients. Although PCR-based methods are currently considered the gold standard, large-scale datasets using these methods are seldom available. To circumvent the need for separate experiments to determine these genotypes, several in silico NGS-based HLA genotyping methods have been developed. However, the scientific community has yet to reach a consensus on which tool performs best. We performed whole genome sequencing and molecular HLA typing for HLA-A and HLA-B alleles, on 46 healthy individuals of Bidayuh ethnicity. We used novoHLA and T1K *in silico* HLA typing pipelines to perform HLA typing on WGS data and benchmarked against molecular HLA typing results. We aimed to evaluate the precision and accuracy of *in silico* HLA typing tools in identifying HLA-A and HLA-B. Our assessment showed that there is a high concordance between *in silico* HLA typing tools and molecular HLA typing. The results of *in silico* HLA typing pipelines performance assessment on Bidayuh WGS dataset will be presented.

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Microbiome, Race and Breast Cancer: Is there an Asian-specific gut microbiome that may affect breast cancer?

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ABSTRACT

Race has long been considered a contributive factor affecting breast cancer, and in recent years, microbiome diversity. The efforts to identify race-specific microbiomes have grown in recent years with the idea of personalized medicine, yet such studies claiming race-specific microbiomes in health and disease continue to be divided. Thus, we conducted a systematic review to address the question: "Is there an Asian-specific gut microbiome that may affect breast cancer?" We conducted a literature search on PubMed, Embase, Cochrane, and Web of Science. Publications included were limited to original research studying breast cancer and gut microbiomes on Asian populations. At first glance, literature seems to suggest that there is a racial difference in the microbiome of breast cancer patients. However, there is a great lack of Asian representation in international breast cancer and microbiome studies with Caucasian cohorts outnumbering Asian cohorts by up to fifty times. In-depth investigation of these studies revealed gross overgeneralization of racial groups. Heterogenous sub-groups of Asian, Latin American, and mixed-race participants were categorized into inappropriate racial groups. Findings from such multi-racial studies in breast cancer were repeated widely across literature without scrutiny and eventually accepted as dogma. The impact of lifestyle factors, such as socioeconomic status and unique behaviors caused by diet and cultural differences, on microbiome and breast cancer profiles were repeatedly acknowledged but remain unaccounted for. Moving forward in studying the effect of a race-specific microbiome in breast cancer, our findings highlight that Asians should not be studied as a homogenous group as it may result in inaccurate conclusions affecting real-world applications such as precision medicine. We hope that an improved understanding of Asian cohorts may improve on the granularity of the effect of race on microbiomes and breast cancer.

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Navigating Cardiovascular Risk in Type 2 Diabetes: Potential Roles of Lipid Peroxidation and Cardiometabolic Markers

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ABSTRACT

Type 2 diabetes poses an elevated risk of cardiovascular disease, contributing to higher morbidity and mortality rates. Identifying individuals at stake in the early stages of CVD is crucial for effective intervention. In this regard, lipid peroxidation and cardiometabolic markers offer insights into the intricate interplay between diabetes and cardiovascular risk, aiding in early detection and risk assessment. This study aims to synthesize evidence on lipid peroxidation and cardiometabolic markers associated with the early onset of cardiovascular disease, shedding light on their potential clinical utility. A systematic search was conducted in Pubmed, Web of Science and Scopus to identify relevant studies published between January 2017 and August 2022. We included studies investigating lipid peroxidation and cardiometabolic markers implicated in the early progression of cardiovascular disease among type 2 diabetes patients. This systematic review has been registered with PROSPERO: CRD42022356566. The search yielded 76 studies,

of which seven that met the criteria were finally included. Through meticulous analysis, malondialdehyde was identified as the promising candidate for lipid peroxidation markers in type 2 diabetes with cardiovascular risk. Other relevant markers investigated include fluorescent damage products of lipid peroxidation, lipid peroxides, thiobarbituric acid reactive substances, oxidized-LDL, arylesterase PON1, and ferric-reducing ability of plasma. The selected studies were of good quality, presenting a median score of 18 (13-25) based on the Downs and Black risk assessment checklist. This systematic review highlights the role of lipid peroxidation and cardiometabolic markers in unveiling the early cardiovascular risk among type 2 diabetes individuals. These biomarkers hold the potential for early detection and risk stratification of cardiovascular disease as well as improving the management of cardiovascular complications in the vulnerable population. Further research is essential to validate and establish the clinical utility of these biomarkers within diverse type 2 diabetes populations.

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The Effect of ZFAS1 Silencing on Cell Viability in Glioblastoma Cell Lines LN18 (PTEN Wildtype) and U87MG (PTEN Mutant)

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ABSTRACT

Recent research has revealed the multifaceted role of ZNFX1 antisense RNA 1 (ZFAS1) in cancer, where it can function as either a tumor suppressor or an oncogene, exerting a critical influence on various cellular processes. Intriguingly, ZFAS1 exhibits upregulated expression in numerous cancers, such as colorectal and ovarian cancers, but is downregulated in breast cancer. Glioblastoma (GBM) demonstrates elevated ZFAS1 expression relative to normal brain cells. Nevertheless, emerging evidence suggests that the expression of ZFAS1 may vary based on cancer cell types and traits. This study aims to elucidate the impact of ZFAS1 silencing on GBM cancer cells, considering their PTEN (phosphatase and tensin homolog) status, focusing on cell viability and clonogenicity. Real-time quantitative PCR (RT-qPCR) was utilised to quantify ZFAS1 expression in Normal Human Astrocytes (NHA) and GBM cell lines (LN18 and U87MG) before siRNA transfection. si-ZFAS1 transfection efficiency was validated by RT-qPCR and siGLO fluorescence imaging. Cell viability was assessed using the MTT assay, and clonogenicity was evaluated. Despite the evidence on ZFAS1 being overexpressed in GBM relative to normal brain cells, our findings indicate that NHA cells exhibit higher ZFAS1 expression compared to LN18 and U87MG. Notably, LN18, a PTEN wild-type cell line, demonstrated significantly higher ZFAS1 expression than U87MG, a PTEN-mutated cell line. LN18 si-ZFAS1

silencing efficiency was 90.22%, and U87MG was 62.77%. Cell viability assays at 24-, 48-, and 72-hours post-siRNA transfection showed reduced LN18 cell viability (75.74%, 78.39%, and 59.68%) and moderate effects on U87MG (103.83%, 87.48%, and 92.96%) compared to non-targeting controls. In conclusion, ZFAS1 silencing significantly reduces cell viability, particularly in LN18 (PTEN wild-type), suggesting ZFAS1's oncogenic role in GBM development, with its impact influenced by GBM subtype or characteristics. Further investigation on the mechanism of action of ZFAS1 in GBM cells is warranted.

