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Cancer Immunotherapy in 2022 - End of the Beginning

Assoc. Prof. Dr. Toh Han Chong

In the past over 10 years, the renaissance of cancer immunotherapy and its impact on cancer lives saved has been unprecedented. The first immune checkpoint inhibitor (ICI) anti-CTLA4Ig was approved in 2011 and the first CAR T cell therapy approved in 2017. Since then, 6 more ICIs have been approved across many cancer indications. Tumour mutational burden and microsatellite instability form critical biomarkers for the use of ICIs. In 2022, we are seeing a rise in combination therapies involving ICI.

In cell therapy, CAR T cell therapy has made early important inroads in blood cancers. In solid tumours, challenges exist in reaching optimal clinical efficacy. Still, we see glimmers of hope. T Cell Receptor and tumour infiltrating lymphocyte T cell therapy have shown some striking benefit in some patients refractory to further treatments with little further alternatives. Other exciting developments are in the therapeutic areas of T cell engager, antibody drug conjugates and therapeutic cancer vaccines. – where clinical benefits have been achieved. Central in the development of new immunotherapies and generally cancer treatment is a deeper dissection and understanding of the tumour microenvironment, to better elucidate mechanisms, biomarkers, resistance and new targets.

Immunotherapy now forms a vital pillar of cancer therapy with a trend towards more combinations showing greater impact. Challenges remain for cost of treatment and manufacturing capabilities. With more supersurvivors and complete remissions with such treatment even in advanced disease, we will surely see a better tomorrow for cancer patients.

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Targeting Treatment Resistance in Head and Neck Cancer through Unravelling Molecular Mechanisms

Prof. Dr. Goh Boon Cher

Head and neck cancer is a common malignancy worldwide and carries a poor prognosis when it is diagnosed late. Treatment itself often carries significant morbidity and impairs quality of life. There have been few actionable targets for squamous cell carcinoma of the head and neck, notably anti-epidermal growth factor receptor monoclonal antibody cetuximab and antiPD1 immunotherapy that have reached clinical use. We studied a polymorphism in the extracellular semaphorin domain of C-MET in the context of SCC head/neck and lung, uncovering a novel mechanism conferring biological aggressiveness and potential for therapeutic intervention. TP53 mutations are the most common somatic mutations in SCCHN and we studied the gain of function mutation p53R158G in the S4 strand of the DNA binding domain, covering the mechanism of carcinogenicity. In South East Asia including Malaysia and Singapore, nasopharyngeal carcinoma is associated with EBV and is the commonest head and neck malignancy, with a propensity for recurrence and metastases. We have interest in development of anti-vascular endothelial growth factor therapy as a means to overcome treatment resistance, and some of this work will be described in this presentation.

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The Identification of a Group of Self-Assembling, Trimetallic Cryptands with Multi-Kinase Inhibitor Activity Using a Phenotypic Approach to Drug Discovery

Prof. Dr. Roger Phillips

Despite significant advances in the development of targeted anti-cancer drugs, the much hoped for paradigm shift in cancer survival has been blunted by the emergence of new mechanisms of drug resistance. The development of drugs with multiple mechanisms of action is one approach that has been adopted to circumvent this problem and in this talk, the use of a phenotypic approach to drug discovery based on potency and selectivity in vitro has been used to identify compounds that have multiple mechanisms of action. This talk will specifically focus on the discovery, preclinical activity and mechanism of action of self-assembling trimetallic cryptands that have potent but selective activity against cancer cells in vitro and in vivo.

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Precision Medicine in Lymphoid Malignancies

Prof. Dr. Richard Rosenquist Brandell

With the introduction of high-throughput sequencing technologies, the molecular landscape of major lymphoid malignancies was rapidly unraveled. While a few lymphoid neoplasms were characterized by a predominant gene mutation (e.g. Waldenström's macroglobulinemia and hairy cell leukemia), the majority demonstrated a highly diverse genomic landscape with a high number of recurrent mutations. These studies have not only furthered our understanding of the onset and evolution of lymphoid malignances, but also provided us with new biomarkers that can improve diagnostics, prognostication as well as guide clinical decision-making in lymphoid malignancies. In this lecture, I will highlight clinically relevant diagnostic, prognostic and predictive biomarkers that are used today in clinical diagnostics of chronic lymphocytic leukemia (CLL), one of the most frequent lymphoid malignancies, also as a basis for treatment decisions on

novel targeted therapies. I will also highlight future directions in this rapidly evolving field.

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ATLAS Project and the Future of International Collaborative Clinical Research in Asia

Dr. Mitsumi Terada

National Cancer Center Japan has initiated ATLAS (Asian Clinical Trials Network for Cancers) project since 2020 with financial support from AMED that is a Japanese public funding agency. The goals of ATLAS are to strengthen multilateral cooperation with ATLAS member institutions and improve the cancer clinical research capabilities; establish robust infrastructure for conducting oncology trials with high quality clinical data; deliver novel drugs and practice changing research results to cancer patients. To achieve this goal, construction of well-organized governance as a cooperative group such as EORTC, SWOG, or JCOG is urgently needed. We have just started to prepare the establishment of the ATLAS Board that is the guardian of ATLAS's sustainability.

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Developing Affordable Cancer Therapeutics in Low and Middle Income Countries

Dr. Yolanda Augustin

Over half the world's cancers occur in Low-and-Middle-Income-Countries (LMICs), where cancer is a neglected disease. Despite significant advances in systemic anticancer therapy, many of these treatments are simple not available to the majority of the world's cancer patients. For example, immunotherapy has changed the treatment paradigm for many cancer patients in developed countries, significantly prolonging survival and maintaining patient quality of life but at a cost that is currently unaffordable for many public health systems and patients in LMICs. We urgently need better solutions to treat patients more effectively, humanely and equitably.

Drug discovery and novel drug development takes on average 10-15 years to go from bench to bedside at an average cost of USD1 billion. Repurposing of 'old' drugs for new indications can shorten this pathway substantially with significant cost savings. One strong drug repurposing candidate is artesunate, an antimalarial derived from traditional Chinese medicine that also displays anticancer properties. This drug is currently the subject of our drug repurposing programme which includes a number of cancers relevant to Malaysian needs including colorectal cancer, cervical cancer, nasopharyngeal cancer and Acute Myeloid Leukaemia.

Alongside drug repurposing, platform technologies such as molecular pharming – using plants to grow cancer drugs, in particular immunotherapies and targeted monoclonal antibodies can also reduce the cost of cancer drug development significantly. Malaysia has the opportunity to lead on healthcare priorities facing LMICs, creating reverse linkage collaborations between Muslim countries through entities such as the Islamic Development Bank (which now has a Center of Excellence for Science, Technology and Innovation) in Kuala Lumpur and local governments. This also represents opportunities for technology transfer and halal sector manufacturing. Collaboration is urgently needed between academia, clinicians, patient advocate groups, governments, non-governmental stakeholders, regulators and responsible pharma to ensure equitable access to affordable cancer therapeutics for patients globally.

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Triple Negative Breast Cancer: Tumour Biology and Optimisation of Clinical Outcome

Dr. Mastura Md Yusof

Triple negative breast cancer are breast cancer types that are difficult to treat because they lack expression of the estrogen receptor (ER), progesterone receptor (PR) and Human Epidermal Receptor 2 (HER2) gene amplification.

Intensive research, analysis by the Cancer Genome Atlas (TCGA) Research Network and the advent of high-throughput technology tools has expanded the classification of TNBC tumors into subgroups according to its gene expression profiles in order to identify the different molecular subtypes, novel TNBC biomarkers that can play both predictive and prognostic roles and enhance therapeutic strategies.

The "immune-activated," subtype or tumours with defective BRCA pathway are amongst initial TNBC group with established genetic vulnerabilities that has allowed the addition of promising therapeutic approaches, including DNA-damaging agents (PARP inhibitors, platinum) as well as immunotherapy. The treatment of metastatic NBC (mTNBC) is currently transforming rapidly with better outcomes observed in clinical trials.

The recent success with immune checkpoint inhibitors (ICIs) targeting the programmed cell death receptor 1 and programmed death ligand 1 (PD-L1) and PARP inhibitors for germline BRCA mutation-associated breast cancers as well as other novel strategies in mTNBC treatment will change the course of this unique cancer subtype in the future.

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Updates on Treatment of Gastroesophageal Cancers

Prof. Dr. Ho Gwo Fuang

Management of gastric cancer has undergone significant changes over the past few years. TCGA classified gastric cancer into four molecular subtypes: chromosomal instability (50% of cases), MSI-H (21%), genomically stable (20%) and EBV-positive (9%). Molecular profiling is recommended prior to the start of systemic

treatment, testing predictive markers such as HER 2-expression, microsatellite instability status, programmed death ligand-1 expression (combined positive score, CPS), and Epstein-Barr virus expression.

CheckMate 649 trial established the role of immunotherapy in advanced gastric cancers. Adding nivolumab to chemotherapy in the first line setting resulted in significantly improved OS and PFS in all randomly assigned patients, with the greatest magnitude seen in patients with high PD-L1–expressing tumours. In the PD-L1 CPS ≥ 5 population, median overall survival was 14.4 months with nivolumab/chemotherapy vs 11.1 months with chemotherapy (HR = 0.71, P < .0001)

Destiny-Gastric 01 trial demonstrated the efficacy of antibody-drug conjugate, trastuzumab deruxtecan, for patients with pretreated HER2-positive gastric or gastroesophageal junction adenocarcinomas, showing a response rate of 51% and median OS 12.5 vs 8.4 months (HR = 0.59, P=0.01) when compared to the physician's choice of therapy.

In the phase 2 FIGHT trial, mFOLFOX6 plus bemarituzumab, a humanized IgG1 monoclonal antibody against FGFR-2b, showed a trend towards improved PFS (9.4 vs 7.4 month; HR 0.68, p=0.073) versus mFOLFOX6 plus placebo. This regimen is now being investigated in a phase 3 trial.

The recently announced SPOTLIGHT trial demonstrated efficacy of zolbetuximab, an IgG1 monoclonal antibody against CLDN18.2, a transmembrane protein. Zolbetuximab plus mFOLFOX6 showed improved PFS and OS in CLDN18.2+ve gastric cancer patients compared to placebo plus mFOLFOX6 in the first line setting. The results of GLOW trial (zolbetuximab combined with CAPOX) is being awaited.

Immunotherapy has also changed the treatment landscape for oesophageal cancers. CheckMate 648 trial showed first line nivolumab regimen improved survival in advanced oesophageal squamous cell carcinomas, both among patients with tumour-cell PD-L1 expression of 1% or more (15.4 vs 9.1 months; HR 0.54, P<0.001) and in the overall population (13.2 vs 10.7 months; HR 0.74; P=0.002). In Keynote 590, pembrolizumab regimen demonstrated improved survival in oesophageal carcinoma patients with PD-L1 CPS of 10 or more (13·9 vs 8·8 months; HR 0·57, p<0·0001) and in all randomised patients (12.4 vs 9.8 months; HR 0·73, p<0·0001).

Many ongoing trials are investigating combination targeted and immunotherapies in gastroesophageal cancers, and will continue to change the treatment landscape in the coming years.

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Insights into Advanced Non-Small Cell Lung Cancer Patients with MET Alterations

Emeritus Prof. Dr. Liam Chong Kin

The receptor for hepatocyte growth factor, a tyrosine kinase that is encoded by the mesenchymal epithelial transition factor (MET) oncogene, plays a crucial role in cancer growth, invasion and metastasis. Oncogenic MET alterations can act as a primary driver of tumorigenesis, with tumor dependence on MET signaling for cancer initiation and progression, a phenomenon that is called 'oncogene addiction'. MET exon 14 (METex14) skipping mutations and MET amplification have been identified as alterations that can convert MET into a primary oncogenic driver in non-small cell lung cancer (NSCLC). MET amplification is also an acquired resistance mechanism in 10-15% of patients with epidermal growth factor receptor (EGFR)-mutated non-small cell lung cancer (NSCLC) treated with EGFR tyrosine kinase inhibitors (TKIs). Targeted therapies are available or are in development to target NSCLC harbouring METex14 skipping mutations or MET amplification. Both of these MET alterations are biomarkers predictive of sensitivity to MET inhibition in NSCLC. The level of MET amplification potentially has an impact on the efficacy of targeted treatment, with patients with highly amplified tumours expected to show a better response.

METex14 skipping mutation is now an actionable oncogenic driver in advanced/metastatic NSCLC with selective MET inhibitors such as tepotinib and capmatinib showing durable clinical activity in advanced NSCLC patients with this MET alteration.

Testing for MET amplification often requires tissue analysis using fluorescent in-situ hybridisation because tissue and especially circulating tumour DNA next-generation sequencing underestimates MET amplification. Combining a selective MET inhibitor with first- or third-generation EGFR TKI on development of acquired EGFR TKI resistance due to MET amplification is a treatment strategy with promising efficacy.

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Exploiting New Cancer Drug Targets that Drive Drug Resistant and Metastatic Disease

Prof. Dr. Andrew D Westwell

Continuing progress in the field of cancer therapeutics will require the development of novel agents against previously unexplored drug targets driving resistant and metastatic disease. In a collaboration between the School of Pharmacy and the European Cancer Stem Cell Research Institute at Cardiff University (Wales, U.K.), we have focused on drug discovery against the transcription factor complex protein Bcl3. As an emerging cancer drug target, driving cancer hallmarks in diseases of unmet medical need such as metastatic colorectal cancer, Bcl3 is ripe for therapeutic exploitation [1]. We have used computational drug design methods to virtually screen for inhibitors at the interface between

Bcl3 and partner protein p50. This has led to the identification of a hit compound (JS6) demonstrating on-target anti-metastatic and growth inhibitory activity within a range of preclinical in vitro and in vivo models [2]. Development of a preclinical lead compound suitable for daily oral administration and with a wide therapeutic window will be described further, alongside plans for first-in-class clinical trials of a novel small molecule Bcl3 inhibitor.

[1] Legge et al. (2020). The role of B-Cell Lymphoma-3 (BCL-3) in enabling the hallmarks of cancer: implications for the treatment of colorectal carcinogenesis. Carcinogenesis, 41, 249-56.

[2] Soukupova et al. (2021). The discovery of a novel antimetastatic Bcl3 inhibitor. Mol. Cancer Therap. 20, 775-86.

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Emerging Immuno-cellular Therapy in the Treatment of Diffuse Large B-cell Lymphoma

Prof. Dr. Bee Ping Chong

Diffuse large B cell lymphoma (DLBCL) is the most common aggressive lymphoma in adults. The R-CHOP immunochemotherapy protocol has been the first-line standard of care for DLBCL patients for decades and is curative in approximately two-thirds of patients. However, 30–40% of patients are refractory or relapsing and they second-line salvage therapy that consisted of platinum-based chemotherapy regimens followed by autologous hematopoietic stem cell transplantation with curative intent for transplant-eligible patients or palliative chemotherapy for transplant-ineligible patients. The overall response rate is ranged between 40 to 60%. The median survival is approximately 13 months and the prognosis is even poorer for those who do not respond to salvage chemotherapy. Therefore, there is still an unmet need for these patients. Better understanding of both molecular biology of lymphoma cells and the tumor microenvironment have initiated clinical trials exploring targeted therapy based on driver genetic alterations. In recent years there have been several new therapeutic agents approved for the treatment of relapsed/refractory DLBCL. These agents include monoclonal antibodies such as polatuzumab vedotin (targeting CD79a), tafasitamab and loncastuximab tesirine (targeting CD 19), Selinexor (SINE inhibitor), anti-CD19 chimeric antigen receptor T-cell therapies and bispecific antibodies.

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Cancer-Associated Fibroblasts as Potential Therapeutic Targets in Head and Neck Cancer

Prof. Dr. Ian Paterson

It is now recognised that non-malignant components within the tumour microenvironment (TME) also influence tumour development and progression. Cancer-associated fibroblasts (CAFs) are often the most abundant cell type within the tumour stroma. Here, they actively participate in the reciprocal communication between tumour cells and other host cells in the TME to create a tumour-permissive microenvironment in a number of epithelial tumours, including those of the head and neck. CAFs share many characteristics with fibroblasts found within healing wounds and demonstrate a perpetually "active," alpha-smooth muscle actin positive phenotype. In this talk, I will describe the functional role of CAFs in head and neck cancers (oral and nasopharyngeal carcinomas) and discuss the signalling pathways involved in CAF activation. Finally, the possibility of targeting CAFs therapeutically will be discussed.

