Abstracts for Poster Presentation: Advanced Medical Research

AMR-1-21

Eradication of Hepatocellular Carcinoma by Augmenting Immunotherapy Efficacy via Cell Cycle Related Kinase (CCRK) Inhibition

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Introduction and Project Objectives: Hepatocellular carcinoma (HCC) is a sexually dimorphic cancer associated with elevated male hormone androgen, inflammation and obesity. Obesity-promoted HCC was reported to be dependent on the enhanced production interleukin 6 (IL-6) that also play roles in linking oncogenic pathways and tumor-associated immunosuppression. Our previous study demonstrated that androgen receptor (AR)/cell cycle-related kinase (CCRK) signaling can activate β-catenin which has been shown to trigger inflammation and promote immune escape in HCC. However, the role of CCRK in immunoregulation remains undefined.

Methods: The role of CCRK was determined in a carcinogen-dietary induced non-alcoholic-fatty-liver-disease (NAFLD)- and orthotopic-HCC mouse models followed by tumorigenicity and immunophenotype analysis. Mechanistic studies on the relationship of CCRK/IL-6 and myeloid-derived suppressor cell (MDSC) were determined by flow cytometry, expression/correlation analyses in co-culture system. Further studies were conducted in liver-specific CCRK-inducible transgenic (TG) mice and orthotopic HCC models using CRISPR/Cas9-mediated Ccrk depletion to determine the role of CCRK/IL-6/MDSC in tumorigenicity.

Results: Our results showed that lentivirus-mediated CCRK ablation in liver of male mice fed with high-fat-high-carbohydrate(HFHC) diet abrogated obesity-related HCC development associated with reduced polymorphonuclear (PMN)-MDSC expansion. Mechanistically, hepatoma CCRK stimulated immunosuppressive CD11b+CD33+HLA-DR− MDSC expansion through upregulating IL-6. At molecular level, CCRK activated nuclear factor-κB (NF-κB) via enhancer of zeste homologue 2 (EZH2) and facilitated NF-κB-EZH2 co-binding to IL-6 promoter. Consistently, hepatic CCRK induction in TG mice activated the EZH2/NF-κB/IL-6 cascade, leading to accumulation of PMN-MDSCs with potent T-cell suppressive-activity. In contrast, inhibiting tumorous CCRK or hepatic IL-6 increased interferon (IFN)-γ+tumour necrosis factor(TNF)-γ+CD8+T-cell infiltration and impaired tumorigenicity, which was rescued by restoring PMN-MDSCs.

Conclusion: Our results delineate an immunosuppressive mechanism of the hepatoma-intrinsic CCRK signaling and highlight an overexpressed kinase target whose inhibition might suppress hepatocellular carcinogenesis. By taking both cancer biology and tumor immunology into consideration, CCRK is an exploitable druggable target for cancer therapy.

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Project No. : 03141376

AMR-2-40

Impact of Haemostatic Sealant versus Electrocoagulation on Ovarian Reserve After Laparoscopic Ovarian Cystectomy of Ovarian Endometriomas: A Randomised Controlled Trial

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Introduction and Project Objectives: Bipolar coagulation is used for haemostasis in laparoscopic ovarian cystectomy of endometrioma, but it may damage the ovarian reserve. This study was designed to determine the effect of a haemostatic sealant, FloSeal©, compared to bipolar coagulation on the ovarian reserve after laparoscopic cystectomy for endometriomas.

Methods: This was a prospective, patient-blinded, randomised controlled trial conducted in a university-affiliated tertiary hospital. Women aged 18 to 40 years with clinical and ultrasound diagnosis of 3-8cm unilateral or bilateral endometriomas, with no previous ovarian surgery, were randomised to haemostasis by the application of haemostatic sealant, FloSeal©, or standard care. Primary outcome was the effect on the antral follicular count (AFC) 6 months after the operation as it captures the effect in the ovary subjected to treatment. Secondary outcomes included the change in anti-mullerian hormone (AMH), follicular-stimulating hormone (FSH), peak systolic velocity, normal ovarian volumes and peri-operative outcomes, including haemostasis, complications, pain and satisfaction scores. Repeated measure analysis of variance was used to analyse between the two groups.

Results: A total of 94 patients aged 32.36 ± 4.92 years underwent laparoscopic cystectomy for ovarian endometriomas. The average diameter of the endometrioma was 4.21± 1.38 cm. The increase in AFC of the affected ovaries at 6 months in the intervention group was significantly ($p=0.018$) higher than that in the control group. Repeated measures analysis of variance revealed significant effect with time ($p=<0.001$) and of interaction of group x time ($p=0.028$) for affected ovary AFC. No significant difference was noted between the two groups with regards to other the secondary outcomes.

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Conclusion: Applying FloSeal© after laparoscopic cystectomy of ovarian endometriomas produced a greater increase in AFC 6 months after surgery than the control group. FloSeal© should be considered for haemostasis during laparoscopic ovarian cystectomy in those women who wish to preserve fertility.