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Hyperglycemic Induced Pluripotent Stem Cell (iPSC) Reprogramming: Unveiling the Bittersweet Epigenetic Memories in iPSCs and their Differentiated Endothelial Cells

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ABSTRACT

Background: Induced pluripotent stem cell (iPSC) offers great promise in regenerative medicine due to its pluripotency and indefinite self-renewal capabilities. However, most if not all iPSC lines were reprogrammed and maintained under hyperglycemic conditions, which raises safety concerns. Despite hyperglycemia is known to be detrimental to adult cells, its impact on iPSC has not been well-studied. Hence, this study aimed to elucidate the impact of hyperglycemic iPSC reprogramming on the DNA methylation profile and functions of the resulting iPSCs and their differentiated endothelial cells (EC). **Methods:** Human umbilical vein endothelial cells were first isolated and expanded from a healthy donor in normoglycemic (LG-i-EC) or hyperglycemic (HG-i-EC) conditions. LG-i-EC and HG-i-EC were then reprogrammed into iPSC under normoglycemic (LG-iPSC) and hyperglycemic (HG-iPSC) conditions, respectively. Following successful reprogramming, HG-iPSC was expanded under normoglycemic condition (HGLG-iPSC) to elucidate the epigenetic memory retained after cessation of hyperglycemic exposure. iPSCs (LG-iPSC, HG-iPSC, and HGLG-iPSC) were then differentiated into endothelial cells (LG-iPSC-EC, HG-iPSC-EC, HGLG-iPSC-EC) in their respective conditions. All iPSCs and their differentiated ECs were characterized by flow cytometry, immunocytochemistry staining of cell-specific markers and were subjected to global DNA methylation microarray analysis and functional assessment. **Results:** All iPSCs expressed pluripotent markers SSEA-4, TRA-1-60, TRA-1-81 and OCT4, and proliferate at similar rates despite differences in glucose levels. However, differential

methylation analysis revealed 2400 CpG sites differentially methylated in HGLG-iPSC compared to LG-iPSC, with over 70% of the 2400 epigenetic memories inherited by their differentiated ECs. Molecular pathway analysis implicated alterations in the transendothelial leukocyte migration pathway in HGLG-iPSC-EC. Functional assays demonstrated increased transendothelial monocyte migration activity in HGLG-iPSC-EC compared to LG-iPSC-EC, indicative of endothelial dysfunction. **Conclusion:** In conclusion, iPSC reprogramming in normoglycemic, not hyperglycemic, conditions is crucial, to avoid persistent epigenetic memories that lead to dysfunctional ECs, rendering them unsuitable for clinical applications in regenerative medicine.

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Abstract for 9th Regional Conference on Molecular Medicine (RCMM)
in Conjunction with 5th National Conference for Cancer Research (NCCR)
14-15th October 2023, Bangi Resort Hotel, Selangor

Hsa-miR-181a-5p, hsa-miR-182-5p and hsa-miR-26a-5p as New Biomarkers for Undetermined *BCR-ABL1* in Chronic Myeloid Leukemia Patients Treated with Tyrosine Kinase Inhibitors

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ABSTRACT

Background: Chronic Myeloid Leukemia (CML) patients are treated with tyrosine kinase inhibitors (TKIs) and showed a high success rate. However, molecular relapse occurred 50% after discontinuation, with 80% occurring within the first six months. The molecular response is monitored and guided by *BCR-ABL1* levels. The gold standard RT-qPCR measures gene expression (mRNA), and the new digital PCR has improved gene-level (DNA) sensitivity. Therefore, a complementary method is needed. In a previous study, we identified and validated three potential miRNAs (for translational repression / mRNA degradation) as therapeutic biomarkers for monitoring CML. Hsa-miR-181a-5p at below current limit of detection, hsa-miR-182-5p, and hsa-miR-26a-5p for the substantial presence of *BCR-ABL1* in CML adults treated with TKIs at the molecular response. Additionally, we have mRNA NGS data from our CML patients treated with TKIs. The study objective is to demonstrate the miRNA-mRNA interactions and thus verify the three validated miRNAs as biomarkers for monitoring CML treated with TKIs. **Methods:** mRNA NGS data was analysed using bioinformatics analysis. Targets of the three validated miRNAs were

predicted using molecular prediction tools and databases. **Results:** Differential expression gene analysis between cases and controls showed that inframe insertion was observed only in non-responsive patients. Interestingly, insertions included three similar genes that were *PPIP5K2, POLE3, and NADK*. Duplication was observed with Lysine on *PPIP5K2* and *POLE,* while Glutamic acid on *NADK*. These genes were listed on the Proteomic and Genomic Data Commons (PDC/GDC), a repository for tumors and cancer. Moreover, prediction tools predicted these genes were among the targets of the validated miRNAs. Thus these verified the interactions between the three validated miRNAs and mRNA in CML patients treated with TKIs. **Conclusion:** These showed that the three miRNAs could be used as new biomarkers in monitoring CML to complement current detection methods. Further analysis of the interactions will continue.

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Abstract for 9th Regional Conference on Molecular Medicine (RCMM)
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14-15th October 2023, Bangi Resort Hotel, Selangor

Polygenic Risk Score Significantly Predicted Coronary Heart Disease Death among those with Type 2 Diabetes in The Malaysian Cohort

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ABSTRACT

Polygenic risk scores (PRS) have been widely researched to measure the risk of coronary heart disease (CHD), however, they are mainly optimised for the Caucasian population. There is a lack of PRS specifically targeting the Malaysian population. This study was aimed at developing a PRS to predict CHD death risk among type 2 diabetes (T2D) participants in The Malaysian Cohort project (TMC). In Phase I, T2D TMC participants (224 "T2DM+CHD" as cases, 625 "T2DM" as controls) were genotyped using genomic microarray followed by genetic imputations using GenomeAsia reference panel to identify single nucleotide polymorphisms (SNP) associated with CHD death and to construct the PRS. In Phase II, T2D participants without known CHD during recruitment (n=806) and followed-up until their deaths, were analysed based on their PRS risk categories. The outcome of interest was CHD deaths in both phases. From the 56 CHD-related SNPs with suggestive genome-wide significance identified in Phase I (P<5.00 10^{-5}), PRS was constructed using 15 SNPs genotyped from the microarray. Compared to "T2DM", the "T2DM+CHD" had higher PRS scores across ethnicities while the area under the receiver operating curve (AUC) showed the ability of PRS to classify CHD death. Using the cut-off point of 0.8493 (AUC=0.762, sensitivity=64.7%, specificity=73.1%), the odds of CHD death in the high-risk category were 6.96, 7.90, and 22.58 for Malay, Chinese, and Indian, respectively. In Phase II, survival analysis showed that mean survival years were shorter in high-risk than the low-risk category among Malays

(6.60 vs 8.92 years), Chinese (6.20 vs 8.66 years), and Indians (7.21 vs 8.60 years). Meanwhile, among high-risk participants, the CHD death risk was 3.32, 3.36, and 12.72 times higher among Malays, Chinese, and Indians, respectively. The potential use of PRS to predict CHD death among T2D patients could assist in the long-term management and risk identification, subsequently enhancing the precision medicine practice in Malaysia.