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Biomarkers in Clinical Trials: From Cancer Researcher and Cancer Patient Perspective

Dr. Magdalena B. Wozniak

Biomarkers have been used for diagnosis, assessment of disease risk, prognosis, prediction, as well as treatment response and safety at virtually every stage of drug discovery and development. In an era of targeted therapies, and precision medicine, clinical trials have adapted their designs to bring new medicines to the right patient population and improve treatment outcomes. In my presentation, I will provide an overview of the landscape of biomarkers discovery at various stages of drug development with specific examples to illustrate diagnostics and therapeutic monitoring. Biomarkers also play an important role in the detection and management of patients with breast cancer. Biomarkers that aid in the diagnosis, prognosis, and prediction of breast cancer are crucial for early detection and appropriate disease control throughout treatment. As a breast cancer survivor, I will share my experience of how biomarkers affected my treatment decision making during my cancer journey and discuss challenges in biomarker and clinical development from cancer researcher and cancer patient perspectives.

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ODD01

Targeted Delivery of Cisplatin Using RGD-Modified ZIF-90 as Delivery Vehicle

Emilia Abdulmalek[1,2,*], Adamu Abubakar[1,3], Kyle. E. Cordova[4], Mohd Basyaruddin Abdul Rahman[1,2,5]

[1] Integrated Chemical BioPhysics Research, Faculty of Science, Universiti Putra Malaysia (UPM), 43400 UPM Serdang, Selangor, Malaysia

[2] Department of Chemistry, Faculty of Science, UPM, 43400 UPM Serdang, Selangor, Malaysia

[3] Department of Chemistry, Taraba State University, Jalingo P.M.B 1167, Taraba State, Nigeria

[4] Materials Discovery Research Unit, Advanced Research Centre, Royal Scientific Society, Amman 11941,
Jordan
[5] Foundry of Reticular Materials for Sustainability (FORMS), Materials Synthesis and Characterization
Laboratory, Institute of Advanced Technology, UPM, 43400 UPM Serdang, Selangor, Malaysia
* Corresponding author's email: emilia@upm.edu.my

Introduction: Chemotherapy is one of the most effective therapy available to treat cancers, but the side effects make it less favourable. Therefore, we aimed to effect selective and targeted delivery of cisplatin to cancer cell via covalent-modification of zeolitic imidazolate framework-90 (ZIF-90) with RGD peptide, a tripeptide that was known for its ability to recognise integrins on cell surface. Due to overexpression of integrins by cancer cell, RGD peptide on the surface of ZIF-90 would be able to guide the delivery of the cancer drug (cisplatin) encapsulated in the pores of ZIF-90, to the intended recipient. Thus, side effect to normal cells will diminish. **Methods:** Nano-sized ZIF-90 encapsulated cisplatin (RGD@Cis⊂nZIF-90) was prepared by in-situ encapsulation followed by covalent modification with RGD peptide. The drug loading amount and drug released was profiled, and MTT assay on MRC-5 (human fetal lung fibroblast cells) and A549 (lung adenocarcinoma) cells was conducted. **Results:** Powder XRD confirmed the formation of ZIF-90 and covalent bond to RGD peptide was confirmed with NMR and IR showing formation of imine bond between imidazolcarbaldehyde linker of ZIF-90 and the RGD peptide. The cisplatin loading was measured to be 24.8% using UV-vis and sustained released behaviour at pH 5 was observed with maximum released (92%) observed after 24 hours. The MTT assay showed that RGD@Cis⊂nZIF-90 nanoparticle was more toxic towards A549 (IC50 8.79 μgmL-1) than MRC-5 (IC50 31.07 μgmL-1). The selectivity index was found to be 3.5 suggesting that there is selectivity towards A549 cell in the presence of RGD peptide. **Conclusion:** RGD-modified nano-sized ZIF-90 encapsulated cisplatin has been successfully synthesized and determined to have good selectivity and toxicity towards lung cancer cell over normal cell. This may lead to more selective and improve performance of cancer therapy with little side effect.

Keywords: ZIF-90, RGD peptide, cisplatin, selective chemotheraphy, cancer drug delivery
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ODD02

Targeting Tropomyosin Receptor Kinase C Expressing Cancer Cells through Synthetic Ligand Conjugate and Cyclophosphamide for Immunotherapy

Siti Nursyahirah Bakar[1], Kue Chin Siang[1,*]
[1] Faculty of Health and Life Sciences, Management and Science University, Seksyen 13, 40100 Shah Alam,
Selangor, Malaysia
* Corresponding author's email: cskue@msu.edu.my

Introduction: Tropomyosin receptor kinase-C (TrkC) has been reported to be overexpressed in cancer and regulates its survival and metastasis. In addition, tumour microenvironment is immunosuppressive and associated with high expression of immunosuppressive mediators including regulatory T cells (Tregs), TGF-β and myeloid-derived suppressor cells. This study was aimed to study the antitumour efficacy of TrkC-targeting dinitrophenol (DNP) conjugate (IYIY-DNP) combined with Cyclophosphamide (CYP) for immunotherapy. CYP is an FDA-approved anticancer drug and has been reported to selectively suppress Treg cells at low dose. **Methods:** Female Balb/c mice was immunised with DNP-KLH (Keyhole Limpet Hemocyanin) to stimulate anti-DNP antibodies. After immunisation, TrkC expressing-4T1 cells were implanted, and tumour bearing mice at the size of 60-80mm^3 were randomly divided to group of 10 mg/kg IYIY-DNP (I.V), 25 mg/kg CYP (I.P), 10 mg/kg IYIY-DNP + 25 mg/kg CYP for treatment on every alternative day, for five cycles (n=8 for each group including control saline). Tumour size were recorded using calliper on every two days, for 30 days. **Results:** No toxicity was observed on mice treated with all groups throughout 14 days of observation. Mice treated with low dose of 25 mg/kg CYP displayed delayed tumour growth by 6.29%, however, mice treated with 10 mg/kg IYIY-DNP + 25 mg/kg CYP delayed tumour growth by 36.14%, both compared to saline-treated mice. The average area under the tumour growth curve vs. day post treatment for 10 mg/kg IYIY-DNP + 25 mg/kg CYP group is significantly smaller compared with saline-treated group ($P<0.05$). **Conclusion:** These findings suggest that combination therapy of IYIY-DNP and CYP is able to decrease the tumour growth preclinically and have a potential to be developed to become new immunotherapy treatment strategy.

Keywords: Tropomyosin Receptor Kinase C (TrkC), 4T1 cells, Cyclophosphamide (CYP), Immunotherapy
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ODD03

Molecular Modelling Analysis of Squamocin as Potential BCL-XL Anti-apoptotic Protein Inhibitor

Kaynat Khimani[1], Mohd Faiz Abdul Ghani[1,2], Rozana Othman[3], Noraziah Nordin[1,*]
[1] Basic Medical Sciences 1, Faculty of Medicine & Health Sciences, University Sains Islam Malaysia, 55100,
Kuala Lumpur, Malaysia.
[2] School of Pharmacy, KPJ Healthcare University College, Kota Seriemas, 71800, Nilai, Negeri Sembilan,

Malaysia.
[3] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University Malaya, 50603, Kuala Lumpur,
Malaysia.
* Corresponding author's email: noraziahnordin@usim.edu.my

Introduction: BCL-XL is one of the prosurvival proteins that is overexpressed in certain cancers and implicated as a chemoresistance factor. It is known from *in-vitro* studies that certain acetogenins, including squamocin (SQ), have potent anticancer properties. However, its inhibitory effect towards BCL-XL has not been established. In this study, we evaluated the interaction and binding affinity of SQ into BCL-XL protein in molecular docking and dynamics. **Methods:** Autodock Vina was used to perform molecular docking of SQ with BCL-XL protein. The tested ligand was also compared with ABT-737, a known BCL-XL inhibitor. The best conformation with lowest binding energy and interaction with key active residues of BCL-XL receptor was chosen. The complex was further subjected to a molecular dynamic study for 100ns simulation using FF19SB forcefield in Amber 22. Molecular mechanics/Poisson-Boltzmann surface area (MM/PBSA) energy calculations, Root mean square deviation (RMSD) and Fluctuation of protein conformation (RMSF) of the complex were performed. **Results:** Interaction of SQ with the active site of BCL-XL protein revealed a better binding affinity of -11.9 kcalmol^{-1} compared to -9.179 kcalmol^{-1} of ABT-737. The SQ/BCLXL complex was found to be stable throughout 100ns simulation time. Furthermore, hydrogen bonding patterns between key residues of ARG139 and SER 109 with SQ ligand were detected, and energy profiles reflect the stability of the bound complex. **Conclusion:** Squamocin is a potential inhibitor of the BCL-XL protein based on the in-silico study. However, a further experimental study is needed to validate its action toward the development of a new drug for cancer therapy.

Keywords: Cancer, Squamocin, BCL-XL protein, Molecular docking, Molecular dynamic
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ODD04

Cytotoxicity Effects of Selected Flavonoids in Human OvarianCancer Cells

Mohd Faiz Abd Ghani[1,2], Marjanu Hikmah Elias[1], Kaynat Khimani[1], Noraziah Nordin[1,*]
[1] Department of Basic Medical Sciences 1, Faculty of Medicine & Health Sciences, Universiti Sains Islam
Malaysia, 71800, Nilai, Negeri Sembilan Malaysia
[2] KPJ Healthcare University College, PT 17010 Kota Seriemas, 71800 Nilai, Negeri Sembilan, Malaysia
* Corresponding author's email: noraziahnordin@usim.edu.my

Introduction: Ovarian cancer is the most lethal gynaecological malignancy in women. The use of chemotherapy for ovarian cancer treatment is effective in many patients, but it has been associated with serious side effects. Therefore, attention is given to natural compounds, including flavonoids as alternative drugs in cancer treatment and prevention. **Objective:** This study aims to determine the cytotoxicity of seven flavonoids, namely apigenin, biochanin A, flavone, fisetin, galangin, myricetin and 6- hydroxyflavone against two epithelial ovarian cancer cell lines (CAOV-3 and SKOV- 3). **Method:** The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was carried out to determine the cell viability after being treated with selected flavonoids and carboplatin at 24, 48 and 72 h. The experiment was then followed by an AO/PI double staining assay using a fluorescent microscope to elucidate the morphological changes of cell death. **Result:** A total of four flavonoids, namely flavone, galangin, 6-hyroxyflavone and biochanin A showed highly cytotoxic against CAOV-3 cells at 24 h with lower IC50 value, ranging from 33.9 to 37.91 μgml^{-1} compared with carboplatin 41.63 μgml^{-1}. Galangin has the lowest IC50 value among allat 48 h (26.16 μgml^{-1}) and 72 h (23.9 μgml^{-1}). Meanwhile, apigenin, 6-hydroxyflavone, flavone, biochanin A and myricetin exhibited less IC50 value than carboplatin (69.13 μgml^{-1}) of 36.55, 38.67, 38.78, 39.61 and 57.48 μgml^{-1}, respectively, at 24 h of SKOV- 3 cells treatment. Apigenin and biochanin A were detected to be highly cytotoxic in SKOV-3 cells at 48 and 72 h, respectively. The morphological changes of treated cells with flavonoids exhibited the presence of cell membrane blebbing and chromatin condensation indicating induction of apoptosis. Meanwhile, secondary necrosis was also seen in all flavonoids treatment after 24 h. **Conclusion:** This study shows that apigenin, myricetin, flavone, 6-hydroxyflavone and biochanin A can be potential therapeutic drugs in ovarian cancer.

Keywords: Flavonoid, CAOV-3, SKOV-3, Ovarian cancer
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ODD05

Elucidating the transcriptional regulation and chemosensitivity efficacy of Terminal Differentiation Induced Non-Coding RNA (TINCR) driver regulator in Triple-Negative Breast Cancer (TNBC)

Afreena Afiqah A[1], Nurul Nadiah AD, Chin Siok Fong[1], Norfadilah R[2], Reena Rahayu MZ[3], Rohaizak M[4], Norlia A[4], Nani Harlina ML[4], Ezanee Azlina MH[1,*]
[1] UKM Medical Molecular Biology Institute, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia
[2] Centre For Healthy Aging & Wellness, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Kuala
Lumpur, Malaysia

[3] Pathology Department, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia
[4] Surgery Department, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia
*Corresponding author's email:
ezanee.azlina.mohamad.hanif@ppukm.ukm.edu.my

Introduction: Previous baseline analysis (GSE58812) displayed dysregulation of TINCR in TNBC metastasis and death patients and high TINCR expression was shown to have shorter disease-free survival in TNBCs (HR: 2.69, p-value: 0.0044). However, the underlying mechanism of TINCR in driving poor outcome TNBC is unclear. Therefore, the study aimed to elucidate the transcriptional regulation and potential chemo- sensitisation effects of TINCR in TNBC cell line model. **Methods:** Ensembl, PROMO, KM plotter and Promoter 2.0 Prediction Server databases was utilised to predict TINCR promoter region and transcription binding sites. TINCR promoter region was predicted by input query FASTA TINCR sequence into Promoter 2.0 Server. The highest score of the predicted site was chosen for PROMO putative transcription factors (TFs). Predicted TFs were analysed further of their prognostic effects in TNBC on KM plotter. TNBC cell line model MDA-MB-231 were transduced with shTINCR and shNT via lentiviral transduction packaging vectors. Functional assays such as growth, proliferation, migration, wound-healing, and drug sensitivity assay (MTT) were carried out. **Results:** Three TFs were prognostically relevant in TNBCs such as the CEBPB, ELK1, and ARNT. RqPCR showed downregulation of CEBPB and ARNT whereas ELK1 was upregulated in TINCR-depleted MDA-MB-231 cell line. Characterisation of MDA-MB-231 shTINCR cell line showed slower rate of cell proliferation, growth rate, and migration of cells compared to MDA-MB-231 shNT control. This preliminary analysis narrowed down three potential driver mechanism of TINCR in TNBC however, RqPCR revealed contradicting outcome for ELK1. MTT drug assay showed 2-fold efficacy chemo-sensitisation to Cyclophosphamide by TINCR. **Conclusion:** Silencing TINCR and potential TINCR-TFs may suppress tumour progression and chemoresistance. Therefore, TINCR and TINCR-TFs expression may potentially serve as a significant predictive value and prognostic marker for TNBC patients. Further insights into the functional and clinical implications of the molecular cross talk between TINCR, CEBPB, ARNT, and ELK1 may also provide therapeutic benefit for TNBC patients.

Keywords: Triple negative breast cancer, LncRNA, TINCR, Transcription factors
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ODD06

Phytochemical Analysis and Evaluation of Antioxidant and Antibacterial Activities of Crude and Fractionated Flower Extracts of *Clitoria ternatea*

Kamran Ashraf[1,*], Nur Fatin Adlin[1], Aina Nabila Basri[1], Wasim Ahmad[2], Mohammad Humayoon Amini [1]
[1] Faculty of Pharmacy, Universiti Teknologi MARA(UiTM), Cawangan Selangor, Kampus Puncak Alam,
42300 Bandar Puncak Alam, Selangor Darul Ehsan, Malaysia
[2] Department of Pharmacy, Mohammed Al-Mana College for Medical Sciences, Dammam 34222, Saudi Arabia
* Corresponding author's email: kamranashraf2@gmail.com/ kamran1368@uitm.edu.my

Introduction: *Clitoria ternatea* (CT), (fam: Fabaceae), also known as Butter pea flower, is a medicinal plant used to treat various diseases. This plant contains a variety of plant metabolites including flavonoids. The phytochemicals present in flower extracts have been reported to possess various pharmacological activities that are beneficial for protecting the body against various diseases. The aim of the present work was to analyse, separate, and identify the phytochemicals and evaluate the antioxidant potential and antibacterial activities of flower extracts of CT. **Methods:** CT flower was dried, powdered and macerated in methanol to obtain methanolic crude extract. The process continued by successive fractionation using solvents of different polarities to obtain n-hexane fraction, chloroform fraction, ethyl acetate fraction, and aqueous extract fraction. The phytochemicals present in the extracts were separated by TLC and HPLC methods. Antioxidant and antibacterial activities were evaluated by DPPH and *in vitro* well diffusion methods, respectively. **Results:** Phytoconstituents present in CT were separated and some flavonoids were also identified in chloroform and ethyl acetate fractions. Antioxidant and antibacterial results showed that ethyl acetate fraction was found to exhibit good antioxidant activity (IC50 value 0.11 mg/ml) and border antibacterial effect. **Conclusion:** The ethyl acetate extract of CT was found to be potent and could be used to isolate pure compounds that might be helpful in the development of antibacterial pharmaceutical entities in the future.

Keywords: *Clitoria ternatea,* Butter pea flower, Antibacterial, Antioxidant, Extract
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ODD07

Asymmetric Total Synthesis of (-)-Swainsonine from Inexpensive and Commercially Available Starting Material, Ascorbic Acid

Zheng Yang Lee[1], Mohd Fazli Mohammat[2], Agustono Wibowo[3], and Jhi Biau Foo[1,4*]
[1] School of Pharmacy, Faculty of Health & Medical Sciences, Taylor's University, 1, Jalan Taylors, 47500,

Subang Jaya, Selangor, Malaysia
[2] Organic Synthesis Laboratory, Institute of Science, University Teknologi MARA (UiTM), 40450, Shah Alam,
Selangor, Malaysia
[3] Faculty of Applied Science, University Teknologi MARA (UiTM) Pahang, Jengka Campus, 26400, Bandar
Tun Abdul Razak Jengka, Pahang, Malaysia
[4] Centre for Drug Discovery and Molecular Pharmacology, Faculty of Health & Medical Sciences, Taylor's
University, 1, Jalan Taylors, 47500, Subang Jaya, Selangor, Malaysia
* Corresponding author's email: jhibiau.foo@taylors.edu.my

Introduction: Swainsonine is a natural and synthetic alpha-mannosidase II inhibitor which has been shown to inhibit various types of cancer growth *in vitro* and *in vivo*. Our initial study was to synthesise (-)-swainsonine from commercially available D-isoascorbic acid, yet unsuccessful, although following published protocol. Hence, we envisioned this challenge associated with aldehyde-lactol tautomerism by exploiting another novel route. **Methods:** Acetonide protected diol 1 was first prepared from D-isoascorbic acid via a three-step procedure. It was then selectively acetylated, and further Swern oxidation yielded aldehyde 2. Wittig olefination was carried out to afford olefinic ester 3 with ease. Subsequent deacetylation and azide substitution gave imino ester 6, which readily produces (-)-swainsonine in 4 steps. Purification of the products was carried out using the classical column chromatography Si-gel G60 (230-400 mesh, Merck). The ^{1}H and ^{13}C NMR spectra were registered in $CDCl_3$ with Joel Resonance ECZ400S 400 MHz (^{1}H) and 100 MHz (^{13}C) using TMS as the internal standard. **Results:** The imino ester 6 was obtained as a colourless oil with an overall yield of 2% from D-isoascorbic acid. **Conclusion:** The present study illustrated the total synthesis route of (-)-swainsonine.

Keywords: Swainsonine, Cancer, Swern oxidation, Wittig olefination, Aldehyde-lactol tautomerism
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ODD08

Development and Characterisation of Zerumbone-Superparamagnetic Iron Oxide Nanoparticle Co-Loaded Nanostructured Lipid Carriers as A Potential Treatment for Breast Cancer

Li Kar Stella Tan[1], Jhi Biau Foo[1,2,*], Yong Sze Ong[3], Chee Wun How[3]
[1] School of Pharmacy, Taylor's University, Subang Jaya, Selangor, Malaysia
[2] Centre for Drug Discovery and Molecular Pharmacology (CDDMP), Faculty of Health & Medical Sciences,
Taylor's University, 1, Jalan Taylors, 47500, Subang Jaya, Selangor, Malaysia
[3] School of Pharmacy, Monash University, 47500 Bandar Sunway, Selangor, Malaysia
* Correspondence: jhibiau.foo@taylors.edu.my

Introduction: Zerumbone (ZER), having significant medicinal value, is poorly soluble in water, and this poor solubility of ZER has been overcome by loading into nanostructured lipid carriers (NLC), but clinical trials with this compound have been rarely reported due to lack of selectivity. Hence, using superparamagnetic iron oxide nanoparticles (SPION) could be a potential approach to achieve specific delivery in various treatments. This study aims to develop and characterize an improved drug delivery system for ZER. **Method:** ZER and SPION were loaded into NLC (ZSN) using hot ultrasonication. This formulation was characterised by particle diameter, polydispersity index (PDI), zeta potential, encapsulation efficiency, loading capacity, state of lipid modification, lipid interaction, morphology, in vitro drug release, haemocompatibility, and stability. ZSN was also evaluated on its *in vitro* cytotoxicity, cellular uptake, internalisation, and magnetic targeting. **Results:** ZSN was milky brownish with an average diameter of 140.32 ± 1.14 nm, PDI of 0.18 ± 0.02, and zeta potential -13.37 ± 0.61 mV. ZSN was shown to be homogeneous, spherical, uniformly dispersed, haemocompatible, and stable over 3 months of storage. Over 72 hours, 38.88 ± 6.61% and 41.25 ± 4.90% of ZER were released from acidic and physiological conditions, respectively. At 48 hours of treatment, ZSN was time-dependently cytotoxic and apoptotic, with half maximal inhibitory concentration (IC_{50}) values of 1.94 ± 0.19 and 2.55 ± 0.93 μg/mL in MCF-7 and MDA-MB-231 cells, respectively. The apoptotic effect was more significant in MDA-MB-231. In terms of the internalisation pathway, ZSN was internalised primarily by caveolae-mediated endocytosis and active energy-dependent process in MCF7 and MDA-MB-231 cells, respectively. The process was time-dependent with half maximum uptake (Km) value of 25.19 ± 1.50 minutes for MCF-7 cells and 6.07 ± 0.54 minutes for MDA-MB-231 cells. The magnetic characteristics of SPION were sufficient to direct ZSN. Under a 2T magnetic field, localised targeting occurred at the cellular level, resulting in a 19.85% ZSN uptake in MCF-7 and 40.66% in MDA-MB-231 cells than non-magnetic targeted cells. **Conclusion:** ZSN is a potential magnetic guiding multi-modal therapeutic system for breast cancer treatment.

Keywords: Zerumbone, Superparamagnetic Iron Oxide Nanoparticle, Nanostructured Lipid Carrier, Nanoparticle, Specific Targeting
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ODD09

The Effects of Repeated Exposure of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) on Bhas 42 Cell Line at Human Physiologically Relevant Doses

Thayvee Geetha Bharathi Silvaragi[1], Azman Seeni[1], Siti Nazmin Saifuddin[1,*]

[1] Department of Toxicology, Advanced Medical and Dental Institute, Universiti Sains Malaysia, 13200 Kepala Batas, Penang, Malaysia

* Corresponding author's email: sitinazmin@usm.edu.my

Introduction: Heterocyclic amines (HCAs) are among the major groups of food carcinogens produced by high-temperature cooking of proteinaceous food. This study aims to examine the effects of two of the most abundant HCAs, 2-amino-1-methyl-6-phenylimidazo-[4,5-b]pyridine (PhIP) and 2-amino-3,8-dimethylimidazo[4,5- f]quinoxaline (MeIQx) on Bhas 42 cells' viability and proliferation upon repeated exposure at human physiologically relevant concentrations. **Methods:** Cytotoxicity and cell proliferation of PhIP- and MeIQx-treated Bhas 42 cells were assessed using the alamar blue assay for 48 hours and 8 days, respectively. In both assays, Bhas 42 cells were exposed to PhIP or MeIQx at concentrations of 10^{-7}, 10^{-8}, 10^{-9}, and 10^{-10} M, respectively, which were diluted in three different vehicle concentrations of DMSO, namely 0.003%, 0.003%, and 0.1%. In the cell proliferation assay, the cells were repeatedly exposed to all sets of treatments every 48 hours for 8 days. **Results:** For cytotoxicity assay, all concentrations of PhIP exhibited a significant toxic effect in 0.003% and 0.03% DMSO with reductions of cell viability up to 19.1% and 75.6 %, respectively whereas MeIQx showed toxic effects only in 0.03% DMSO, specifically at the two highest doses with cell viability reduced up to 90.4% and 75.0%, respectively. In the cell proliferation test, all concentrations of PhIP and MeIQx in all vehicle concentrations did not cause any toxic effects on Bhas 42 cells. **Conclusion:** The current study shows that at human physiologically relevant concentrations, the toxic effect of PhIP and MeIQx on Bhas 42 cells decreased when the cells were exposed to these carcinogens for a longer duration compared to when exposed in a short period of time regardless of DMSO concentrations. Therefore, further research on the carcinogenic potential of those HCAs may need to take these findings into account for better elucidation of their mechanism of action.

Keywords: Cytotoxicity, Heterocyclic amines, PhIP, MeIQX, Bhas 42 cells

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ODD10

RNA Sequencing Analysis of NTCU-Induced Lung Squamous Cell Carcinoma in Mice

Muhammad Asyaari Zakaria[1], Amnani Aminuddin[2], Nor Fadilah Rajab[3], Siti Fathiah Masre[1,*], Eng Wee Chua[2,*]

[1] Centre for Toxicology and Health Risk Studies, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, 50300 Kuala Lumpur, Malaysia

[2] Centre for Drug and Herbal Development, Faculty of Pharmacy, Universiti Kebangsaan Malaysia, 50300 Kuala Lumpur, Malaysia

[3] Centre for Healthy Ageing and Wellness, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, 50300 Kuala Lumpur, Malaysia

* Corresponding authors' email: sitifathiah@ukm.edu.my; cew85911@ukm.edu.my

Introduction: Lung squamous cell carcinoma (SCC) is associated with a high mortality rate because of its assemblage of DNA mutations causing poor therapeutic responses. Therefore, elucidating the molecular events that underpin the pathobiology of lung SCC is essential to the development of effective therapies. **Methods:** We induced lung SCC in N-nitroso-tris-chloroethylurea (NTCU)-treated mice and RNA sequenced the resultant lung tumours. Cleaned sequence reads were aligned to the *Mus musculus* genome (GRCm38/mm10) using Spliced Transcripts Alignment to a Reference (STAR). Then, DESeq2, an R package, was used to normalise per-gene read counts before Gene Set Enrichment Analysis (GSEA). The functional impact of single nucleotide polymorphisms (SNPs) was assessed using the Protein Variation Effect Analyzer (PROVEAN) and Sorting Intolerant From Tolerant (SIFT). Pathogenic DNA variants were defined as having SIFT and PROVEAN scores <0.05 and -2.5, respectively. The final list of SNP-affected genes was analysed using g:Profiler to pinpoint overrepresented pathways. Adjusted *p*-values <0.05 were considered statistically significant. **Results:** The transcriptomic analysis revealed that the top five pathways potentially driving lung SCC development were cholesterol biosynthesis, keratinization, activation of gene expression by SREBF (SREBP), formation of the cornified envelope, and neutrophil degranulation. These pathways are plausibly involved in cancer development; for instance, cholesterol is an activator of tumorigenic signaling pathways and is required for increased membrane synthesis during cell proliferation. However, the association of the pathways with lung SCC has not been extensively reported. The SNP analysis revealed 'homophilic cell adhesion via plasma membrane adhesion molecules' as the most enriched gene ontology. The dysregulation of homophilic cell adhesion is a well-known contributor to cancer cell migration and metastasis. **Conclusion:** Our combinatorial analysis of differentially expressed genes and deleterious SNPs revealed novel, interacting biological pathways that may contribute to lung SCC

development. These pathways are potential targets for effective lung SCC therapy.

Keywords: Lung squamous cell carcinoma (SCC), RNA sequencing, Single Nucleotide Polymorphism (SNP), Cholesterol, Homophilic cell adhesion

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OTM01

ADAR1 Dependency in Oral Squamous Cell Carcinoma

Pei San Yee[1], Annie Wai Yeeng Chai[1], Shi Mun Yee[1], Shiyin Ooi[1,3], Siew Kit Ng[2], Sok Ching Cheong[1,3,*]

[1] Translational Cancer Biology Research Unit, Cancer Research Malaysia, No. 1, Jalan SS12/1A, 47500 Subang Jaya, Selangor, Malaysia

[2] Advanced Medical and Dental Institute, Bertam, Universiti Sains Malaysia, 13200 Kepala Batas, Pulau Pinang, Malaysia

[3] Faculty of Dentistry, University of Malaya, 50603 Kuala Lumpur, Malaysia

* Corresponding author's email: sokching.cheong@cancerresearch.my

Introduction: Our recent genome-wide CRISPR knockout screen revealed that the Adenosine deaminase acting on RNA-1 (*ADAR1*) gene is essential for the survival of a majority of oral squamous cell carcinoma (OSCC) cell lines. Given that deleting ADAR1 caused severe lethality in OSCC, targeting ADAR1 could have therapeutic benefits. ADAR1 catalyzes Adenosine-to-Inosine RNA editing, and suppresses dsRNA sensing-triggered cell death. ADAR1 has two isoforms, the constitutively expressed P110 and the interferon-inducible P150. It is also an interferon-stimulated gene (ISG) that is highly expressed in cancers including OSCC. In this study, we aimed to determine the molecular mechanisms underlying ADAR1 dependency which may afford an opportunity to develop better treatment strategies for OSCC. **Methods:** ADAR1 dependency is validated by competitive co-culture assay, apoptosis and colony formation assay using single-guide RNA (sgRNA) knockout in OSCC cell lines (ORL-48, ORL-214, ORL-174, ORL-195, SCC9 and BICR10). Changes in protein expression upon gene(s) knockout are determined by western blotting. **Results:** We confirmed that ADAR1 depletion significantly increased apoptosis by flow cytometry and inhibited colony formation in selected cell lines. For cell lines that are ADAR1-less dependent (BICR10 and ORL-174), IFN-β treatment increased the expression of ADAR1 and other ISGs, sensitizing these cells to cell death. Notably, overexpression of ADAR1-P150 but not ADAR1-P110, significantly rescued cell lethality in ADAR1 depleted cells suggesting that the ADAR1-P150 is important in the survival of OSCC. We also showed that cell death is mediated by dsRNA sensors protein kinase R (PKR) and melanoma differentiation-associated protein 5 (MDA5) whereby knocking-out either one of these genes did not reverse cell lethality, but co-deleting both genes partially rescued cell death in ADAR1-dependent cell lines. **Conclusion:** The present findings revealed that the interferon-inducible P150 of ADAR1 is essential for OSCC survival. Activation of the dsRNA-sensing pathways such as PKR and MDA5 underlies this dependency.

Keywords: ADAR1 dependency, dsRNA sensing, CRISPR knockout, Interferon-stimulated gene, OSCC

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OTM02

Identification and Evaluation of Tumour-homing Peptides Specifically Targeting Metastatic Breast Cancer Cells

Dhayaalini Bala Gopal[1,*], Yin Quan Tang[1]

[1] School of Biosciences, Faculty of Health and Medical Sciences, Taylor's University Lakeside Campus, 47500 Subang Jaya, Selangor, Malaysia

* Corresponding author's email: dhayaalini.taylors@gmail.com

Introduction: Molecular probes are crucial for early breast cancer diagnosis. Metastatic breast cancer is associated with high mortality and recurrence rates. An active-targeted strategy using homing peptides is an effective approach to diagnose and treat breast cancer as it encompasses prominent specificity and activity through cellular membranes and have lesser toxicity than the vastly used small molecule inhibitors. **Method**: Bioinformatics analysis was carried out to find peptides homing breast cancer cells and to predict the peptide sequence binding to receptors found on metastatic breast cancer cells. Total of 4 peptides were designed based on their binding affinity with highly expressed receptors by breast cancer cells and synthetised for imaging detection purpose. These tumour-homing peptides (THPs) were labelled with fluorescein isothiocyanate (FITC) and were tested on metastatic breast cancer cell lines (MDA-MB-231, MDA-MB-453, T47D & MCF-7) and a normal human breast cell line (MCF-10A). MTT assay was performed to evaluate FITC-labelled THPs and potency in cells exposed to these peptides for up to 24 hours. The binding efficacy of the THPs was then confirmed by fluorescence imaging and detection in human metastatic breast cancer cells. **Results**: Identified positive THPs were termed as MBC-BT1, MBC-BT2, MBC-BT3 & MBC-BT4. After incubation with increasing concentrations of the FITC-labelled THPs for MTT assay, the dose selected for succeeding tests were fixed at 12.5 µg/mL. FITC-labelled MBC-BT1, MBC-BT2, MBC-BT3 & MBC-BT4 peptides bind to MDA-MB-231 breast cancer cells based on fluorescence imaging after 30 minutes of incubation of these peptides with MDA-MB-231 cells. These peptides had no binding to normal human breast cancer cell line MCF-10A. **Conclusion**: The findings of this study produce high potential peptide-based

diagnostic tracers for metastatic breast cancer detection and identification of unique cell surface profile of highly metastatic breast cancer cells, thereby provide new targets for therapeutic intervention.