Keywords: CHD death; coronary heart disease; polygenic risk score; single nucleotide polymorphism; The Malaysian Cohort Project; type 2 diabetes mellitus

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14-15th October 2023, Bangi Resort Hotel, Selangor

Revealing Uncharted Depths of the Dark Proteome: A Re-examination of Mass Spectrometry Data Uncovers New Insights into Colorectal Cancer Microproteins

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ABSTRACT

Background: Cancer proteogenomic projects like the Clinical Proteomics Tumor Analysis Consortium (CPTAC) concentrate on identifying and quantifying those ~20,000 proteins sourced from annotated open reading frames (ORFs). However, these initiatives inadvertently overlook non-canonical proteins emerging from unconventional ORFs. Nonetheless, increasing experimental evidence has pointed to previously inactive regions that now yield steady, functional proteins—spanning alternate proteins (with over 30 amino acids) and their subset, microproteins (below or equal to 100 amino acids). **Methods**: We downloaded a dataset of TMT-10plex MS-based proteomics data CPTAC acquired from colorectal cancer (CRC) tissues (PDC000116). We then re-analyzed this dataset using FragPipe harboring MSFragger, Philosopher, IonQuant and TMT-Integrator. MS data were searched against an in-house protein FASTA comprising human SwissProt, OpenProt and *in silico* translated, verified RIBO-Seq sequences. FragPipe Analyst was used for subsequent statistical analysis. **Results**: In PDC000116, we identified 10,634 proteins from all 100 samples, whereby 9482 were canonical proteins and 501 were alternate proteins. Out these 501 non-canonical proteins, 205 were 100 amino acids. Divided by the stages of CRCs, we found 10, 13, 10 and 18 upregulated microproteins for Stage 1, 2, 3 and 4 CRC respectively, whereas 34, 31, 27, 21 downregulated ones. Particularly, IP_613259, IP_650627 and IP_573056 were found to be significantly upregulated in

all stages of cancer compared to normal. Conversely, IP_671454, IP_690979 and IP_624921 was found to be significantly downregulated in all cancer stages. Also, members of the S100 protein family were found in different stages of CRC, such as S100P, S100A8, S100A12, S100B and S100A1. **Conclusion**: The analysis revealed differentially expressed novel microproteins and alternate proteins, across colon cancer stages, highlighting their potential significance in cancer development.

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Demethylbelamcandaquinone B from *Marantodes pumilum* var. alata (Blume) Kuntze Inhibits Osteoclast Differentiation in RAW264.7 cells

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ABSTRACT

Background: A delicate balance between bone-forming osteoblast and bone-resorbing osteoclast activities keeps bone remodelling stable. Excessive bone resorption by osteoclasts, on the other hand, causes bone loss illnesses such osteoporosis, periodontitis, rheumatoid arthritis, and bone cancer. Positive tartrate-resistant acid phosphatase (TRACP) staining distinguishes osteoclast bone-resorbing cells, which are produced from hematopoietic progenitor cells of the monocyte/macrophage lineage. The purpose of this study was to determine *M. pumilum var. alata* crude aqueous extract and its active components reduced osteoclast activity and cytokine production in osteoclast precursor cells triggered by RANKL via the oestrogen receptor (ER) signalling pathway. **Method:** RANKL was used to develop RAW264.7 macrophages into osteoclast-like cells. They were then given 10 g/mL crude aqueous extract of *Marantodes pumilum var. alata*, 5 g/mL dichloromethane fraction, 0.6 g/mL Dmcq B, and 0.06 g/mL estradiol. This study determined TRACP staining, TRACP 5b colometric assays, and bone-resorbing pits. Pro-inflammatory cytokine gene and protein expression (TNF-α and IL-6) as well as ERα and ERβ

protein expression were assessed. **Results:** When compared to a normal control, *Marantodes pumilum var. alata* crude aqueous extract and Dmcq B suppressed RANKL-stimulated osteoclast differentiation as demonstrated by size reduction of large multinucleated osteoclast cells, decreased TRACP 5b activity, and subsidence of resorbed pit area. Furthermore, they lowered TNF-α and IL-6 gene and protein expression. Treatments with *Marantodes pumilum var. alata,* Dmcq B, and estradiol enhanced ER and ER protein expression in osteoclasts. **Conclusion:** *Marantodes pumilum var. alata* and its active compound, Dmcq B suppressed osteoclast differentiation.

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14-15th October 2023, Bangi Resort Hotel, Selangor

GPC3 Alterations and Interactions in Lung Adenocarcinomas: A Comprehensive Data Mining Study of Publicly Available Datasets

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ABSTRACT

Background: Glypican-3, encoded by the *GPC3* gene, is a membrane-bound heparan sulfate proteoglycan commonly implicated in human lung adenocarcinomas (LUADs). Nevertheless, the function, mutational profile, epigenetic regulation, co-expression profile, and clinicopathological significance of the *GPC3* gene in LUAD progression are not well understood. In this study, we aimed to elucidate the above parameters by mining cancer microarray datasets from publicly available databases. **Methods:** Oncomine, GEPIA2, and UALCAN databases were used for analysis of the *GPC3* expression and methylation. The correlation between *GPC3* expression and patient survival was then evaluated using PrognoScan. In addition, the correlation between *GPC3* expression and immunological infiltration was investigated using TIMER. Mutational and CNA profiles of the *GPC3* gene were analyzed using the cBioPortal platform. miRNAs and TFs interacting with *GPC3* were identified using NetworkAnalyst v3.0. **Results:** We observed significant downregulation of *GPC3* in LUAD tissues compared to their normal counterparts, and this downregulation was associated with shorter overall survival (OS) and relapse-free survival (RFS). Nevertheless, no significant differences in the methylation pattern of *GPC3* were observed between LUAD and normal tissues, although lower promoter methylation was observed in male patients. *GPC3* expression was also found to correlate significantly with infiltration of B cells, CD8+, CD4+, macrophages, neutrophils, and dendritic cells in LUAD. In addition, a total of 11 missense mutations were identified in LUAD patients, and ~1.4-2.2% of LUAD patients had copy number amplifications in *GPC3*. Seventeen genes, mainly involved in dopamine receptor-mediated signaling pathways, were frequently co-expressed with *GPC3*. We also found 11 TFs and 7 miRNAs interacting with *GPC3* and contributing to disease progression. Finally, we identified three potential inhibitors of *GPC3* in human

LUAD, namely heparitin, gemcitabine and arbutin. **Conclusions:** *GPC3* may play an important role in the development of LUAD and could serve as a promising biomarker in LUAD.

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Unraveling AGR2 Regulation in Breast Cancer: Insights from RNA-Seq Analysis of CRISPR/Cas9 Mediated Targeting

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ABSTRACT

Anterior Gradient Protein-2 (AGR2), a member of the protein disulphide isomerase family, plays a pivotal role within the endoplasmic reticulum (ER) by managing the folding and maturation of client proteins as they navigate the secretory pathway. AGR2 has gained prominence as a key contributor to tumorigenesis and metastasis, with its upregulation observed in various cancer types, further fueling malignant progression. Interestingly, despite its primary residence in the ER, this intriguing protein possesses the ability to extend its influence beyond cellular confines and assume an oncogenic role in the extracellular environment. Nonetheless, the intricate mechanisms governing the functions of AGR2 and its secretion have remained enigmatic. Here, we unveiled the dual presence of AGR2 protein expression, both within breast cancer cells' intracellular compartments and in the extracellular space. Using the metastatic breast cancer cell line 1833-BoM as our experimental model, we have demonstrated that AGR2 secretion lacks glycosylation and partly involves unconventional protein secretion, and this was evidently pronounced under conditions of tunicamycin-induced ER stress. By employing lentivirus-delivered CRISPR-Cas9 technology to target AGR2, we have witnessed a reduction in cancer cell fitness, leading to impaired proliferation, adhesive capacity, invasion capabilities. Through comprehensive RNA-seq analysis, we have identified 603 significant differentially expressed genes in two isogenic AGR2-targeted cells. Functional enrichment analysis has shed light on the involvement of these genes in critical cellular processes, such as receptor signaling, oxidative stress response, cell

adhesion, and vesicle trafficking. Further exploration via protein-protein interaction network analysis has revealed hub genes, including ESR1, AR, LCK, CDH1, and CD55, which have the potential to modulate AGR2 intracellular signaling and secretion. Our findings provide substantial evidence supporting the role of AGR2 in driving breast cancer progression, thereby highlighting its potential as a therapeutic target for interventions aimed at disrupting AGR2-associated pathways in breast cancer.

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14-15th October 2023, Bangi Resort Hotel, Selangor

Integrative Pipeline to Analyze Immune Cells in Digital Pathology Images

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ABSTRACT

Conventional digital pathology image analysis involves basic tasks such as cell detection, classification and quantification. While these may seem straightforward, their application to whole slide images is not simple, requiring intricate AI-driven image processing techniques that can be difficult for researchers to access. This study presents an integrative semi-automated pipeline to quantify immune cells on digital pathology images and derive predictive spatial features. Immunohistochemical (IHC) staining for CD3, CD4 and CD8 immune markers was performed on samples of breast cancer patients. After digitization, 43 images with a large tumour area were selected for further analysis. Immune cell detections were carried out on two different bioimaging software, QuPath and Fiji. Immune cells were detected, categorized and quantified in two areas (invasive front and tumour center) within tumour region using QuPath. Cell detection based on color threshold and region of interest (ROI) was conducted using Fiji. The ROI were divided based on their predicted area sizes into two levels – individual cells and groups of cells. The filtered ROI were used as inputs for CytoMAP to calculate distances between immune markers and local density of immune markers at both levels. Combining both results, a total of 48 spatial features derived from IHC immune markers were used to train a machine learning model to identify spatial features that were predictive of gene expression-based immune scores. Based on the machine learning prediction, we determined that the best predictive spatial features were the number of CD4 hotspots in tumor center and the distance between CD8 and other markers. Our results also suggest that immune features within the tumour center are more predictive than within the invasive front. These results provide insights into the use of off-the-shelf imaging software tools to analyze digital pathology images to make useful predictions in lieu of more complicated image processing techniques.

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14-15th October 2023, Bangi Resort Hotel, Selangor

KCNN4 Mutation Presenting with Non-Immune Hydrops Haemolytic Anaemia

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ABSTRACT

Background: Dehydrated hereditary stomacytosis (DHSt) is a rare autosomal dominant hemolytic anaemia disorder. The two genes reported in DHSt are PIEZO 1 gene and KCNN4 gene. In DHSt, the gene mutations cause dysfunctional membrane protein with the imbalance of potassium and sodium influx, resulting in the loss of water that leads to red blood cell dehydration and hemolysis. To date, there is no case reported in Asia. **Case description:** We report a case of an 8-year-old boy with DHTs who presented with hemolytic anaemia and non-immune hydrops at birth with strong family history of hemoglobinopathy with G6PD deficient Viangchan phenotypes. Antenatally, he was diagnosed as fetal hydrops with polyhydramnios at 28-week gestational age. He was noted to have anaemia at birth with a haemoglobin level of 7.3 g/dL and reticulocytes of 14% and required two times packed cell transfusions during neonatal period. His initial laboratory investigations for hemolytic anemia showed negative results for TORCHES and parvovirus screening but a mild increase in osmotic fragility test. He had a strong family history of anaemia involving his maternal side and siblings. His mother and maternal aunt had a history of cholecystectomy at 24 and 16 years old respectively. His mother and elder brother and sister had anaemia and confirmed to be heterozygous G6PD Viangchan. His parents were non-consanguineous. He had hepatosplenomegaly with poor growth due to chronic hemolytic anemia and presented with intermittent episodes of crisis with headache and jaundice. His brain imaging revealed dural venous malformation in magnetic resonance imaging and partial venous obstruction in cerebral angiogram. A whole exome sequencing

showed positive KCNN4 gene mutation. **Conclusion:** DHSt is a rare hereditary disorder with no specific treatment. The mainstay treatment of DHSt is supportive with pack cell transfusion when indicated. Splenectomy should be avoided in DHSt as it increases the risk of thrombosis.

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14-15th October 2023, Bangi Resort Hotel, Selangor

Risk Factors of Long COVID in Klang Valley Malaysia: A Snapshot from the COVGEN study

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ABSTRACT

Background: Long COVID is a condition where prolonged symptoms, after Covid-19 infection, that continue for at least three months after the initial infection and persisting for at least two months without any other explanation, A significant number of patients with COVID-19 experience this condition, however few studies have investigated the characteristic of this population, particularly in outpatient settings. **Methods:** We recruited 1,561 participants from the baseline infection who had previously contracted COVID-19 from the Klang Valley area, specifically at Hospital Canselor Tuanku Muhriz (HCTM), UKM Medical Molecular Biology Institute (UMBI), and Cheras district's COVID-19 Assessment Centre (CAC), from August 11, 2021, to September 30, 2022. We followed them up at 3, 6, and 12 months. Only participants who had at least one follow-up at any of these time points were included in this study. Among them, only 872 met the eligibility criteria for the final analysis. Self-reported questionnaires and blood samples were

collected from participants both at baseline and during the follow-up sessions after obtaining their consent. **Results:** Out of 872 participants, 227 (26.0%) were still experienced prolonged symptoms. Most of the participants were aged 30-50 (59.5%), female (59.1%), and Malay (88.1%). More than 90% of the Long COVID participants completed two doses of vaccination and most of them received BNT162b2 vaccine. Having respiratory disease, high diastolic blood pressure, high haemoglobin A1c, and presenting with more than five COVID-19 symptoms during infection were significantly associated with long COVID. The most common symptoms that remained persistent were neurological and cognitive function, such as brain fog, memory problems, difficulty concentrating, decreased alertness, sleep issues, confusion, and speech difficulties regardless of whether it was 3, 6, or 12 months after their initial COVID-19 infection. While the most newly developed diseases due to COVID infection diagnosed by medical doctors were hypertension, cardiovascular disease, and diabetes. A phenomenon of diabetes regression was also observed during the 12 months follow-up among the Long COVID patient. **Conclusion:** Understanding the characteristics and effects of Long COVID will help facilitate the healthcare needs for the patients including monitoring and treatment.

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Development of Breast Cancer Spheroids as a Model for a Pre-clinical Evaluation using Vitamin C as Anti-Cancer Agent

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ABSTRACT

Background: Breast cancer is a highly prevalent type of cancer that affects women globally, resulting in a significant number of fatalities. The treatment of breast cancer continues to pose significant challenges. Therefore, the development of effective therapeutic models is of utmost importance in order to improve treatment strategies and provide significant benefits to patients. **Methods:** MCF-7 and MDA-MB-231 were cultured as monolayers until they reached 70–80% confluency. A 96-well plate coated with agarose was utilized to generate a multi-cellular breast cell spheroid. The morphology of spheroids formation was examined and observed daily until day 3 under an inverted microscope and images were analyzed using ImagJ software (USA). The stock solution of vitamin C was diluted. The time and dose of Vitamin C were optimized. The CellTiter-Glo® 3D Cell Viability Assay (Promega, USA) and Annexin V-FITC/PI Apoptosis Detection Kit (BD, Franklin Lakes, NJ, USA) were employed to assess the cytotoxicity effect on the spheroids. **Results:** The results showed the formation of spheroids in MCF-7 and MDA-MB-231. Both exhibited unique morphological features that correspond to the behavior of these different types of breast cancer. Both apoptosis and cytotoxicity activities were higher in MCF-7 compared to MDA-MB-231 with vitamin C treatment.