Keywords: Homing, Peptide, Metastatic breast cancer
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OTM03

An Effort to Establish the Concept of Adaptive Therapy *In Vitro* Using Non-Small Cell Lung Cancer Cells Treated with Afatinib

Amir Imran Faisal Hamdi[1], Jonathan Lim Chee Woei[1], Ummi Nadira Daut[1], How Soon Hin[2], Johnson Stanslas[1,*]

[1] Department of Medicine, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia

[2] Kulliyyah of Medicine, International Islamic University Malaysia

* Corresponding author's email: jstanslas@yahoo.co.uk

Introduction: Drug resistance in cancer becomes a barrier to cancer cure and they are insensitive towards a drug, which requires patients to change to a new set of treatments. It was reported that minor pre-existing drug resistance is present in patients during diagnosis. Although changing treatment options is the only way to eradicate drug-resistant cells, a new theory called Adaptive Therapy (AT) focuses on survival competition between sensitive and resistant cells in treatment-free period. During this period, sensitive cells will outgrow resistant cells for space. Thus, AT possesses the potential to delay the emergence of resistance. This *in vitro* study was done with sensitive and resistant non-small cell lung cancer (NSCLC) cell lines against afatinib. Continuous (96 hours exposed) and intermittent treatment (24 hours, followed by a 72-hour treatment-free period) in a direct co-culture of 0.1% and 0.5% resistance cells. **Methods**: Separate cell viability assays over 96 hours for sensitive and resistant NSCLC cell lines were evaluated. Cell counting was done at 96- and 192-hours of experiment and quantitative PCR (qPCR) with specific primers against sensitive and resistant cells was performed to determine the remaining population. **Results:** The sensitive cell line has a lower IC_{50} value than the resistance cell line (0.3 nM against 300 nM). At 96- and 192-hours, concentrations at 1.0 and 2.0 nM showed higher cell counts for intermittent than continuous by 5000 cells, and qPCR showed gene expression of resistance cells are higher than sensitive cells. **Conclusion:** This preliminary study showed that AT was not able to be established to delay the emergence of NSCLC resistance *in vitro*. One of the shortcomings is that the resistant cells proliferate 24 hours faster than sensitive cells, and this does not reflect the reported scenario in patients and opposes one of the parameters to achieve AT. Thus this study gave resistant cells the advantage to be dominant.

Keywords: Drug resistance, Continuous therapy, Adaptive therapy, Non-small cell lung cancer
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OTM04

Gene Signature for Predicting Homologous Recombination Deficiency in Triple-Negative Breast Cancer

Jia-Wern Pan[1], Pei-Sze Ng[1], Muhammad Mamduh Ahmad Zabidi[1,2], Putri Nur Fatin[1], Jie-Ying Teo[1], Siti Norhidayu Hasan[1], Cheng Har Yip[3], Pathmanathan Rajadurai[3,4], Lai-Meng Looi[5], Nur Aishah Mohd Taib[6], Oscar M. Rueda[7], Carlos Caldas[7,8], Suet Feung Chin[7], Joanna Lim[1], Soo-Hwang Teo[1,9,*]

[1] Cancer Research Malaysia, No. 1, Jalan SS12/1A, 47500 Subang Jaya, Malaysia

[2] Roche (Malaysia), The Pinnacle, Bandar Sunway, 47500 Subang Jaya, Malaysia

[3] Subang Jaya Medical Centre, No. 1, Jalan SS12/1A, 47500 Subang Jaya, Malaysia

[4] Jeffrey Cheah School of Medicine & Health Sciences, Monash University Malaysia, Jalan Lagoon Selatan, Bandar Sunway, 47500 Subang Jaya, Malaysia

[5] Department of Pathology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

[6] Department of Surgery, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

[7] Cancer Research UK, Cambridge Institute & Department of Oncology, Li Ka Shing Centre, Robinson Way, Cambridge CB2 0RE, UK

[8] NIHR Cambridge Biomedical Research Centre and Cambridge Experimental Cancer Medicine Centre, Cambridge University Hospital NHS Foundation Trust, Cambridge, UK

[9] University Malaya Cancer Research Institute, Faculty of Medicine, University Malaya, 50603 Kuala Lumpur, Malaysia

* Corresponding author's email: soohwang.teo@cancerresearch.my

Introduction: Recently, PARP inhibitors have been approved for treatment of triple negative breast cancer in patients with germline or somatic alterations in BRCA1 or BRCA2. Whilst many mechanisms of action have been proposed, it is likely that the response in this group is due to deficiencies in the homologous recombination repair pathway. Thus, a biomarker that is able to identify patients who may have deficiency in homologous repair despite a lack of mutations in BRCA1 or BRCA2 would have significant clinical utility. **Methods:** We designed a nearest-centroid classifier for homologous recombination deficiency (HRD) in Asian TNBCs from the Malaysian Breast Cancer (MyBrCa) cohort using an RNA-seq gene expression dataset of 100 genes (HRD100). The classifier was trained on data from 94 TNBC samples from the MyBrCa cohort, and validated in an

additional 35 and 87 TNBC samples from MyBrCa and TCGA. The classifier was also validated on the NanoString nCounter platform, as well as using FFPE instead of fresh frozen tissue. **Results:** The HRD100 classifier identified samples with strong HRD mutational signature at an AUROC of 0.892 in the MyBrCa training dataset, as well as 0.783 and 0.713 in MyBrCa and TCGA validation datasets, respectively. Analysis of the 100 genes in the HRD100 classifier using the NanoString nCounter platform showed a concordance rate of 98% (CI: 95-100%) with RNA-seq gene expression analyses, and a concordance rate of 87% (CI: 73-100%) between FFPE and fresh frozen tissue. **Conclusion:** Taken together, gene expression using these 100 selected genes may identify triple-negative breast cancer patients with homologous recombination deficiency who may benefit from treatment with PARP inhibitors or platinum chemotherapy.

Keywords: Triple-negative breast cancer, Homologous recombination deficiency, Gene expression signature, PARP inhibitors

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OTM05

Induction of *in vitro* Cytotoxicity in High-Risk Oral Leukoplakia Using a Cancer Vaccine

Chai Phei Gan[1,2,*], Hany Ariffin[1], Sok Ching Cheong[2,3], Kue Peng Lim[2]

[1] Department of Paediatrics, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia.

[2] Cancer Immunology and Immunotherapy Research Unit, Cancer Research Malaysia, 47500 Subang Jaya, Selangor.

[3] Department of Oral and Maxillofacial Clinical Sciences, Faculty of Dentistry, University of Malaya, 50603 Kuala Lumpur, Malaysia.

* Corresponding author's email: chaiphei.gan@cancerresearch.my

Introduction: Patients with oral leukoplakia diagnosed with moderate-severe oral epithelial dysplasia (OED) have an increased risk of developing oral cancer. However, chemoprevention agents targeting epithelial cells are ineffective in preventing malignant transformation and disease recurrence. Emerging evidence indicates that host immunity can impact premalignant disease progression, but the immune profile of oral leukoplakia has not been studied. In this study, we characterized the immune profile of high-risk oral leukoplakia with a long-term aim of identifying immunotherapeutic strategies to intercept cancer development. **Methods:** The immune profile of moderate-severe OED was determined by transcriptomic analysis of 125 immune signatures reported in cancer. To determine the utility of a cancer vaccine targeting MAGED4B, we evaluated MAGED4B expression by immunohistochemistry on oral leukoplakia tissues. Then, *in-vitro* T cell-based immunogenicity studies were performed using patients' blood samples to evaluate antigen-specific immune responses. **Results:** Immune profiling demonstrated the induction of both immune surveillance and immune suppression mechanisms in moderate-severe OED. Notably, three distinct immune subtypes were identified: (1) immune cytotoxic; (2) non-cytotoxic; and (3) non-immune reactive. Patients progressed to cancer appear to lack cytotoxic T cells responses, suggesting that restoring T cell immunity via cancer vaccine may intercept malignant development. Next, we evaluated the feasibility of activating patients' immune responses using a cancer vaccine targeting MAGED4B. We demonstrated that moderate-severe OED significantly over-expressed MAGED4B. Our T cell-based immunogenicity studies showed that MAGED4B-specific $CD8^+$ T cells could be expanded *in-vitro* and become activated as indicated by increased interferon gamma secretion and CD38 expression. Importantly, these $CD8^+$ T cells demonstrated antigen-specific killing of tumour cells expressing MAGED4B. **Conclusion:** Our series of studies demonstrated that discrete immune responses are present in high-risk leukoplakia, and antigen-specific immune response can be harnessed to induce cytotoxic responses in these lesions.

Keywords: Oral leukoplakia, Immune profile, Oral epithelial dysplasia, Cancer vaccine

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OTM06

Characterisation of Primary Cilia (PC) Expression in Oral Cancer Cell Lines with Different Metastatic Potentials

Sakinah Syed Gulam[1,3], Nazia Abdul Majid[1], Siti Amalina Inche Zainal Abidin[2,3,*]

[1] Institute of Biological Sciences, Faculty of Science, Universiti Malaya, 50603 Kuala Lumpur, Malaysia

[2] Department of Oral & Craniofacial Sciences, Faculty of Dentistry, Universiti Malaya, 50603 Kuala Lumpur, Malaysia

[3] Oral Cancer Research & Coordinating Center, Faculty of Dentistry, Universiti Malaya, 50603 Kuala Lumpur, Malaysia

* Corresponding author's email: sitiamalina@um.edu.my

Introduction: Primary cilia (PC), a sensory organelle that exists on the surface of most cells are found in various cancers including prostate, renal, lung, ovarian, and breast cancer. PCs are critical signalling hubs for the maintenance of cell homeostasis through various signalling pathways including Hedgehog, Wnt, Hippo, and ROR2 pathways. Dysregulation of these pathways, therefore, lead to tumorigenesis. While most PC studies focused on cancer related molecular pathway, little is known about intraflagellar-transport 20 (IFT20), a component of IFT machinery that is required

for ciliogenesis. Therefore, PC changes and its associated ciliary signalling in oral cancer cells were investigated. **Methods:** Immortalized normal oral keratinocytes (OKF6- TERT2 cell) and two OSCC cell lines, HSC-2 (non-metastatic OSCC) and HSC-3 (highly metastatic OSCC) cells were cultured in media containing low serum (2%) and high serum (20%) concentrations of foetal bovine serum (FBS). Cells were subjected to serum-starvation for 24, 48, 72 hours and 5 days. For each culture condition, the incidence, length and association of PC with cell proliferation were assessed by fluorescence microscopy. Further, the expression of the ciliogenesis gene, IFT20, was determined by quantitative-RT-PCR. **Results:** An increase in the percentage of ciliated HSC-2 and HSC-3 cells cultured in low and high serum media was observed compared to OKF6-TERT2 cells. Cilia length was longer in HSC-2 and HSC-3 cells compared to OKF6-TERT2 cells, suggesting cilia length abnormalities in oral cancer cells. The number of ciliated cells was increased, and proliferation was decreased in both HSC-2 and HSC-3 cells cultured in low serum and high serum media at all time points. Furthermore, mRNA transcript levels of IFT20 increased in HSC-2 and HSC-3 cells as compared to OKF6-TERT2 cells. **Conclusion:** These findings indicate that an increase of PC and the presence of ciliary defects in oral cancer cells is associated with the upregulation of IFT20.

Keywords: Primary Cilia (PC), Oral Squamous Cell Carcinomas (OSCCs), Oral Cancer, Intraflagellar-transport 20 (IFT20)
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OTM07

The Expression of YKL-40 Protein in Oral Potentially Malignant Disorders (OPMD) and Oral Squamous Cell Carcinoma (OSCC)

Nur Fatinazwa Mohd Faizal[1], Karen-Ng Lee Peng[2], Zuraiza Mohamad Zaini[1,2,*]

[1] Department of Oral and Maxillofacial Clinical Sciences, Universiti Malaya, Kuala Lumpur, Malaysia

[2] Oral Cancer Research and Coordinating Centre, Universiti Malaya, Kuala Lumpur, Malaysia

* Corresponding author's email: zuraiza@um.edu.my

Introduction: Oral squamous carcinoma cell (OSCC) is the most common type of oral cancer, which results in significant morbidity and mortality. In OSCC, the progression from normal mucosa to different grades of dysplasia and to invasive carcinoma is characterized by an increased formation of angiogenesis. There is much evidence that indicated YKL-40, a secreted glycoprotein, was highly elevated in cancers and associated with tumour angiogenesis. Being a potent angiogenic factor capable of stimulating development and vascularization of tumour during carcinogenesis, the role of YKL-40 in oral dysplasia and its progression remains unknown. Hence, this study aims to determine the expression of YKL-40 in OSCC and its potential role in the oral carcinogenesis. **Methods:** Secreted YKL-40 protein levels were screened by enzyme-linked immunosorbent assays (ELISA) in serum samples from 16 healthy donors, 14 oral precancer and 22 OSCC patients as well as conditioned media collected from oral epithelial dysplasia (OED) and OSCC cell lines. In addition, we employed immunohistochemical (IHC) detection in 75 oral tissue samples consisting of 20 normal oral mucosa (NOM), 35 OED and 20 OSCC cases. To determine the effect of YKL-40 on vascular endothelial angiogenesis, migration assay and tube formation assay of human umbilical vein endothelial cell (HUVEC) was performed. **Results:** YKL-40 protein expression in serum was the highest in OSCC followed by oral dysplasia and normal samples. Similar trend was seen in cell lines whereby OSCC exhibited higher YKL-40 expression as compared to dysplasia cell lines. IHC results showed that YKL-40 was highly expressed across the epithelium in OSCC, and OED compared to NOM tissue. Additional of exogenous recombinant YKL-40 prompted HUVEC migration and tube formation compared to control. Notably, YKL-40 also enhanced migration of HUVEC induced by conditioned medium of OSCC cells. **Conclusion:** These findings provide novel insights into angiogenic activities of YKL-40 in oral cancer development.