Conclusion: The spheroids models were developed and characterized. High-dose Vitamin C showed the ability to induce cytotoxicity. This highlights the usefulness of the breast cancer spheroid as a preclinical model for drug screening platforms.

Keywords: Apoptosis; breast cancer; development; spheroid; Vitamin C

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14-15th October 2023, Bangi Resort Hotel, Selangor

Advancements in Amber Suppression Technology: A Window into Molecular Interactions of Human Viruses

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ABSTRACT

Amber suppression technology, inspired by genetic code expansion (GCE), is a molecular tool that facilitates the precise incorporation of noncanonical amino acids into a site-specific target protein within cellular environments. The technique requires several components: a target gene, photoactivatable unnatural amino acids, and an amber suppressor tRNA ($tRNA_{CUA}$)/aminoacyl tRNA synthetase (aaRS) orthogonal pair. A primary challenge associated with this technology is that it involves the delicate task of optimizing a combination of key components to maximize successful gene code expansion. Here, we demonstrate that amber suppression technology unveils crucial residues within a Herpes Simplex Virus 1 (HSV-1) viral protein that photocrosslink with a host protein complex. This interaction implies that HSV-1 viral protein may be involved in inhibiting the transcription elongation of cellular genes, potentially serving as a pivotal factor in HSV-1 pathogenesis. Building upon the successful optimization of this molecular tool, we are currently extending its application to various other human viruses, including SARS-CoV-2, H5N1, and HIV-1. In conclusion, our research underscores recent advancements that emphasize the promising potential of amber suppression technology in a wide range of medical biotechnological applications.

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14-15th October 2023, Bangi Resort Hotel, Selangor

Metabolomic Fingerprinting and Footprinting analyses on TNBC sh-TINCR cell lines

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ABSTRACT

47.9% breast cancer cases in Malaysia were detected at late stage which 29% of the cases represented by the triple negative breast cancer (TNBC) subtype. The affected pathway of the long noncoding RNA (TINCR), which promotes proliferation and metastasis of cancer cells is poorly understood. In this study the TINCR-knocked-out TNBC cell lines were subjected to metabolomic analysis with the aim to identify key metabolites and discover metabolic pathways responsible for the poor prognosis of this cancer subtype. MDA-MB-231 (n=3) and HS578T (n=3) cells were knocked down via lentiviral delivery of shRNA vector. Cell lysates and culture medium were harvested and subjected to NMR 600 MHz analysis. NMR spectra were calibrated, baselined, bucketed and spectra bin file was exported to Metaboanalyst 5.0 for statistical analysis. Spermine and Nicotinic acid metabolites in MDA-MB-231 cell while Cystathionine and Pyridoxal metabolites in HS578T cell were found to be perturbed by the knocked-out process. This suggests that biosynthesis of cofactors metabolic pathway (KEGG:hsa01240) took place in the TNBC cell lines progression. Downstream analysis was done using protein-protein and gene-gene interactions database indicated that 2 proteins/genes to be the main factor in the process which are PNP and BHMT gene. Further analysis on these 2 genes could have a potential to shed some light to the discovery of prognostic biomarkers and effective target for therapeutic intervention for TNBC cancer patients.

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14-15th October 2023, Bangi Resort Hotel, Selangor

Evaluation and Optimisation of Adipogenic Differentiation Protocol for the Human Adipocyte Tissue-derived Stem Cells.

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ABSTRACT

Background: *The in vitro* adipocyte cell line model is often used to provide insight into fundamental aspects of adipocyte physiology. Although most studies used murine adipose cells, human adipose tissue-derived stem cells (ASC) isolated from various adipose depots have noteworthy translational significance and relevance for adipocyte mechanistic studies. However, the differentiation method using the ASC cell line is inconsistent across different studies. This study aimed to evaluate and determine the optimum media composition for mature adipocyte differentiation from the ASC cell line. **Methods:** Undifferentiated hTERT-immortalized human adipose-derived stem cell line (ASC52Telo, ATCC) was grown in four different adipogenic differentiation media for 18-21 days. The media composition was (i) M1: ATCC premade differentiation media; (ii) M2: Published basic cocktail media without vitamins and hormones; (iii) M3: Published complete cocktail media without fetal bovine serum (FBS); (iv) M4: Modified M3 media supplemented with FBS and glutamax; and (v) stem cell media (undifferentiated, CON). Adipocyte differentiation was confirmed by the formation of lipid vesicles stained by Oil Red O. Expressions of adipocyte markers were measured via the Real-time quantitative PCR method. **Results:** M4 media have the highest differentiated mature adipocytes (92.7%) compared to CON (0%), M1 (45.0%), M2 (10.3%), and M3 (38.6%) (P-value <0.0001). Expressions of three adipocyte markers (*FABP4*, *SREBP1C*, and *PPARG2*) were increased following M4 media treatment compared to the CON (P-value <0.001). For M1 media treatment, only *FABP4* marker expression was higher (P-value <0.05). The stem cell marker *THY1* expression was reduced only following M4 media treatment compared to the CON (P<0.0001). These marker expressions

did not change after the M2 and M3 media treatment. **Conclusion:** This study provides the optimum media composition for mature adipocyte differentiation from the human ASC cell line. Establishing optimum *in vitro* adipogenic differentiation protocol is vital for the future fundamental research on obesity.

Keywords: Adipose tissue-derived stem cells (ASC); adipogenesis; differentiation; obesity; optimisation

Acknowledgment: This study is sponsored by the Fundamental Research Grant Scheme (FRGS) from the Ministry of Higher Education (FRGS/1/2019/SKK08/UKM/03/8).

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in Conjunction with 5th National Conference for Cancer Research (NCCR)
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SLC6A14 Expression and Methionine Uptake Increased in Advanced Colorectal Cancer Cells Stage

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ABSTRACT

Backgrounds: Data from our previous metabolomics study reported that l-methionine was significantly increased in the more advanced stages of colorectal cancer cells (CRC). Upregulated amino acids transporter in cancer cells may affect the uptake of methionine into cells. In this study, we aim is to determine the association between the SLC6A14 expression and methionine uptake in CRC at different stages. **Methods**: Normal colon cell lines (CCD 841 CoN) and CRC cell lines; HT 29 (early stage) and HCT 116 (late stage) were cultured to determine the expression of SLC6A14 by western blot analysis. SLC6A14 inhibits with alpha-methyltryptophan (α-MT), 4.5 mM for 72 hours was performed. The viability of cells was also determined by MTS assay, and apoptosis assay. The l-methionine level was measured with and without inhibition of α-MT by L-methionine fluorescence assay. The data was analysed using GraphPad Prism Version 6. **Results:** The results show SLC6A14 expression level in CRC cells at early and late stages were significantly upregulated compared to normal. The inhibition of SLC6A14 with α-MT (4.5 mM) leads to significant decreased in cell viability and associated with increased apoptosis activity and decreased intracellular level of methionine. **Conclusion**. This study conclude that increased level of methionine observed in the previous metabolomics study is due to increase expression of SLC6A14 in CRC cells that is involved in the uptake of methionine into cells for their growth and proliferation.

Keywords: Colorectal cancer; methionine; SLC16A4; stages

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14-15th October 2023, Bangi Resort Hotel, Selangor

Optimization of Annealing Temperature for Dengue Virus Serotyping Using Multiplex Polymerase Chain Reaction (PCR) Technique

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ABSTRACT

Malaysia is a dengue hyper-endemic country with all four serotypes of dengue virus (DENV) circulating simultaneously. All four serotypes of DENV have similar clinical manifestations, but the homology among serotypes is less than 80% at the amino acid level. Dengue serotyping using polymerase chain reactions (PCR) technique is widely used to determine the serotype of DENV due to fast turnover time. Multiplex PCR techniques were selected due to their feasibility in detecting all four serotypes of DENV in a single-tube reaction compared to the conventional PCR technique for DENV serotyping. In this study, we aimed to determine the optimum annealing temperature for multiplex PCR for all four primer sets to simultaneously detect all four serotypes of DENV. We used the universal forward primer (UFP DENV) and four different dengue virus-specific reverse primers (D1-TR1DENV1, D2-TR2DENV2, D3-TR3DENV3, D4-TR4DENV4), each with a final concentration of 200 µM. Viral RNA of DENV-1, DENV-2, DENV-3, and DENV-4 cultured in BHK cells were extracted using SpinStar Nucleic Acid Extraction Kit 1.0. Then, cDNA was synthesized using Super Script III First-Strand Synthesis Super Mix and PCR was performed using EasyTaq PCR reagent. Gradient PCR was performed to determine the optimum annealing temperature using two sets of temperatures: 55˚C to 60˚C

and 58°C to 60°C. The results show that the optimum annealing temperature for DENV-1, DENV-2 and DENV-3 was 59.2°C with an apparent band size of 482 bp for DENV-1, 119 bp for DENV-2 and 290 bp for DENV-3. However, we were not able to get any amplification for DENV-4. Thus, we aimed to design a new primer for DENV-4. In conclusion, the optimum annealing temperature to be used in the multiplex PCR was 59.2°C. Therefore, we decided to use this temperature for validation with clinical samples.

Keywords: Annealing temperature; dengue virus; multiplex PCR; optimization; serotyping

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14-15th October 2023, Bangi Resort Hotel, Selangor

Current Status of Predictive Serum Biomarkers for Polyps and Colorectal Cancer

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ABSTRACT

Early detection of colorectal cancer (CRC) is crucial to enhance the disease treatment and prognosis of patients. Colonoscopy remains a golden method for CRC detection which requires trained personnel with expensive tools. Other non-invasive methods available such as faecal occult blood test (FOBT), however, still have a problem with high false positive rates. Currently, serum metabolites biomarker has been discovered to be potentially used to discriminate patients with polyps and CRC. This systematic review focuses on identifying the most commonly detected predictive serum metabolites for polyps and colorectal cancer. A systematic search of the Web of Science, PubMed, and Cochrane Library databases was conducted using PRISMA guidelines. Nine studies investigating serum metabolite biomarkers of colorectal cancer and polyps using different analytical platforms and study populations were included. QUADOMICS tool was used to analyze the quality of included studies. All metabolites detected for polyps and CRC was enriched into pathways using MetaboAnalyst 5.0. Metabolites detected by three studies and more might be potentially used as a predictive biomarker for polyps and CRC. A review of these studies revealed several potential signature metabolites overlapped between studies including Tyrosine, Lysine, Cystine, Arabinose, and Lactate for CRC, and Lactate and Glutamate for polyps. The most affected pathways related to CRC were the urea cycle, glutathione metabolism, purine metabolism, glutamate metabolism, and ammonia recycling, while the urea cycle, glutamate metabolism, glutathione metabolism, arginine and proline metabolism, and carnitine synthesis were found to be affected in the polyps. However, the differences between altered pathways

in polyps and CRC, other external factors, and their effects on the regulation level, sensitivity, and specificity of each identified metabolite still remained unclear. Further research is needed to validate this finding and would benefit from large cohort studies, standardized analysis instruments, and well-defined subject groups.

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High-throughput Genotyping of Asian-specific Genetic Variants Associated with Colorectal Cancer Risk in the Malaysian Population: A Pilot Study

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ABSTRACT

Background: Identification of genetic variants associated with colorectal cancer (CRC) risk has shown promise in preventing the disease. Although it is well-established that the influence of genetic variants on CRC risk varies by populations and environmental factors, the association of genetic variants with CRC risk in the Malaysian population has not been studied using high-throughput genotyping method. In this work, we aimed to conduct a pilot genome-wide association studies (GWAS) on Malays using a genotyping panel developed specifically for the Asian population, with the aim to identify genetic variants associated with CRC risk. **Methods:** A case-control study was performed on 95 CRC patients and 95 healthy controls. Genotyping of all samples was performed with the Illumina Infinium Asian Screening Array using DNA obtained from peripheral blood of the study subjects. Genetic data were first subjected to preliminary analysis using Plink and R software to identify the association between individual polymorphisms and CRC risk. Logistic regression was performed using SPSS to determine the significance of the polymorphisms, along with epidemiological data, in predicting CRC risk. **Results:** Our preliminary analysis identified 10 suggestive significant polymorphisms (rs76997593, rs79946793, rs10483660, rs77350431, rs79883457, rs10870101, rs12434275, rs7815453, rs6471236, and rs5747442) were statistically significant for CRC risk prediction in the genetic models. When epidemiological data (age, BMI, and marital status) were incorporated into the prediction model, only rs76997593 and rs10870101 maintained their significance with regard to CRC risk. However, the statistical significance diminished when TNM staging and histological grading were included in the prediction model. **Conclusion:** The results obtained suggests that rs76997593 and rs10870101 could serve as potential markers for predicting the risk of CRC in Malays, although further studies with a larger sample size are required to clarify this point.

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Novel Ryr2 Receptor Gene Mutation Presenting with Cardiac Tumour and Refractory Arrythmias: Case Report and Systemic Review

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ABSTRACT

Background: Ryanodine Receptor 2 (RYR2) gene mutation causing cathecholaminergic polymorphic ventricular tachycardia (CPVT) is one of the identified causes of sudden death in adult and children. **Case description:** We report a case of novel Ryanodine Receptor 2 (RYR2) gene mutation presented with cardiac arrest and recurrent syncopal attack with accidental finding of cardiac tumour. **Methods:** Case was reported based on retrospective review of information via electronic medical record, digital laboratory system, and digital radiological, image and reports in Hospital Pakar Kanak-Kanak (HPKK), Universiti Kebangsaan Malaysia. For systemic review, three large databases; PubMed, Ovid and Scopus were used to find articles with keywords "RYR2 gene mutations" and "CPVT" up to December 2022. Selected articles based on title and abstract included are: (i) case reports and articles on children 18 years old and below with (ii) treatment response on CPVT and (iii) written in English only. We excluded reports of asymptomatic patients with RYR2 gene mutations and articles written in other languages. **Results:** 14 studies were chosen and reviewed together with our reported patient. Most of the patients presented initially with syncopal attack and developed cardiac arrest later. Some of them presented with both syncopal attack and seizures precipitated by exercise or stress. We found that 43.8% of patients shared similar variants or coding effects in RYR2 gene mutation. Demographically, the mean age at presentation is 11 years old with 53% of reported cases were male. **Conclusions:** Refractory arrythmias with cardiac arrest not responded to adrenaline should raise the suspicion towards RYR2 gene mutations. Recognition of this condition is important as it affects the outcome of resuscitation. Untimely diagnosis of RYR2 gene mutations with appropriate use of pharmacological agent during resuscitation is important to ensure a better outcome.