Keywords: Oral squamous carcinoma cell, Oral epithelial dysplasia, Angiogenesis
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OTM08

Preliminary Study of BRF2 Expression in Subset of Head and Neck Cancers

Noor Akmar Nam[1,*], Siti Nur' Aqilah Halimi[2], Muhammad Iqbal Mustaqim Abd Razak[2], Muhd Shamsuzzaman Ilham Fitri[3], Tuan Norhayati Tuan Mahmood[3], Asfizahrasby Mohd Rasoul[4]

[1] Department of Basic Sciences, Faculty of Dentistry, USIM, Pandan Indah, Ampang, Kuala Lumpur

[2] Faculty of Dentistry, USIM, Pandan Indah, Ampang, Kuala Lumpur

[3] Faculty of Applied Sciences, UiTM Shah Alam, Selangor

[4] Department of Oral and Maxillofacial Surgery, Pathology and Medicine, Faculty of Dentistry, USIM, Pandan Indah, Ampang, Kuala Lumpur

* Corresponding author's email: noorakmar@usim.edu.my

Introduction: Head and neck cancers (HNC) are abnormal cell proliferation in the mucosal surfaces involving several anatomical sites such as oral cavity, sinonasal cavity, pharynx, and larynx. In Malaysia, HNC especially involving the nasopharyngeal cancer is the fourth most common cancer, with total new cases of 2,222 detected in 2020. Determination of the potential biomarkers for

early diagnosis of this disease is crucial. Transcription factor II B (TFIIB)-related factor 2 (BRF2) is one of the important transcription factors, regulating the RNA Polymerase III (Pol III) gene transcription. Dysregulation of Pol III transcription mediated by BRF2 lead to abnormal cell growth and cancer progression. This study aimed to compile and analyse data on BRF2 expression related to HNC using online database and immunohistochemistry (IHC) analysis. **Methods**: Information regarding BRF2 protein and specific mRNA transcripts were retrieved from Human Protein Atlas (HPA), Oncomine, GEPIA, and cBioPortal databases. IHC analysis was performed on tissue microarrays (TMA) containing normal and a subset of HNC involving the lip tissues using BRF2 and PCNA antibodies as comparison. **Results**: HPA revealed overexpression of BRF2 protein in the nuclear region, detected in 25-75% cancer cells of all HNC cases (n=499) with staining intensity ranging from moderate to strong. In Oncomine, BRF2 shown differential expression between normal vs tumour tissues whereby 4/8 datasets showed upregulation of BRF2 transcripts. GEPIA also reported mRNA overexpression for BRF2 (8.6 vs 5.32) in head and neck squamous cell cancer (HNSC) compared to normal tissue. IHC staining revealed varying degree of BRF2 expression in HNC involving the lip as compared to normal tissue controls. **Conclusion:** Compiled data suggest that BRF2 is commonly overexpressed in HNC studied. Further study is essential to evaluate BRF2 expression in more HNC subsets as tissue distribution is heterogenous and involved large anatomical sites

Keywords: RNA Polymerase III, BRF2, Head and Neck Cancers, Oral Cancers, Biomarkers

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PDD01

Synergistic Growth Inhibitory Effect of Hydroxyurea and SRJ23 Combination on T-ALL Jurkat Cells

Laith Marashdeh[1], Johnson Stanslas[1], Thilakavathy Karuppiah[2,3], Bahariah Khalid[1,*]

[1] Department of Medicine, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

[2] Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

[3] Genetics and Regenerative Medicine Research Group, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

* Corresponding author email: bahariah@upm.edu.my

Introduction: T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive haematologic neoplasm that accounts for about 15% and 25% of children and adults, respectively of all types of acute lymphoblastic leukemia. Treatment has not shown positive curative sustenance and is cost-effective. SRJ23, a bicyclic lactone, is a novel semi-synthetic derivative of andrographolide while hydroxyurea (HU) is a known inexpensive anticancer agent causing suppression of DNA synthesis by inhibiting ribonucleoside diphosphate reductase and inhibits GDP-GTP exchange to induce apoptosis. We aim to evaluate the inhibitory effect of HU in combination with SRJ23 on the Jurkat cell line and determine its efficacy. **Methods:** T-ALL (Jurkat cells) were seeded and treated with different concentrations of SRJ23 (0.1, 1, 10, 100 μM) and HU (7.8, 15.6, 31.25, 62.5, 125, 250, 500, and 1000 μM). After 96 hours of incubation, the MTT assay was used to assess the *in vitro* growth inhibition of the combination towards the cells. An apoptosis assay was also performed by flow cytometry technique using Annexin V-FITC and Propidium Iodide (PI) double staining assay. **Result:** The dose-response curves showed a reduction of cell viability with a synergistic effect with a combination of concentration of 10 μM SRJ23 with 25 μM HU (combination index of 0.53) or 250 μM HU (combination index of 0.40). The combination induced enhanced apoptosis when compared with single-agent alone. **Conclusion:** The combination of HU and SRJ23 had a remarkable synergistic effect inhibiting T-ALL Jurkat cells by inducing apoptosis. The combination needs to be tested further both *in vitro* and *in vivo* in order to assess how well it works as a potential novel targeted therapy.

Keywords: T-ALL, SRJ23, Hydroxyurea, Synergistic effect, MTT, Apoptosis

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PDD02

The *In Vitro* Cytotoxic Evaluation and Apoptotic Effects of Histone Deacetylase (HDAC) Inhibitory Drugs on Anaplastic Large Cell Lymphoma (ALCL) Cell Lines

Mutaz Jama[1] Al-Khreisat[1], Abdul Aziz Mohamed Yusoff[2], Faezahtul Arbaeyah Hussain[3], Muhammad Farid Johan[1,*]

[1] Department of Haematology, School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian, Malaysia

[2] Department of Neurosciences, School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian, Malaysia

[3] Department of Pathology, School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian, Malaysia

* Corresponding author's email: faridjohan@usm.my

Introduction: Anaplastic large cell lymphoma (ALCL) is a subtype of aggressive peripheral T-cell lymphoma (PTCL). ALCL develops from T-lymphocytes (T-cells) and approximately 10-20% of all T-

non-Hodgkin lymphomas (T-NHL). CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or CHOP-like anthracycline-containing combination chemotherapy is common treatment used in treating T-NHL including ALCL. The response to these regimes varies in ALCL patients. Hence, it is hypothesised that the disease may exhibit variable molecular biological behavior, which would affect how it responds to the conventional treatment. Alternative drugs with low toxicities and affecting epigenetic behavior like histone deacetylase need to be explored as new drug option to ALCL. Our study aimed to assess the cytotoxicity and apoptotic effects of histone deacetylase (HDAC) inhibitory drugs (Trichostatin A and Panobinostat) on ALCL cell lines. **Methods:** ALCL cell lines (SU-DHL-1, Ki-JK, and DL-40) were treated with different concentrations of Trichostatin A and Panobinostat to determine the cytotoxicity. 5-Azacytidine, an epigenetic drug that inhibits DNA methylation was used as positive control. Half maximal inhibitory concentration (IC50) values were determined by MTS cell viability assays after 48h of treatment and calculated with GraphPad Prism Software version 3 using a dose–response inhibition curve. Apoptosis assays were performed using Annexin V-FITC binding assays and analysed by flow cytometry. **Results:** Trichostatin A and Panobinostat were cytotoxic against all ALCL cell lines and Ki-JK were the most sensitive cell lines with IC50 211.7± 7.2 nM and 15.24± 3.4 nM, respectively. Trichostatin A and Panobinostat induced apopotosis in all ALCL cell lines with significantly higher apoptotic induction in Ki-JK ($p<0.05$). **Conclusion:** HDACs drugs have significance effects on all ALCL cell line as the dose concentration were low, no cytotoxicity, and have a great impact on apoptosis within a short period of time.

Keywords: ALCL, Lymphoma, Epigenetics, Trichostatin A, Panobinostat

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PDD03

Preliminary Study of Structure-Activity Relationship of Xanthone and Jacareubin Derivatives in Inhibiting Aromatase Activity via in Vitro and in-Silico Approaches

Salsabiilaa M. Razib[1,*], Nadiah Mad Nasir[1,*], J Stanslas [2], Muhammad A M. Latif [3], Nur Qurratu Ain A. Nordin[1], Pavithren Devakrishnan[1]

[1] Department of Chemistry, Faculty of Sciences, University Putra Malaysia, Selangor, Malaysia

[2] Department of Medicine, Faculty of Medicine and Health Sciences, University Putra Malaysia, Selangor, Malaysia

[3] Centre of Foundation Studies, University Putra Malaysia

* Corresponding authors' email: salsabiilaarazib@gmail.com & nadiahmadnasir@upm.edu.my

Introduction: Jacareubin is a plant-derived natural xanthone that has a strong inhibitory effect on human breast cancer cells. According to a previous study, Jacareubin is less toxic and more effective than the conventional medication, 5-Fu (IC50 17.01±0.23), with an IC50 of 6.28±0.47. (Sun et al., 2016). Strong interactions were discovered in the binding pocket of aromatase (3EQM) by an initial molecular docking study on Jacareubin. However, there is still a dearth of knowledge regarding how this structure would affect the binding strength of these compounds. We propose that changes to the main structure of Jacareubin with a more varied array of substituents will significantly affect inhibitory activity of the xanthonoids. **Methods:** The Grover, Shah, and Shah (GSS) reaction is used to synthesis xanthonoids in a single pot. It involves phenol-benzoic acid condensation followed by direct cyclization of the benzophenone intermediate to produce the xanthonoid product. Nuclear Magnetic Resonance (NMR), Gas Chromatography-Mass Spectrometry (GC-MS), and Fourier Transform Infrared Spectroscopy (FTIR) are used to analyze newly synthesized compounds. The biological activity of the synthetic xanthonoids is evaluated against breast cancer cell lines via MTT assay. **Results:** There are nine xanthones and seven Jacareubin derivatives that have been synthesized. The molecular docking study shows that the derivatives have higher binding energy (-8.3 kcal/mol to -9.8 kcal/mol) than the Jacareubin (-9.4 kcal/mol) which the value of IC50 is 6.28±0.47μM. The IC50 value for 8-fluoro-1,3-dihydroxy-9H-xanthen-9-one (-8.3 kcal/mol) and 9-fluoro-5- hydroxy-2,2-dimethylpyrano[3,2-b] xanthen-6(2H)-one (-9.8 kcal/mol) are 3 μM and 60 μM respectively. **Conclusion:** The preliminary study showed that the IC50 value of 9- fluoro-5- hydroxy-2,2-dimethylpyrano[3,2-b] xanthen-6(2H)-one was higher than the Jacareubin due to how intensely this derivative interacts with aromatase binding sites (3EQM). Aromatase and ligand have a binding energy of -9.8 kcal/mol. Thus, this Jacareubin derivative may prevent human breast cancer from spreading throughout the body.

Keywords: Xanthone, Jacareubin, Gemcitabine, Breast cancer, Aromatase.

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PDD04

Synthesis of PEGylated Liposome Co-loaded with Doxorubicin Hydrochloride and Tumour Suppressor miR-145 Mimics for Its Anti proliferative Effect Against Triple Negative Breast Cancer *In vitro*

Chu Xin Ng[1], Chee Wun How[2], Pei Pei Chong[1], Sau Har Lee[1,*]

[1] School of Biosciences, Faculty of Health and Medical Sciences, Taylor's University, Lakeside Campus,
Selangor, Malaysia
[2] School of Pharmacy, Monash University Malaysia, Selangor, Malaysia
* Corresponding author's email: sauhar.lee@taylors.edu.my

Introduction: Doxorubicin hydrochloride (Dox-HCl) is a widely prescribed anti-cancer drug for the treatment of breast cancer. However, the clinical application of Dox-HCl is constrained, especially in triple-negative breast cancer (TNBC) due to its toxicity and the aggressiveness of the tumour. Meanwhile, microRNA-145 (miR-145) is known to be a tumour suppressor miRNA (tsmiRs) exhibiting anti-proliferative and anti-metastasis effect against various cancers. However, there is no evidence delineating the combination use of miR-145 mimics with Dox-HCl co-loaded in nanoparticles that increases the therapeutic efficacy on TNBC when compared to individual or combinational treatment without nano-carrier. The aim of this study is to synthesise and characterise Dox-HCl and miR-145 mimics co-loaded in PEGylated liposome and to investigate its *in vitro* anti-proliferative activity against MDA-MB-231 cells. **Methods:** Dox-HCl and miR-145 mimics co-loaded in PEGylated liposomes were formulated according to composite central design. Response models were developed to investigate the correlation of formulation parameters toward nanoparticle size (d.nm) and encapsulation efficiency (EE%) of both Dox-HCl and miR-145 mimics. The ideal liposome formulation was further characterised for its *in vitro* stability, drug release, cellular uptake, and cellular toxicity. **Results:** Statistical analysis of the response models indicated that formulation 6 (F6) exhibited the highest desirability function (D=0.934) with optimum nanoparticle size (137.1±0.4d. nm) and high EE% for both Dox-HCl (67.31±3.76%) and miR-145 mimics (87.85±4.60%). F6 displayed great stability over 60 days at 4°C with stable nanoparticle size and zeta potential (p<0.05), while the EE% of Dox-HCl and miR-145 mimics were 94.97±0.53% and 51.96±2.67%, respectively. At 48 hours, F6 displayed increased *in vitro* cellular uptake at 1.3 folds along with maximal drug release, which correlate to its higher toxicity (IC50=0.58±0.02μM) against MDA-MB-231 cells than the free regimen (IC50=1.03±0.07μM). **Conclusion:** The current findings suggest that PEGylated liposome shows potential in targeted delivery of anti-cancer drugs and therapeutic miRNAs into tumour cells, hence warrant further investigation.

Keywords: Breast cancer, Tumour suppressing miRNAs, Dox-HCl, Liposome, Nanoparticles
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PDD05

Anti-pancreatic Cancer Stem Cell Activity of a Semi-synthetic Andrographolide Derivative

Michelle Dass[1], Dineshwar Sugumaran[2,+], Chee-Woei Lim[2], Johnson Stanslas[2,*], Audrey Chee-Hui Yong[1,*]
[1] Department of Medicinal Chemistry, Faculty of Pharmacy, MAHSA University, Selangor, Malaysia.
[2] Pharmacotherapeutics Unit, Department of Medicine, Faculty of Medicine and Health Sciences, Universiti
Putra Malaysia, UPM Serdang, Selangor, Malaysia
+Presenting author
* Corresponding authors' email: audrey@mahsa.edu.my and rcxjs@upm.edu.my

Introduction: Pancreatic cancer is one of the deadliest cancers, with a high fatality rate approaching 95-100%. The development of pancreatic cancer is strongly affected by cancer stem cells (CSCs), which are a small subset of the cancer population. Current treatment strategies have shown drawbacks in combating pancreatic cancer due to the presence of these cancer stem cells. Moreover, pancreatic cancer cells easily acquire resistance to gemcitabine, standard chemotherapy. Studies have indicated that andrographolide, a naturally occurring agent, exhibits various pharmacological properties, including anti-cancer and anti-inflammatory activities. In this study, the anti-pancreatic cancer stem cell activity of andrographolide and its derivative, Raspholide was evaluated *in vitro*. **Methods:** Pancreatic cells Capan-2 were treated with andrographolide, raspholide, as well as dimethylsulfoxide, gemcitabine, sodium chloride, vismodegib (positive control for anti-CSCs), and benzimidazole (anti-Ras agent). The effect of the treatment was assessed via a tumoursphere assay and flow cytometry analysis. **Results:** It was found that raspholide had a significant effect on the tumorsphere number reduction with a p-value less than 0.05 compared to andrographolide. Raspholide was also able to target the main cancer stem cell markers, CD24+, CD44+, and CD133+ with a value of 4.3%, comparable to vismodegib. **Conclusion:** Raspholide exhibits anti-cancer activity against pancreatic cancer stem cells.

Keywords: Anti-pancreatic cancer stem cells, Andrographolide, Raspholide, Tumoursphere assay, CD markers
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PDD06

Transforming Growth Factor-β (TGF-β) may Regulate Progression of Triple Negative Breast Cancer Intrinsic Subtypes via Differential Activation of TGF-β Isoforms

Ezanee Azlina MH[1,*], Afreena Afiqah A[1], Nurul Nadiah AD[1], Siok-Fong C[1], Nor Fadilah R[2], Reena Rahayu MZ[3], Rohaizak M[4], Norlia A[4], Nani Harlina ML[4]
[1] UKM Medical Molecular Biology Institute (UMBI), Universiti Kebangsaan Malaysia (UKM), Cheras,

Malaysia
[2] Centre for Healthy Aging & Wellness, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia.
[3] Faculty of Medicine (Pathology Department), Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia.
[4] Department of Surgery, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia.
* Corresponding author's email: ezanee.azlina.mohamad.hanif@ppukm.ukm.edu.my.

Introduction: Transforming growth factor-β (TGF-β) is known to play significant dual roles in cell development and pathophysiology but very cell-specific dependent. Due to these features, it has been concomitantly associated in the development of triple negative breast cancer (TNBC) via activation of TGF-β signaling pathway. In house baseline studies directed the involvement of TGF-β signaling in TNBC via differentially expression analyses and may be regulated by SETDIA, an epigenetic regulator. This study aimed to identify differentially expressed genes by SETD1A knockdown and elucidate the molecular mechanism of TGF-β signaling across isoforms. **Methods:** Two TNBC cell line models (FEC-sensitive MDA-MB-468 and FEC-resistant Hs578T; FEC – 5-flurouracil, epirubicin, cyclophosphamide chemo cocktail) were transfected by SETDIA siRNA and RNA were subjected for the Nanostring Cancer Progression Panel. Differentially expressed mRNA and pathway analysis were performed using the nSolver Software. RqPCR were carried out to confirm downstream regulated genes. Wound scratch, colony forming, invasion assays were conducted to complement the gene mechanisms. **Results:** Differentially expressed genes (TGF-β1, SMAD1, ID2, ZEB1) narrowed down TGF-β signaling among top hit pathways. Each gene was found to be differentially expressed between the MDA-MB-468 and Hs578T cell lines. TGF-β1, TGF-β3, SMAD1, SMAD2 and ID2 genes were upregulated and TGF-β2 was suppressed by SETD1A knockdown in FEC-resistant TNBC (Hs578T). These genes were inversely expressed in MDA-MB-468 cell line. SETD1A knockdown inhibited proliferative effects in TNBC cell lines, complemented by downregulation of Ki-67 by colony forming assay. Reduction wound closure were observed upon SETD1A knockdown. Induction of invasion were seen in TGF-β2 and TGF-β3 in a recovery setup. **Conclusion:** TGF-β signaling maybe a key regulator of TNBC development via activation of epigenetic regulator SETD1A. TNBC may be regulated via intrinsic mechanisms differing between FEC-sensitive and FEC-resistant cells, indicating a potential personalised therapeutic intervention in the two intrinsic TNBC groups. However, further evaluation is warranted to extend the understanding of TNBC progression by TGF-β signaling activation.