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Integrin Beta 1/CD29: A Preliminary Investigation into a Possible GBM EV Surface Marker

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ABSTRACT

Background: Glioblastoma multiforme (GBM), is both malignant and hard to detect yet extracellular vesicles, facilitates disease detection via liquid biopsy. In this study, we elucidated exosomal surface epitopes of GBM clinical and cell lines' EVs and offered insights into a potential GBM EV surface marker. **Methodology**: GBM derived EVs were collected from clinical (5 GBM patients vs 5 healthy controls) and cell line (A172, LN18, U251MG, KNS42) settings via the precipitation and size exclusion chromatography combination strategy. After BCA quantitation, isolated EVs characterized for their particle size (Zetasizer), morphology (transmission electron micrography) and surface exosomal epitopes (MACSPlex Exosome Kit, human). 6 µg EVs were incubated with capture beads before staining with APC-conjugated antibody cocktails, followed with flow cytometry detection. Positive surface epitopes were run through RNA and proteomics dataset to determine their RNA and protein level in the cell. Literature search was performed to determine literature relevant to epitope expression. **Result:** GBM derived EVs displayed typical round morphology within 30-200 nm exosomal range. MACSPlex analysis revealed varying exosomal surface epitopes expression for both clinical and cell lines' EVs: CD63 being the most expressed tetraspanins for clinical EVs while CD81 is most prominent among GBM cell lines' EVs. Interestingly, GBM markers were among the exosomal surface epitopes list (CD29, CD44 and CD146). Clinical EVs displayed higher expression of CD146 compared to healthy controls, while cell line EVs has higher expression of CD29 and CD44. Downstream analyses on positive epitopes revealed ITGB1 as a cell surface protein on U-251MG, LN-18 and ex vivo GBM sample (Cell Surface Protein Atlas) while Human Protein Atlas, ProCan-DepMapSanger and CCLE mass spectrometry data revealed ITGB1 is more expressed in RNA and protein level across tested GBM cell lines **Conclusion:** ITGB1 as a GBM invasion driver is also expressed on GBM EV surface.

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Radioactive Iodine Refractory Papillary Thyroid Cancer: ABC Transporter Genes Under the Microarray Lens

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ABSTRACT

The mortality rates in cases with papillary thyroid cancer (PTC) are notably elevated when characterised by lymph node positivity (LN+) and resistance to radioactive iodine (RAI-R) ablation. ATP-binding cassette (ABC) transporters, a class of transmembrane proteins, have been closely associated with drug resistance. However, the relationship and roles of these transporters in LN+ and RAI-R PTC have not been determined. To address this knowledge gap, our study aimed to identify differentially expressed ABC transporter genes in RAI-R PTC using microarray analysis. A total of five thyroid tumour tissues without lymph node metastasis (LN-) and with RAI avidity (RAI-A), five thyroid tumour tissues with LN+ and RAI-R, and four adjacent normal thyroid tissues were collected from PTC patients operated at Hospital Canselor Tuanku Muhriz UKM (HCTM). Histopathological evaluations were performed on the collected tissues before the extraction of total RNA. Subsequently, we conducted mRNA profiling using the Agilent SurePrint G3 Human Gene Expression v3 8x60K Microarray. Finally, AltAnalyze and NetworkAnalyst software were utilised to conduct probeset expression analysis. The defined criteria for the identification of differentially expressed ABC transporter genes are p-value < 0.05 and fold change (FC) ≥ 2 or ≤ -2. In the analysis of 47 ABC transporter genes, it was observed that three genes, namely *ABCB4*, *ABCB7*, and *ABCF2*, exhibited differential expression exclusively in RAI-R PTC. The expression of *ABCB4* (FC = 2.41) and *ABCB7* (FC = 2.03) genes were significantly upregulated in RAI-R PTC compared to normal thyroid tissue. In contrast, the expression of the *ABCF2* gene

was significantly downregulated in RAI-R PTC relative to both normal thyroid tissue (FC = -2.79) and RAI-A PTC (FC = -2.44). In conclusion, our findings suggest that the *ABCB4*, *ABCB7*, and *ABCF2* genes may play pivotal roles in RAI-R PTC. However, comprehensive investigations are warranted to elucidate their involvement in thyroid cell iodine uptake.

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CovGen Study: Dissecting COVID-19 Symptom Severity and Risk Factors

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ABSTRACT

The COVID-19 pandemic, stemming from coronavirus SARS-CoV-2, has emerged as a profound global health crisis. This study investigates the association between risk factors and the severity of COVID-19 symptoms. Conducted between August 2021 and October 2022, this study encompassed laboratory-confirmed COVID-19 patients admitted to the Hospital Canselor Tuanku Muhriz, Universiti Kebangsaan Malaysia in Cheras, Kuala Lumpur. We classified stage I to III as mild, while stage IV and V as severe, according to ISARIC - WHO Case Report Form. Among the 554 participants, 413 (74.5%) were admitted to general wards, with 223 (97.4%) encountering mild symptoms and 190 (58.5%) facing severe symptoms. Notably, 141 (25.5%) required ICU admission, with 6 (2.6%) displaying mild symptoms and 135 (41.5%) experiencing severe symptoms. Hospitalization demonstrated a strong association with symptom severity ($p<0.001$), with an adjusted odds ratio of 15.36

for ICU admission (p=0.009). Age also held significance (p<0.001), as those over 55 exhibited an adjusted odds ratio of 2.85 (p=0.009) for severe symptoms. Ethnicity emerged as a significant factor (p<0.001), with Malay, Indian, and other groups exhibiting milder symptoms compared to the Chinese patients. Comorbidities such as hypertension and obesity showed a notable link to severe symptoms (p=0.005, p=0.034 respectively). Vaccination demonstrated a mitigating effect on symptom severity while the vaccine brand correlated with symptom severity. The Delta variant showed greater severity compared to the Omicron variant (adjusted OR = 4.05, p=0.005). Symptoms like systemic, cardiovascular, musculoskeletal, and certain neurological symptoms indicated higher risk (p<0.05). Abnormal laboratory parameters—ALT, total protein, albumin, LDH, lymphocyte count, neutrophil count, CRP, and D-dimer—linked to severe symptoms (p<0.05). Elevated HbA1c levels were tied to greater severity (p=0.030, OR=1.10), while lower calcium levels indicated higher risk (p<0.001, OR=0.02). This study underscores the multifaceted factors influencing COVID-19 symptom severity, offering insights for patient management and tailored interventions based on risk profiles.

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High Glucose Level Reduces Cisplastin Chemosensitivity in Cervical Cancer Cells

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ABSTRACT

Cervical cancer is the fourth most common cancer in women worldwide. Recent evidence suggests that metabolic syndrome and diabetes may influence cancer prognosis. Studies have shown that patients with cervical cancer and pre-existing diabetes have a worse prognosis than those without. Currently, chemotherapy is the main treatment for inoperable or advanced postoperative cervical cancer. The effectiveness of cisplatin to treat advanced or recurring cervical cancer may, however, be severely hampered by the development of cisplatin resistance. The aim of this study is to investigate the effect of high glucose condition on the response of cervical cancer cells to chemotherapy. Using Hela cells, we investigated the effect of different glucose conditions: 5-, 9- and 25-mM glucose on cells' response to chemotherapy drug cisplastin at concentration of 10 µM and 60 µM. Trypan Blue dye-exclusion assay was used to determine the cell death. We found a significant reduction of cisplastin-induced cell death on Hela cells grown in 9 mM and 25 mM glucose conditions compared to Hela cells grown in normal glucose conditions (5 mM). This finding suggested that high glucose condition induces resistance to chemotherapy drugs in cervical cancer cells. These findings may indicates the importance of personalized treatment and management of cervical cancer patients with pre-existing diabetes.