Keywords: TNBC, TGF-β, SETD1A, Chemoresistance, Epithelial-Mesenchymal-Transition, Triple negative breast cancer
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PDD07

Differential Cell Cycle Arrest in Dihydroorotate Dehydrogenase (DHODH) Inhibition of Different Subtypes of Breast Cancer Cell Lines

Muhammad Aiman Akmal Shahhiran[1,2], Mohamad Fairus Abdul Kadir[3], Nurshamimi Nor Rashid[1,2], Shatrah Othman[1,2,*]
[1] Department of Molecular Medicine, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur,
Malaysia
[2] Drug design and Development Research Group, University of Malaya, 50603 Kuala Lumpur, Malaysia
[3] Aurigene Discovery Technology (M) Sdn Bhd, Level 2 Research Management and Innovation Complex,
University of Malaya, 50603 Kuala Lumpur, Malaysia
* Corresponding author's email: shatraho@um.edu.my

Introduction: Dihydroorotate dehydrogenase (DHODH) inhibitors hold a great potential for breast cancer treatment especially in combinatorial therapy due to their antitumour capability. Brequinar and teriflunomide, the two potent DHODH inhibitors, showed cell cycle arrest in several cancer cells. These inhibitors target DHODH, the key enzyme in the *de novo* pyrimidine biosynthesis pathway that generates nucleotides essential for cells to proliferate. While the treatment for breast cancer is closely associated with the molecular subtyping (ER/PR and HER2 receptors) that has major implications on the survival of breast cancer patients, yet, the effect on the distinct hormone receptor status by these inhibitors has not been fully investigated. Thus, this study aimed to investigate the differential effects of breast cancer subtypes on the phases of cell cycle preferentially arrested by DHODH inhibitors. **Methods:** Three breast cancer cell lines with different receptor status were selected to represent each subtype; namely the MCF-7 ($ER/PR^+/HER2^-$), SKBR-3 ($ER/PR^-/HER2^+$), MDAMB-231 ($ER/PR^-/HER2^-$, also referred to as triple negative) and one non-tumorigenic MCF-10A ($ER/PR^-/HER2^-$) cells. Cells were cultured and treated with variable concentrations of inhibitors. Cell cycle analysis was performed by staining with propidium iodide and examined through BD FACSCanto™ II and analysed using Flow Jo software. **Results:** Results showed that MCF-10A, MCF-7 and MDAMB-231 were arrested at G1/S phase of the cell cycle by both inhibitors. Meanwhile, SKBR-3, the only cell line with $HER2^+$, did not exhibit any distinct cell cycle phase arrest between both inhibitors and the control. **Conclusion:** The present study showed that the phase of cell cycle arrested in DHODH inhibition in breast cancer is

independent of the ER/PR status, but may depend on the HER2 status, a finding that warrants further analysis on other cell lines.

Keywords: Breast cancer, DHODH, Brequinar, Teriflunomide, Receptor subtypes

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PDD08

Chemopreventive Effects of Curcumin, 10-Gingerol, 6-Gingerol, 10-Shogaol, and 6-Shogaol on Cervical Cancer Cells HeLa and Siha

Unwaniah Abdull Rahim[1], Marami Mustapa[2], Nur Aishah Che Roos[3], Nursiati Mohamad Taridi[1], Armania Nurdin[4], Yasmin Anum Mohd Yusof[1,*]

[1] Biochemistry Unit, Faculty of Medicine and Defense Health, National Defense University of Malaysia, Kuala Lumpur, Malaysia

[2] Anatomy Unit, Faculty of Medicine and Defense Health, National Defense University of Malaysia, Kuala Lumpur, Malaysia

[3] Pharmacology Unit, Faculty of Medicine and Defense Health, National Defense University of Malaysia, Kuala Lumpur, Malaysia

[4] Department of Biomedical Science, Faculty of Medicine and Health Science, Universiti Putra Malaysia, Selangor, Malaysia

* Corresponding author's email: yasmin.anum@upnm.edu.my

Introduction: Turmeric and ginger are among the most commonly used food condiments that are studied for their use as chemopreventive agents. Bioactive compounds curcumin in turmeric and gingerol and shogaol in ginger have been shown to modulate multiple cell signaling pathways involved in carcinogenesis including apoptosis, cancer cell survival (PI3K/AKT), inflammation (NF-kB, IL-6, and TNF), and proliferation (EGFR and AP-1) pathways. Very few studies have explored the chemopreventive role of these bioactive compounds in cervical carcinogenesis. This research aims to elucidate the chemopreventive properties of curcumin, 6/10-gingerols, and 6/10-shogaols on cervical cancer cells HeLa and SiHa. **Method:** MTT assay was used to determine the cell growth inhibition of curcumin, 6/10-gingerols, and 6/10-shogaols in different concentrations ranging from 3 to 100 μM. **Results**: Curcumin and shogaols are the most potent compounds against both cervical cancer cells. The decreasing order of inhibition effects of curcumin and ginger bioactive compounds on the growth of cervical cancer cells are as follows (mean ± SD, n=3): for HeLa cells; Curcumin (IC50=31.2 ± 1.7μM)> 10-shogaol (IC50=36.3 ± 3.2μM)> 10-gingerol (IC50=65.5 ± 1.5μM)> 6-shogaol (IC50=68.8 ± 2.4μM)> 6-gingerol (IC50=83.3 ± 10.5μM). For SiHa cells; Curcumin (IC50=17.8 ± 2.5μM)> 6-shogaol (IC50=54.5 ± 10.6μM)> 10-gingerol (IC50=69.8 ± 0.8μM)> 10-shogaol (IC50=83.0 ± 9.9μM)> 6-gingerol (IC50=88.2 ± 3.8μM). **Conclusions:** Our study shows that curcumin and 6/10-shogaols have higher inhibitory effects compared to 6/10-gingerols on the growth of both cervical cancer cells, HeLa and SiHa. The chemopreventive effect of 10-shogaol and 10-gingerol is most likely due to longer carbon chain length which plays a significant role in antiproliferative effects and initiating apoptosis. Further investigations will be conducted to determine the optimal formulation of curcumin in combination with 6/10-gingerol, and 6/10-shogaol compounds against cervical cancer cells.

Keywords: Cervical cancer cell, Chemoprevention, Curcumin, Gingerol, Shogaol

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PDD09

In silico* Prediction and Physicochemical Analysis of Mutant Anticancer Short-length Pardaxin 6 Peptide Fragments Derived from *Pardachirus Marmoratus

Sau Har Lee[1,2,*], Yong Hui Wong[1]

[1] School of Biosciences, Faculty of Health and Medical Sciences, Taylor's University, Subang Jaya, Selangor

[2] Centre for Drug Discovery and Molecular Pharmacology (CDDMP), Faculty of Health and Medical Sciences, Taylor's University, Subang Jaya, Selangor

* Corresponding author's email: sauhar.lee@taylors.edu.my

Introduction: Cancer is a worldwide health issue, and conventional therapies are facing obstacles due to drug resistance and a wide range of side effects. Interestingly, peptides have emerged as promising therapeutic alternatives in the pharmaceutical industry, especially in the fight against cancer. Recent research utilised marine-derived sources of lead compounds in the drug discovery field for the treatment of various diseases, including their anti-cancer potential. Herein, we aim to study the anticancer activities of Red Sea Moses sole, *Pardachirus marmoratus*-derived peptides, namely pardaxin 6, in the form of short-length peptides through *in silico* approach. **Methods:** Fragmented peptides ranging from 5 to 15 amino acids were derived from parental peptides. These peptides were further mutated (quote technique), and along with the original fragmented peptides, were predicted for their Support Vector Machine (SVM) scores and physicochemical properties. The top mutant peptides were further examined for their toxicity, hemolytic probability, peptide structures, docking models (state enzyme/ receptor/ pathway) and energy scores using various web servers. The trend of fragmented and mutant peptides SVM scores, hemolytic possibility, and docking energy scores across 5 to 15 amino acid fragments were analyzed. **Results:** Results showed that when the amino acid numbers increased, the original

peptides' SVM score increased, whereas the mutant peptides showed a decreasing trend. Similarly, for the mutant peptides analysis, both the hemolytic probability and docking energy scores towards the FAS receptor showed an increasing pattern as the peptide length built up. **Conclusion:** This *in silico* prediction found shorter length pardaxin 6 peptide fragments derived from *Pardachirus Marmoratus* that could have good potential to be developed as anti-cancer agents in the future.

Keywords: *In silico* prediction, Pardaxin, Anticancer peptides, *Pardachirus marmoratus*-derived peptides, Cancer
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PDD10

Cytotoxic and Anti-angiogenic Effects of Postbiotics Derived from *Pediococcus* spp. against CT26 Mouse Colon Carcinoma Cells

Umi Khalsom Mohd Bajuri[1], Kalavathy Ramasamy[1], Siong Meng Lim[1,*]

[1] Collaborative Drug Discovery Research (CDDR) Group, Faculty of Pharmacy, Universiti Teknologi MARA
(UiTM) Cawangan Selangor, Kampus Puncak Alam, 42300 Bandar Puncak Alam, Selangor, Malaysia

* Corresponding author's email: lim219@uitm.edu.my

Introduction: The current pharmacological treatments against colorectal cancer (CRC) are often compromised by their side effects and cancer resistance. As the majority of CRC are sporadic in nature and associated with diet, current efforts have been directed towards alternative approaches which include probiotics. There is now evidence indicating the strain-dependent usefulness of probiotic-derived bioactive metabolites (i.e., postbiotics) against CRC. There are also reports on *Lactobacillus* and *Bifidobacterium* probiotics that inhibit tumour angiogenesis which drives the growth of CRC. As such, this study aimed at assessing the cytotoxic and anti-angiogenic potentials of cell free supernatant (CFS) fermented by two unique strains of lactic acid bacteria (LAB) isolated from fermented tapioca *in vitro*. **Methods:** LAB identification was performed by sequencing the 16S rRNA gene of the LAB and matching the consensus sequence from GeneBank. Cytotoxicity of the LAB were then screened by using Sulforhodamine B (SRB) assay. Immunocytostaining of CT26 cells treated with LAB-derived CFS was performed at their respective highest subtoxic concentration (IC_{15}) for examination of the anti-angiogenicity potential. Lastly, high performance liquid chromatography (HPLC) analyses was undertaken to determine the concentrations of four major short chain fatty acids (SCFA; i.e., acetate, butyrate, lactate and propionate) in the LAB-derived CFS. **Results:** The LAB were identified as *Pediococcus pentosaceus* LAB3 and *Pediococcus acidilactici* LAB4 (99% similarity), respectively. LAB4 emerged as the most potent *Pediococcus* sp. against CT26 cells with IC_{50}=4.67$\pm$0.3%. Immunocytostaining of CT26 cells treated with LAB-derived CFS (especially LAB4) resulted in downregulation ($\leq$6%) of pro-angiogenic vascular endothelial growth factor (VEGF) and upregulation ($\leq$42%) of anti-angiogenic thrombospondin (TSP-1). HPLC analyses found LAB4-derived CFS to yield the highest concentration for lactate and propionate. **Conclusion:** The present findings imply the potential strain-dependent cytotoxic and anti-angiogenic properties of LAB-derived postbiotics against CRC. The potent cytotoxic and anti-angiogenic effects of LAB4 may be associated with the production of SCFA.

Keywords: Lactic acid bacteria, Colorectal cancer, Cytotoxicity, Angiogenesis, Short chain fatty acids
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PDD11

Evaluation of the Cytotoxic Potential of the Crude Extracts from Marine Microalga *Isochrysis* sp.

Umme Tamanna Ferdous[1], Armania Nurdin[2,3], Saila Ismail[4], Khozirah Shaari[5,6], Zetty Norhana Balia Yusof [1,4,7,*]

[1] Aquatic Animal Health and Therapeutics Laboratory (AquaHealth), Institute of Bioscience, Universiti Putra
Malaysia, 43400 Serdang, Selangor, Malaysia

[2] Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia,
43400 UPM Serdang, Selangor, Malaysia.

[3] Laboratory of UPM-MAKNA Cancer Research (CANRES), Institute of Bioscience, Universiti Putra Malaysia,
43400 UPM Serdang, Selangor, Malaysia.

[4] Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia,
43400 Serdang, Selangor, Malaysia

[5] Natural Medicines and Products Research Laboratory, Institute of Bioscience, Universiti Putra Malaysia,
43400 Serdang, Selangor, Malaysia

[6] Department of Chemistry, Faculty of Science, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

[7] Bioprocessing and Biomanufacturing Research Complex, Universiti Putra Malaysia, 43400 Serdang, Selangor,
Malaysia

* Corresponding author's email: zettynorhana@upm.edu.my

Introduction: Breast cancer is the most diagnosed cancer worldwide. Due to the side effects and drug resistance of chemotherapeutic drugs, a search for alternative anticancer agents from natural sources, especially marine sources, is warranted. *Isochrysis* sp. is a marine microalga that produces a wide range of pharmaceutically important metabolites but there is a lack of data about the cytotoxic activities of this microalga. Hence, this research is planned to investigate the cytotoxicity of marine

Isochrysis sp. crude extracts against a human breast cancer cell line (MCF-7) and to evaluate its mode of cell death. **Method:** Freeze-dried *Isochrysis* sp. biomass was extracted with eight solvents with different polarities through sonication and maceration. The algal extracts were evaluated for their cytotoxic effect at 100 µg/mL concentration against the MCF-7 cell line using MTT assay. The most cytotoxic extract of *Isochrysis* sp. was further investigated for apoptosis induction in MCF-7 cells through flow cytometry and RT-PCR. **Results:** Among eight extracts, the ethanol extract of *Isochrysis* sp. reduced the cell viability of MCF-7 cells to 7.24 ± 0.47% after 72 hours of incubation, at a concentration of 100 µg/ml. The IC_{50} (half maximal inhibitory concentration) value was 13.37 ± 0.59 µg/ml after 24 hours in MCF-7 cells and >100 µg/ml in non-cancerous human lung fibroblast cells, MRC-5. Morphological observation under a light microscope revealed cell shrinkage, condensation of cellular contents, and membrane blebbing in treated MCF-7 cells compared to untreated cells. The Annexin V-FITC and PI staining analysis confirmed that the mode of cell death is mainly apoptosis. Cell cycle analysis revealed the accumulation of cells in the sub-G_1 phase and G2/M arrest. An up-regulation of the proapoptotic *Bax* gene and tumor suppressor *p53* gene was observed through RT-PCR. **Conclusion:** The data suggest that crude ethanolic extract from marine *Isochrysis* sp. has induced apoptosis in MCF-7 and may have potential therapeutic value for human breast cancer.