Keyword: Cervical cancer; chemoresistance; cisplatin; hyperglycaemia

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Chemical Characterization, Antioxidant Potential and *in vitro* Anticancer properties of Rice (*Oryza sativa var* Bajong) from Sarawak

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ABSTRACT

Background: Rice (*Oryza sativa L.*) is an important staple food consumed by almost half of the world's population. Many consumers prefer polished white rice and neglect the nutritional benefits contained in pigmented rice. While many studies had reported on the nutritional and medicinal properties of pigmented rice bran layer, there is a knowledge gap regarding rice germ and endosperm layer. This is the first time the bran, germ and endosperm layer of darkly pigmented *Oryza sativa var* Bajong (Bajong) of Sarawak were assessed for its chemical, antioxidant and anti-cancer properties against human colorectal cancer cell line HCT-116 respectively. **Methods:** Bajong was extracted over 24-, 48- and 72-hour durations in alcohol. The extracts were characterised chemically for their total phenolic content, total flavonoid content and antioxidant potential respectively. The anticancer effects of Bajong extracts on HCT-116 cells were determined by 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) cell viability assay and Annexin-V apoptosis assay respectively. **Results:** The results showed Bajong was rich in total phenolic and flavonoid contents, exhibited strong free-radical scavenging activity, efficacious in promoting antiproliferation and triggering apoptosis in HCT-116 cells in dose-dependent and time-dependent manner respectively. The 72-hour Bajong extract exhibited the strongest growth inhibition in HCT-116 cells than those of 48-hour extract. Additionally, it significantly triggered high population of HCT-116 cells (51.2%) to undergo apoptosis at 72 hours. **Conclusion:** *Oryza sativa var* Bajong is

rich in polyphenols and flavonoids which are pertinent and evident by its strong antioxidant activities. It showed promising efficacy in significantly reducing HCT-116 cell viability and triggering apoptosis in dose- and time-dependent manners. These findings showed Bajong possessed high potential as nutraceutical crop and promising chemopreventive agent against colorectal cancer.

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LRG1 Induces Lysosomal Autophagy via Dysregulation of the mTOR Pathway

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ABSTRACT

Leucine-rich-alpha-2-glycoprotein 1 (LRG1) has emerged as a key player in various malignancies, including colorectal cancer (CRC). LRG1 exhibits a stage-dependent expression in CRC patients, and it correlates with tumour size. Several functions of LRG1 have been revealed in CRC such as proliferation, migration and invasion. Recently, LRG1 has been reported to regulate autophagy during cerebral ischemia/reperfusion injury and acute myocardial infarction. However, the autophagic role of LRG1 in CRC and its signalling pathway remains elusive. Hence, genetic modulation of LRG1 was conducted in CRC cells, HT-29 and COLO320DM to study the function of LRG1 in autophagy. HT-29 was overexpressed with LRG1 while LRG1 depletion was done in COLO320DM. The autophagy activity of the LRG1-modulated CRC cells was measured by evaluating the gene and protein expression of autophagy markers, LC3B and p62, through qPCR, western blot, immunofluorescence and flow cytometry. The activity of mTOR pathway was determined by gene expression of mTOR pathway components (*Akt, mTOR* and *Raptor*) and phosphorylation of ribosomal protein S6 (RPS6). Overexpression of LRG1 increased the transcript level of *p62*, but not *LC3B*, in HT-29 cells. The protein expression of autophagy markers, LC3B and p62, was concomitantly increased with LRG1 overexpression, suggesting the induction of impaired autophagy. Furthermore, LRG1 enhanced the gene expression of mTOR pathway components and phosphorylation of RPS6, indicating activation of the mTOR pathway. In contrast, LRG1 depletion upregulated the gene expression of *LC3B* but not *p62*. Surprisingly, silencing LRG1 promoted the protein expression of both LC3B and p62, similar to the overexpression model. However, LRG1 knockdown suppressed the gene expression of mTOR pathway components and phosphorylation of RPS6, implying the inhibition of mTOR pathway. Taken together, genetic modulation of LRG1 gene in CRC cells impacted on the mTOR pathway, although both overexpression and knockdown resulted in activation of impaired autophagy.

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Abstract for 9th Regional Conference on Molecular Medicine (RCMM)
in Conjunction with 5th National Conference for Cancer Research (NCCR)
14-15th October 2023, Bangi Resort Hotel, Selangor

Chemosensitization of TINCR: Potential Key Regulator to Chemoresistance in Triple Negative Breast Cancers (TNBCs)

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ABSTRACT

Terminal Differentiation-Induced Non-coding RNA (TINCR) proven to be involved in molecular/cellular processes in triple negative breast cancer (TNBC). High TNBC recurrence is common. Therefore, this study aimed to assess TINCR level of expression across breast cancer cell panel and evaluate its prognostic relevance in TNBC. Additionally, to assess chemosensitivity to chemotherapy drugs and migration effects in TINCR-transduced TNBC cells. QPCR was carried out on a panel of 8 breast cancer cell lines (184A1, MCF10A - Normal-like; T47D, MCF7 – Luminal; MDA-MB-468, HCC1143 – Basal A TNBC; Hs578T, MDA-MB-231 – Basal B TNBC). Kaplan-Meier survival curve of TINCR was obtained from KM plotter database to indicate the prognostic values of high/low TINCR status. Two TNBC cells (MDA-MB-231 and Hs578T) were stably transduced via lentiviral transduction of shNT and shTINCR plasmids deliveries. The stably-transduced cells were then subjected to drug response curve (DRC) of chemo-drug treatments (5-fluorouracil (5-FU), epirubicin, cyclophosphamide, FEC; 5-FU, Epirubicin, Cyclophosphamide combination and Taxotere) followed by MTT assay for EC50 determination. TINCR expression was found highly expressed in Basal B TNBC cells compared to other cells. Survival analysis indicated shorter survival in patients with elevated TINCR

expressions (HR = 2.69; p=0.0044). shTINCR cells displayed higher chemosensitivity to 5-FU (EC50= 11.95; p= 0.0001), Cyclophosphamide (EC50= 11.58; p= 0.03), and Taxotere (EC50= 7.13; p=<0.0001) in MDA-MB-231 cells compared to MDA-MB-231-shNT. Hs578T-shTINCR chemosensitized cyclophosphamide (EC50= 9.55; p=0.0007) Taxotere (EC50=0.0537) and FEC (EC50=0.00128; p<0.0001). Reduced migration rate (n=1) were seen in 5-FU, Cyclophosphamide, epirubicin and taxotere in MDA-MB-231-shTINCR compared to shNT. These preliminary data indicated that TINCR has a potential role in chemosensitizing chemo-drugs which may also play part in the migration of cells in tumour setup. By observing DRC individually and combination (FEC), drug efficacy is greater in combination drug plus TINCR inhibition, thus suggesting minimal toxicity in treatment management which however require further assessments.