Keywords: Apoptosis, Cytotoxic, *Isochrysis* sp., Marine microalgae, MCF-7

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PDD12

Phytochemical Composition and Anticancer and Cisplatin-sensitising Activities of Green Macroalgae *Ulva* sp. Extracts against Oesophagal Squamous Cell Carcinoma KYSE-150 Cells

Yuan Seng Wu[1,2,*], Naaif Mohamed[2], Yoon Yen Yow[2], Appalaju Velaga[3], Adrian Mark Masnammany[4], Fathima Zahraa Ozeer[1,2]

[1] Department of Biological Sciences, School of Medical and Life Sciences, Sunway University, 47500 Subang Jaya, Selangor, Malaysia

[2] Centre for Virus and Vaccine Research, School of Medical and Life Sciences, Sunway University, 47500 Subang Jaya, Selangor, Malaysia

[3] Deparment of Medicinal Chemistry, Faculty of Pharmacy, MAHSA University, 42610 Jenjarom, Selangor, Malaysia

[4] Department of Rheumatology, Hospital Selayang, Selayang – Kepong Hwy, 68100 Batu Caves, Selangor, Malaysia

* Corresponding author's email: sengwu_21@yahoo.com

Introduction: Oesophageal squamous cell carcinoma (ESCC) is an aggressive subtype of oesophageal cancer. Cisplatin is a standard chemotherapeutic agent for ESCC with limited treatment efficacy and chemoresistance occurrence. Thus, a novel anticancer candidate with a chemosensitising effect could be a promising ESCC therapy. Green macroalgae have exhibited cytotoxicity against several cancers preclinically, but their anticancer and chemosensiting effects against ESCC are unknown. This preliminary study investigated anticancer and cisplatin-chemosensitising potentials of two unidentified *Ulva* sp. extracts. **Methods:** *Ulva sp.* powder was macerated in ethanol and methanol by shaking for 48 h. The metabolites in the concentrated extracts were identified using phytochemical tests. Half-maximal inhibitory concentration (IC_{50}) was determined using MTT assay after treating KYSE-150 and Vero normal cells with different extract concentrations for 24 h. Caspase 3/7 apoptotic assay and morphological alteration were further evaluated after 6h treatment. Intracellular reactive oxygen species (ROS) levels were also measured using DCF-DA assay. In the chemosensitising study, the IC_{50} of 24 h-cisplatin treatment and 2 h-extract pre-treatment were identified using MTT assay. **Results:** The phytochemical tests showed positive for glycosides, tannins, terpenoids, carbohydrates, fats and oils, proteins and flavonoids. The cytotoxicity induced by ethanolic (IC_{50} = 237.2 µg/ml) and methanolic (IC_{50} = 165.6 µg/ml) extracts in KYSE-150 cells was higher than in Vero cells, with a selectivity index of 1.35 and 1.22 respectively. Both extracts induced an increasing apoptotic event in Caspase 3/7 measurement and cellular morphology observation. Higher ROS levels in KYSE-150 cells than in Vero cells were observed. Elevated ROS levels were observed from 0-200 µg/ml as compared to higher concentrations, indicating early and late stages of apoptosis. Only methanolic extract increased KYSE-150 sensitivity towards cisplatin treatment (from 62.3 µM to 50 µM). **Conclusion:** Both *Ulva* sp. extracts show anticancer activity against KYSE-150 cells by inducing apoptosis via ROS modulation.

Keywords: Anticancer, Chemosensitising, *Ulva* sp., Cisplatin, Oesophageal squamous cell carcinoma

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PDD13

Targeted Delivery of Cisplatin Using RGD-Modified ZIF-90 as Delivery Vehicle

Emilia Abdulmalek[1,2,*], Adamu Abubakar[1,3], Kyle. E. Cordova[4], Mohd Basyaruddin Abdul Rahman[1,2,5]

[1] Integrated Chemical BioPhysics Research, Faculty of Science, Universiti Putra Malaysia (UPM), 43400 UPM Serdang, Selangor, Malaysia

[2] Department of Chemistry, Faculty of Science, UPM, 43400 UPM Serdang, Selangor, Malaysia
[3] Department of Chemistry, Taraba State University, Jalingo P.M.B 1167, Taraba State, Nigeria
[4] Materials Discovery Research Unit, Advanced Research Centre, Royal Scientific Society, Amman 11941, Jordan
[5] Foundry of Reticular Materials for Sustainability (FORMS), Materials Synthesis and Characterization Laboratory, Institute of Advanced Technology, UPM, 43400 UPM Serdang, Selangor, Malaysia
* Corresponding author's email: emilia@upm.edu.my

Introduction: Chemotherapy is one of the most effective therapy available to treat cancers, but the side effects make it less favourable. Therefore, we aimed to effect selective and targeted delivery of cisplatin to cancer cell via covalent-modification of zeolitic imidazolate framework-90 (ZIF-90) with RGD peptide, a tripeptide that was known for its ability to recognise integrins on cell surface. Due to overexpression of integrins by cancer cell, RGD peptide on the surface of ZIF-90 would be able to guide the delivery of the cancer drug (cisplatin) encapsulated in the pores of ZIF-90, to the intended recipient. Thus, side effect to normal cells will diminish. **Methods:** Nano-sized ZIF-90 encapsulated cisplatin (RGD@Cis⊂nZIF-90) was prepared by in-situ encapsulation followed by covalent modification with RGD peptide. The drug loading amount and drug released was profiled, and MTT assay on MRC-5 (human fetal lung fibroblast cells) and A549 (lung adenocarcinoma) cells was conducted. **Results:** Powder XRD confirmed the formation of ZIF-90 and covalent bond to RGD peptide was confirmed with NMR and IR showing formation of imine bond between imidazolcarbaldehyde linker of ZIF-90 and the RGD peptide. The cisplatin loading was measured to be 24.8% using UV-vis and sustained released behaviour at pH 5 was observed with maximum released (92%) observed after 24 hours. The MTT assay showed that RGD@Cis⊂nZIF-90 nanoparticle was more toxic towards A549 (IC50 8.79 μgmL-1) than MRC-5 (IC50 31.07 μgmL-1). The selectivity index was found to be 3.5 suggesting that there is selectivity towards A549 cell in the presence of RGD peptide. **Conclusion:** RGD-modified nano-sized ZIF-90 encapsulated cisplatin has been successfully synthesized and determined to have good selectivity and toxicity towards lung cancer cell over normal cell. This may lead to more selective and improve performance of cancer therapy with little side effect.

Keywords: ZIF-90, RGD peptide, Cisplatin, Selective chemotheraphy, Cancer drug delivery
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PDD14

In silico and Cytotoxicity Studies of Two New Bisbenzylisoquinoline Alkaloids Isolated from *Synclisia scabrida* (Miers) ex Oliv

Ogochukwu Ngozi Nwaefulu[1], Nizar A. Al-Shar'i[2], Lam Kok Wai[3], Mohammad Kaisarul Islam[1], Lim Chee Woei[1], Josephine Omonkhelin Owolabi[4], Sreenivasa Rao Sagineedu[5], Johnson Stanslas[1,*]

[1] Pharmacotherapeutics Unit, Department of Medicine, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia
[2] Department of Medicinal Chemistry and Pharmacognosy, Faculty of Pharmacy, Jordan University of Science and Technology, Jordan
[3] Centre for Drug and Herbal Development, Faculty of Pharmacy, Universiti Kebangsaan Malaysia, 50300 Kuala Lumpur, Malaysia
[4] Pharmacology Laboratory, Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Edo State, Nigeria
[5] Department of Pharmaceutical Chemistry, School of Pharmacy, International Medical University, Kuala Lumpur, Malaysia
* Corresponding author's email: rcxjs@upm.edu.my

Introduction: Natural products remain the best source of novel agents for effective drug development. This study aimed to isolate and identify compounds from *Synclisia scabrida* (Miers) ex Oliv and to evaluate their anticancer potential by *in vitro* cytotoxicity and *in silico* studies. **Methods:** The isolated compounds were identified using spectroscopic methods, and their anticancer activities were evaluated using MTT assay. Furthermore, binding of the compounds to their potential molecular targets were determined via *in silico* computer modeling approach. **Results**: Two compounds were identified and found to be new bisbenzylisoquinoline (BBIQ) alkaloids: SS_C2 (a cosculine analogue) and SS_C4 (a cycleanine analogue). Both were selectively toxic towards HCT-116 (colon cancer) and MCF-7 (breast cancer) cells, respectively. The selectivity was far greater than that of gemcitabine, which showed higher toxicity to normal lung cells (BEAS-2B). Compared with the docked poses of the two ligands, SS_C2 and SS_C4, the simulation trajectories showed that the complexed ligands underwent slight conformational changes that resulted in stronger interactions with PARP1. The main interactions common to the two ligands that are likely to be responsible for the stable binding are electrostatic interactions between the ionised amines and the carboxylate groups of Asp105 and Asp109, a pi-pi stacking interaction with Tyr246, and a pi-pi stacking or pi-alkyl interaction with His201. Those interactions were maintained throughout the simulation time, and there were other intermittent interactions including hydrogen bonding and

other hydrophobic interactions. The *in silico* simulation results showed very high PARP1–ligand complex stability for both compounds. **Conclusion:** SS_C2 and SS_C4 are new potential PARP1 inhibitors, which in turn provide a plausible explanation of their observed anticancer activities.

Keyword: *Synclisia scabrida*, Isolation, Structural elucidation, *In silico* studies, Cytotoxicity, PARP1 inhibitor
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PTM01

MMP-9/MMP-2 Inhibitor Sensitises Human Oesophageal Squamous Cell Carcinoma towards Cisplatin and 5-Fluorouracil in Different Treatment Models

Bernadette Xin Jie Tune[1], Maw Shin Sim[1], Najihah Binti Mohd Hashim[2], Yuan Seng Wu[3,4,*]

[1] Department of Pharmaceutical Life Science, Faculty of Pharmacy, Universiti Malaya, Kuala Lumpur, Malaysia

[2] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universiti Malaya, Kuala Lumpur, Malaysia

[3] Centre for Virus and Vaccine Research, School of Medical and Life Sciences, Sunway University, Selangor, Malaysia

[4] Department of Biological Sciences, School of Medical and Life Sciences, Sunway University, Selangor, Malaysia

* Corresponding author's email: sengwu_21@yahoo.com

Introduction: Oesophageal squamous cell carcinoma (ESCC) is an aggressive type of oesophageal cancer in Asia, with a high mortality rate due to the occurrence of chemoresistance. Upregulation of the endopeptidases matrix metalloproteinase (MMP)-9 and MMP-2 are known to contribute to chemoresistance. MMP-9 and MMP-2 overexpression was observed in ESCC. However, their roles in ESCC chemosensitivity are still elusive. This study evaluated the effect of MMP-9/MMP-2 inhibition on the chemosensitivity of ESCC cells towards cisplatin (CDDP) and 5-fluorouracil (5-FU) using co-treatment and pre-treatment models. **Methods:** ESCC cell line (EC109) was treated with different concentrations of CDDP, 5-FU or MMP-9/MMP-2 inhibitor (MMP-9/MMP-2i) to identify their half-maximal inhibitory concentration (IC_{50}) using MTT assay. Using the IC_{50} of MMP-9/MMP-2i, co-treatment was conducted with the same concentration range of CDDP and 5-FU for 3, 6 and 24h. In pre-treatment model, MMP-9/MMP-2i (IC_{50}) was added to the cells for 3 or 6 h before treating with the same concentration range of CDDP and 5-FU. **Results:** Inhibition of MMP-9/MMP-2 activity enhanced EC109 chemosensitivity towards CDDP and 5-FU, except for CDDP in co-treatment model. In the co-treatment model (24h), MMP-9/MMP-2i decreased the IC_{50} of 5-FU from 1000 μM to 400 μM, while the IC_{50} of CDDP increased from 40 μM to >80 μM. In the pre-treatment model, the IC_{50} of CDDP was also reduced from 40 μM to 20 μM at 3 h pre-incubation and from 40 μM to 5 μM at 6 h pre-incubation. Meanwhile, IC_{50} of 5-FU remained at 1000 μM at 3 h pre-incubation but was reduced to 50 μM at 6 h pre-incubation. **Conclusion:** The inhibition of MMP-9/MMP-2 activity induces EC109 chemosensitivity towards CDDP and 5-FU in the pre-treatment and co-treatment models. These preliminary results indicate the potential use of MMP-9/MMP-2i to induce ESCC chemosensitivity and deserve further identification of the associated molecular mechanisms.

Keywords: Oesophageal squamous cell carcinoma, Matrix metalloproteinase, Chemoresistance, Chemosensitivity
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PTM02

Effects of Nicotinamide and Nilotinib on Telomerase Activity and Telomere Length in K562 Myeloid Cell Line

Sarina Sulong[1,*], Nur Rasyidah Muhammad[1], Siti Norasikin Mohd Nafi[2], Farizan Ahmad[3], Azlina Ahmad[4], Zariyantey Abdul Hamid[5]

[1] Human Genome Centre, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan

[2] Department of Pathology, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan

[3] Department of Neurosciences, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan

[4] School of Dental Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan

[5] School of Diagnostic & Applied Health Sciences, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Kuala Lumpur

*Corresponding author's email: ssarina@usm.my

Introduction: Blast phase of chronic myelogenous leukaemia (CML) has continued to exist as a challenging disease even though the advanced tyrosine kinase inhibitor therapy has been introduced. We study the effect of nicotinamide (an active form of Vitamin B3) on telomerase activity, telomere length and *TERT* expression in the K562 myeloid cell line as an approach to enhance the existing therapy for CML. Yet the role of nicotinamide in tumorigenesis is controversial. We hypothesized that nicotinamide would enhance the effects of Nilotinib on K562 cells, hence reducing tumour growth and/or promoting tumour cell death. **Methods:** K562 cell line was treated with nicotinamide, nilotinib and combination of both for IC_{50} assay. Detection of telomerase activity was carried out using TRAP assay, while qPCR assay was used for detection of telomere length and *TERT* expression. **Results:** This study has shown the effect of nicotinamide, nilotinib

and both substances in exhibiting the anti-proliferation ability on K562 cell line after 48 hours. The implicated mechanism involved to induce such an effect are not yet clear. All treated samples exposed by nicotinamide, nilotinib and both have been assessed as telomerase-positive suggesting that all treatments were most likely not able to repress telomerase activity in K562 cells. Data results in longer telomere length in all groups of treatments except for nicotinamide that have a slight decrease of telomerase activity thus decrease in number of telomere length. Expression of *TERT* in this study suggests that the effect of these substances on telomerase activity is necessarily dependent on its effect on *TERT* expression. However, no study has been done to investigate the effect of nicotinamide in combination with nilotinib on telomerase and telomere regulation. **Conclusion:** The effects of nicotinamide and nilotinib on telomerase-telomere mechanisms may be linked to apoptosis which may give evidence for further investigation, notably in PARP-1 regulation.

Keywords: Nicotinamide, Nilotinib, Telomerase, Telomere, CML
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PTM03

Barriers and Challenges in Chemotherapy-Induced Nausea and Vomiting (CINV) Management: A Systematic Review

Nurul Suhaida Badarudin[1,*], Noraida Mohamed Shah[1], Fuad Ismail[2], Farida Islahudin[1], Nurul Ain Mohd Tahir[1]

[1] Centre of Quality Management of Medicines, Faculty of Pharmacy, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia.

[2] Department of Radiotherapy & Oncology, Universiti Kebangsaan Malaysia Medical Centre, Kuala Lumpur, Malaysia

* Corresponding author's email: soobadarudin@gmail.com

Introduction: Barriers and challenges in chemotherapy-induced nausea and vomiting (CINV) management are essential and must be identified for stakeholders to achieve high-quality cancer care. Unfortunately, these components were not routinely addressed, and to date no systematic review has evaluated these aspects. This review aimed to systematically gather and appraise such evidence. **Methods:** Pubmed, Ovid, Scopus, Cochrane Library, Wiley Online, and Web of Science were searched using the following keywords: challenges, prevention, CINV, chemotherapy, and their alternative keywords' equivalent. Studies involved adult cancer patients receiving chemotherapy exclusively, the caretaker, and healthcare professionals handling cancer patients were included. The studies must be in English language and original primary studies. Data extraction form was developed based on the PRISMA guide. The Joanna Briggs Institute (JBI) Critical Appraisal tool was used to assess the quality of the studies. **Results:** From 1,170 related articles retrieved, 37 articles were included in this review. The worldwide data were predominantly from European countries (9 articles) and United States of America (6 articles). Three qualitative study designs were included, and the rest of the included studies were mainly quantitative studies using questionnaires. All articles met the criterion of the JBI critical appraisal. Barriers and challenges particularly nausea and delayed CINV, failure to adhere to the antiemetic guideline, as well as misconception on CINV and its prevention were among the barriers and challenges reported in the management of CINV. **Conclusion:** Planning and implementing interventions to address the barriers and challenges identified may improve the quality of CINV management.

Keywords: Chemotherapy-induced nausea vomiting, Barriers, Challenges, Systematic review
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PTM04

Cloning of KRAS Oncogene-Targeting Single-Guide RNA in a CRISPR/CAS9 System: A First Step in Generating a Non-Small Lung Cancer Cell Line with KRAS and EGFR Double Mutation

Amir Imran Faisal Hamdi[1], Saiful Effendi Syafruddin[2] and Johnson Stanslas[1,*]

[1] Department of Medicine, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia

[2] UKM Molecular Biology Institute (UMBI), Universiti Kebangsaan Malaysia Medical Centre

* Corresponding author's email: rcxjs@upm.edu.my

Introduction: The concomitant presence of EGFR and KRAS mutations in non-small cell lung cancer (NSCLC) patients is relatively rare and their occurance is believed to be mutually exclusive. However, with advancing detection technologies such as liquid biopsy and next-generation sequencing, approximately 10% of patients with dual concomitant mutations are detected, and this can affect the administration of either EGFR or KRAS tyrosine kinase inhibitors. In order to address this issue, we aimed at generating an isogenic cell line with a dual mutation of EGFR and KRAS. The bacterial type II clustered regularly interspaced short palindromic repeat (CRISPR) and CRISPR-associated (Cas) protein systems were being used to introduce a KRAS G12C mutation into a KRAS wildtype, EGFR-mutant T790M lung cancer cell line and it started with cloning the KRAS-specific single-guide RNA (sgRNA) into an expression plasmid, PX330. **Methods:** Briefly, to clone the KRAS-targeting sgRNA into PX330, the plasmid was digested with *BbsI* restriction enzyme. The top and bottom sgRNA sequences were annealed and phosphorylated with antarctic phosphatase enzyme. They were ligated with the open-end PX330 with T4 PNK enzyme and T4 Ligase to create a complete closed plasmid, followed by transformation into the chemically competent DH5a *Escherichia. coli* (*E.coli*) strain.

Following picking of the *E. coli* colonies, the plasmid was extracted and sent for Sanger sequencing to check for the presence of ligated sgRNA. **Results**: The KRAS-targeting sgRNA was successfully phosphorylated and ligated into an expression plasmid. In addition, it was successfully transfomed into a bacterial host for cloning. The sequencing results showed that the top and bottom sequences of KRAS-targeting sgRNA were successfully cloned into PX330 expression plasmid. **Conclusion:** The present study showed that the molecular cloning of KRAS-targeting sgRNA was a success and completed the first step in generating a dual mutation isogenic cell line.

Keywords: Bacterial transformation, CRISPR-Cas9, KRAS G12C
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PTM05

Identification of the Gene Mutation Profile of Obese-related Colorectal Cancer via TruSight Tumor 15 Analysis

Phei-Ying Ng[1], Siti Norasikin Mohd Nafi[1,*]

[1] Department of Pathology, School of Medical Sciences, Health Campus, Universiti Sains Malaysia, 16150
Kubang Kerian, Kelantan, Malaysia

* Corresponding author's email: snmn@usm.my

Introduction: One of the most common risk factors for colorectal cancer (CRC) is obesity. Obese people are more prone to acquire CRC than non-obese people. However, it is unknown how obesity connects to CRC. CRC progression, on the other hand, has been attributed to solid tumour gene mutations such as KRAS, NRAS, BRAF, PIK3CA, AKT1, ERBB2, EGFR, and others. However, limited studies have connected such gene alterations to obesity-related CRC among Malaysians. The Ilumina targeted gene panel TruSight Tumor 15 (TS15) determines 15 typical solid tumour genes using next-generation sequencing (NGS) technology. The purpose of this study is to identify the gene mutation profile of obese-related CRC using TS15 analysis. **Methods:** DNA was isolated from 12 formalin-fixed, paraffin-embedded (FFPE) tumor tissue samples from CRC patients. The DNAs were then sequenced using the NGS Ilumina Miniseq platform after library preparation with a targeted panel TS15. BaseSpace Variant Interpreter was used for variant annotation. **Results:** This study identified an occurrence of mutations in TP53, KRAS, PIK3CA, ERBB2 and EGFR. TP53 demonstrated the highest frequency of gene mutation (75.0%, 9/12), followed by KRAS (66.7%, 8/12) and PIK3CA (25.0%,3/12). 11 variants were detected in TP53, 6 variants in KRAS, and 2 variants in PIK3CA. EGFR amplification was found in 41.7% (5/12) of CRC cases, whereas ERBB2 amplification was seen in 8.3% (1/12) of CRC cases. **Conclusion:** Overall, the findings reported here demonstrate that TS15-based targeted NGS on FFPE DNA offer a promising approach for detecting genetic mutations that might be employed in the future to diagnose CRC. To better understand the gene mutation profiles of obese-related colorectal cancer, the current findings will be linked to BMI categories and levels of apoB protein expression in the future.

Keywords: Colorectal cancer, FFPE, DNA, Gene mutations, Targeted next-generation sequencing
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PTM06

Profiling of Autoantibodies as Biomarkers in Glioblastoma Multiforme (GBM) Patients

Nadiah Abu[1,*], Johannes Low Jun Wei[2], Soon Bee Hong[2]

[1] UKM Medical Molecular Biology Institute (UMBI), Universiti Kebangsaan Malaysia, 56000 Cheras, Kuala
Lumpur, Malaysia

[2] Department of Surgery, UKM Medical Center, 56000 Cheras, Kuala Lumpur, Malaysia

* Corresponding author's email: nadiah.abu@ppukm.ukm.edu.my

Introduction: Autoantibodies are circulating antibodies generated against self-antigens. In cancer, certain autoantibodies are produced based on cancer antigens that are present on the cancer cells. These antibodies have the potential to become minimally invasive diagnostic and prognostic biomarkers in cancer. In this study, we aim to profile the presence of autoantibodies in the sera of glioblastoma multiforme (GBM) patients. **Methods:** We obtained 20 serum of ten GBM patients and ten healthy participants from UMBI's Biobank. We then subjected the profiling of autoantibodies using the i-OME antibody array by Sengenics Malaysia per the manufacturer's instructions. The slides were scanned using the Agilent Scanner (USA) and the image data was extracted. Data analysis was performed using the Loess normalization and fold change analysis. **Results:** After the normalization, we performed a cut-off analysis at >1.5 or <-1.5 fold change. With these criteria, we obtained 20 upregulated and 2 downregulated autoantibodies. The topmost upregulated autoantibodies include PDCL3, CRYAB, ENO2, HSP90AA1 and CT47A1. Whereas the two downregulated autoantibodies are ASNA1 and FIP1L1. **Conclusion:** Based on our analysis, certain antibodies, such as HSP90AA1 and KRT19 have the potential to become minimally invasive biomarkers for GBM as shown by the ROC analysis.

Keywords: Tumor antigens, Brain cancer, Biomarker, Liquid biopsy
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PTM07

The Hypoxia Model using Dimethyloxalylglycine (DMOG) Promoted the Migration and Invasion of HCT116 Colon Cancer Cells

Nor Ezleen Qistina Ahmad[1,*], Noraina Muhammad Zakuan[1], Nur Fariesha Md Hashim[1], Nurul Akmaryanti Abdullah[1]

[1]Faculty of Medicine and Health Sciences, Universiti Putra Malaysia (UPM)

* Corresponding author's email: neq.ahmad@gmail.com

Introduction: Hypoxic microenvironments are one of the primary causes of metastasis. Hypoxia inducible factor-1 alpha (HIF-1α) protein levels increase in hypoxic environments and may give rise to vascularization, cytoskeletal reorganization, and epithelial-to-mesenchymal transformation (EMT) of the cancer cells. Many studies evaluate the effect of hypoxia in the laboratory using hypoxic workstations, hypoxia chambers or hypoxic incubators. Alternatively, hypoxic conditions can be induced using dimethyloxalylglycine (DMOG), as the hypoxia mimetic agent in a cell culture model. Therefore, this study aims to determine the effects of hypoxia induced by DMOG on colon cancer metastasis, particularly in terms of cell migration and invasion. **Methodology:** To characterise the molecular background of the hypoxia response, total proteins were isolated from HCT116 colon cancer cells after 6, 24, and 48 hours of hypoxia induction with 1 mM DMOG. Using western blot, the expression of HIF-1α proteins was measured at each time point. The HCT116 cells were then subjected to wound healing assays and transwell invasion assays to investigate the effects of hypoxia on cell migration and invasion capacity. Images and data were statistically analysed using ImageJ and GraphPad Prism software version 9.0.1. **Results:** The expression of HIF-1α protein increases after 6 hours of DMOG induction and remains within 24 hours. The migration percentage of hypoxic cells is significantly increased compared to normoxic cells at the 6 hour ($p<0.005$) and 24 hour ($p<0.05$) time points. The transwell invasion assay demonstrated that, within 24 hours, hypoxic cells had significantly higher invasive capabilities ($p<0.001$) than normoxic cells. Thus, the increase in migration and invasion of the cells is similar to the increase in HIF1-α expression at 6 and 24 hours. **Conclusion:** The current data suggest that DMOG induction induces HIF-1α expression, which results in an increase in cell migration and invasiveness in colon cancer cells. Therefore, this model can be used to evaluate the effects of hypoxia on cells in combination with gene knockdown or drug treatment studies.

Keywords: Colon cancer, Hypoxia, HIF-1α, Migration, Invasion

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PTM08

46,XY Female Presenting With Dysgerminoma, Gonadoblastoma And Serous Cystadenoma With Androgen Receptor Mutation: A Swyer Syndrome Coexisting With Androgen Insensitivity Syndrome

Mohd Ridzuan Hamid[1], Engku Husna Engku Ismail[2], Nik Rafiza Nik Muhamad Afendi[2], Noorul Balqis Che Ibrahim[3], Aziati Azwari Annuar[1,*]

[1] Human Genome Centre, School of Medical Science, Health Campus, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia

[2] Department of Obstetrics and Gynaecology, School of Medical Science Health Campus, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia

[3] Department of Pathology, School of Medical Science Health Campus, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia

* Corresponding author's email: draziati@usm.my

Introduction: Swyer syndrome is characterized by female phenotype, complete gonadal dysgenesis with the existence of Mullerian structures specifically the uterus, fallopian tube, and vagina, meanwhile individual with androgen insensitivity syndrome (AIS) demonstrate Wolffian structures internally and presence of male gonads. Both syndromes fall under 46,XY disorders of sex development (DSD) and are at risk of developing gonadoblastoma. The coexistence of Swyer syndrome and AIS occurring simultaneously is an extremely rare occasion, hence we present this case. **Case report:** 19-years-old, single, and nulliparous female presented with secondary amenorrhea at the age of 16-years-old. She attained menarche at the age of 12-years-old. She had one sister who had primary amenorrhea and passed away at the age of 22-years-old due to dysgerminoma. Clinically she is tall with height of 173 cm, normal female secondary sexual characteristics. Hormonal level demonstrated gonadal insufficiency with markedly elevated FSH and LH, and low estradiol and normal testosterone. MRI pelvis revealed small uterus with presence of gonad at right adnexal region. Karyotype confirmed as 46,XY in two occasions. SRY gene were detected using Fluorescence In Situ Hybridisation (FISH) method. Androgen mutation analysis revealed mutation in exon 1 [NG_009014.2:g.6286_6288GC dup$(GCA)_2$]. Laparoscopic gonadectomy were performed and the right gonad was confirmed as dysgerminoma with gonadoblastoma and serous cystadenoma. **Discussion:** To the best of our knowledge this is the first case of coexistence of Swyer and AIS syndrome reported. This case demonstrates to us how crucial the role of karyotype analysis in a situation of a young female with amenorrhea which can give us diagnostic clue and further targeted management. This case may raise a suspicion of possibility of familial or inherited condition which need further evaluation. DSD should be co-managed by multidisciplinary team.

Keywords: Swyer syndrome, Androgen insensitivity syndrome, 46,XY female, Dysgerminoma, Gonadoblastom

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PTM09

ADAR1 Dependency in Oral Squamous Cell Carcinoma

Pei San Yee[1], Annie Wai Yeeng Chai[1], Shi Mun Yee[1], Shiyin Ooi[1,3], Siew Kit Ng[2], Sok Ching Cheong[1,3,*]

[1] Translational Cancer Biology Research Unit, Cancer Research Malaysia, No. 1, Jalan SS12/1A, 47500 Subang Jaya, Selangor, Malaysia

[2] Advanced Medical and Dental Institute, Bertam, Universiti Sains Malaysia, 13200 Kepala Batas, Pulau Pinang, Malaysia

[3] Faculty of Dentistry, University of Malaya, 50603 Kuala Lumpur, Malaysia

* Corresponding author's email: sokching.cheong@cancerresearch.my

Introduction: Our recent genome-wide CRISPR knockout screen revealed that the Adenosine deaminase acting on RNA-1 (*ADAR1*) gene is essential for the survival of a majority of oral squamous cell carcinoma (OSCC) cell lines. Given that deleting ADAR1 caused severe lethality in OSCC, targeting ADAR1 could have therapeutic benefits. ADAR1 catalyzes Adenosine-to-Inosine RNA editing, and suppresses dsRNA sensing-triggered cell death. ADAR1 has two isoforms, the constitutively expressed P110 and the interferon-inducible P150. It is also an interferon-stimulated gene (ISG) that is highly expressed in cancers including OSCC. In this study, we aimed to determine the molecular mechanisms underlying ADAR1 dependency which may afford an opportunity to develop better treatment strategies for OSCC. **Methods:** ADAR1 dependency is validated by competitive co-culture assay, apoptosis and colony formation assay using single-guide RNA (sgRNA) knockout in OSCC cell lines (ORL-48, ORL-214, ORL-174, ORL-195, SCC9 and BICR10). Changes in protein expression upon gene(s) knockout are determined by western blotting. **Results:** We confirmed that ADAR1 depletion significantly increased apoptosis by flow cytometry and inhibited colony formation in selected cell lines. For cell lines that are ADAR1-less dependent (BICR10 and ORL-174), IFN-β treatment increased the expression of ADAR1 and other ISGs, sensitizing these cells to cell death. Notably, overexpression of ADAR1-P150 but not ADAR1-P110, significantly rescued cell lethality in ADAR1 depleted cells suggesting that the ADAR1-P150 is important in the survival of OSCC. We also showed that cell death is mediated by dsRNA sensors protein kinase R (PKR) and melanoma differentiation-associated protein 5 (MDA5) whereby knocking-out either one of these genes did not reverse cell lethality, but co-deleting both genes partially rescued cell death in ADAR1-dependent cell lines. **Conclusion:** The present findings revealed that the interferon-inducible P150 of ADAR1 is essential for OSCC survival. Activation of the dsRNA-sensing pathways such as PKR and MDA5 underlies this dependency.

Keywords: ADAR1 dependency, dsRNA sensing, CRISPR knockout, Interferon-stimulated gene, OSCC

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Induction of *in vitro* Cytotoxicity in High-Risk Oral Leukoplakia Using A Cancer Vaccine

Chai Phei Gan[1,2,*], Hany Ariffin[1], Sok Ching Cheong[2,3], Kue Peng Lim[2]

[1] Department of Paediatrics, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia.

[2] Cancer Immunology and Immunotherapy Research Unit, Cancer Research Malaysia, 47500 Subang Jaya, Selangor.

[3] Department of Oral and Maxillofacial Clinical Sciences, Faculty of Dentistry, University of Malaya, 50603 Kuala Lumpur, Malaysia.

* Corresponding author's email: chaiphei.gan@cancerresearch.my

Introduction: Patients with oral leukoplakia diagnosed with moderate-severe oral epithelial dysplasia (OED) have an increased risk of developing oral cancer. However, chemoprevention agents targeting epithelial cells are ineffective in preventing malignant transformation and disease recurrence. Emerging evidence indicates that host immunity can impact premalignant disease progression, but the immune profile of oral leukoplakia has not been studied. In this study, we characterized the immune profile of high-risk oral leukoplakia with a long-term aim of identifying immunotherapeutic strategies to intercept cancer development. **Methods:** The immune profile of moderate-severe OED was determined by transcriptomic analysis of 125 immune signatures reported in cancer. To determine the utility of a cancer vaccine targeting MAGED4B, we evaluated MAGED4B expression by immunohistochemistry on oral leukoplakia tissues. Then, *in-vitro* T cell-based immunogenicity studies were performed using patients' blood samples to evaluate antigen-specific immune responses. **Results:** Immune profiling demonstrated the induction of both immune surveillance and immune suppression mechanisms in moderate-severe OED. Notably, three distinct immune subtypes were identified: (1) immune cytotoxic; (2) non-cytotoxic; and (3) non-immune reactive. Patients progressed to cancer appear to lack cytotoxic T cells responses, suggesting that restoring T cell immunity via cancer vaccine may intercept malignant development. Next, we evaluated the feasibility of activating patients' immune responses using a cancer vaccine targeting MAGED4B. We demonstrated that moderate-severe OED significantly over-expressed MAGED4B. Our T cell-based immunogenicity studies showed that

MAGED4B-specific $CD8^+$ T cells could be expanded *in-vitro* and become activated as indicated by increased interferon gamma secretion and CD38 expression. Importantly, these $CD8^+$ T cells demonstrated antigen-specific killing of tumour cells expressing MAGED4B. **Conclusion:** Our series of studies demonstrated that discrete immune responses are present in high-risk leukoplakia, and antigen-specific immune response can be harnessed to induce cytotoxic responses in these lesions.

Keywords: Oral leukoplakia, Immune profile, Oral epithelial dysplasia, Cancer vaccine

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