Project No.: 04152656

AMR-3-41

Secreted Stanniocalcin 1 Fosters Metastasis of Hepatocellular Carcinoma via the JNK Pathway

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Introduction and Project Objectives: The hypoxic microenvironment of hepatocellular carcinoma (HCC) is a key contributor to aggressive phenotypes of the tumor. Identification of functional hypoxia-responsive molecular targets is pivotal to understand the tumor biology of HCC. Stanniocalcin 1 (STC1) is a glycoprotein that acts in autocrine, paracrine and endocrine manners. It was found upregulated by hypoxia in some cancer cell lines. In this study published in Cancer Letters, we investigated the significance of secreted STC1 in HCC [1].

Methods: Clinical HCC tissue and serum samples were used to analyze the expression of STC1. Cell motility assay and Matrigel invasion assays were employed to examine the functional effects of STC1 in vitro. Effect of STC1 on metastasis in vivo was investigated by orthotopic injection model with nude mice.

Results: Hypoxia upregulated STC1 mRNA expression and induced the secretion of STC1 protein in HCC cells. Functionally, recombinant human STC1 protein (rhSTC1) enhanced cell migratory and invasive abilities in vitro. The pro-metastatic effects were abrogated by co-treatment with anti-STC1 antibody. Furthermore, silencing of STC1 in HCC cells attenuated extracellular STC1 protein secretion and suppressed lung metastasis in vivo. Mechanistically, secreted STC1 activated the JNK pathway in HCC cells by altering the expression of pJNK and p-cJun. The in vitro functional effects of rhSTC1 could be abolished by JNK inhibitor. From our clinical samples, STC1 mRNA level was upregulated in HCC tissues when compared to the paired non-tumoral liver tissues. The clinical relevance was substantiated by a higher STC1 protein level in the serum of HCC patients. Moreover, a higher serum STC1 level was associated with and worse survival outcome.

Conclusion: Our findings illustrated that secreted STC1 promotes metastasis of HCC through JNK signaling. STC1 is a potential prognostic biomarker and a therapeutic target for HCC.

1. Chan KK, et al…& Lo RC. Secretory Stanniocalcin 1 promotes metastasis of hepatocellular carcinoma through activation of JNK signaling pathway. Cancer Lett. 2017 Sep 10;403:330-338.

Project No.: 03142766

AMR-4-47

Clinical Application of Enumeration and Genomic Characterization for Non-invasive Detection and Real-time Monitoring of Circulating Tumor Cells for Esophageal Carcinoma

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Introduction: Esophageal squamous cell carcinoma (ESCC) is deadly cancer with its highest incidence worldwide amongst Chinese. Despite current upfront chemoradiotherapy (CRT) plus surgical approaches, ESCC patient survival rates are dismal. There is still a need for early predictive markers to track treatment responses. Advances in enrichment and identification of circulating tumor cells (CTCs), which can escape from both primary and metastatic tumors into the blood of cancer patients, allows real-time monitoring of CTC levels at diagnosis/during/after treatment to assess treatment efficacy.

Project Objectives: • Determine the clinical usefulness of non-invasive real-time monitoring of CTCs in ESCC patients during treatment in comparison to current CT/PET imaging • Utilize next-generation sequencing (NGS) targeted gene sequencing to examine genetic changes before/during/after treatment to identify useful biomarkers for metastasis and drug resistance

Identification of key driver genes for metastasis and druggable targets from CTC analysis is expected to improve diagnosis and targeted treatment of metastatic ESCC and improve patient precision care. We investigated the prognostication and risk stratification role of liquid biopsy serial monitoring for ESCC.

Methods: CTCs and plasma cell-free DNA (cfDNA) were isolated from 199 blood samples of 103 advanced ESCC patients treated by CT or CRT/surgery at serial treatment timepoints. CTCs were isolated using size separation on microfluidic chips and enumeration by immunofluorescent staining with antibody cocktails of CD45/EpCAM/CK/MUC1. Kaplan Meier curve, log-rank test, and COX regression analysis were used for statistical analysis of disease relapse and survival.

Results: In 57 ESCC patients receiving palliative CT, high CTC counts at CT pre-cycle III is independently associated with

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response at interim reassessment and progression-free survival (PFS) in multivariate COX analysis. Integration of changes of both baseline pre-cycle III CTC and cfDNA into four risk groups based on the number of favorable/unfavorable changes of CTC/cfDNA, were independently associated with overall survival (OS) by multivariate COX analysis. In 43 loco-regionally advanced ESCC treated by CRT/surgery, high CTC counts at end of CRT/pre-operation significantly associated with early-progression at 10-month PFS.

Conclusion: CTC counts at pre-cycle III and combined changes of CTC/cfDNA are independent prognostic markers for ESCC patients receiving palliative CT. CTC counts at post CRT is predictive for early disease progression for CRT/surgery-treated ESCC patients.

Implications: Longitudinal liquid biopsy serial monitoring provides complementary information for prediction/prognosis for CT responses in advanced ESCC. The CTC/cfDNA blood-based diagnostics have potential clinical utility for non-invasive monitoring of minimal disease burden to better guide clinical treatment.

Project No.: 05160926

AMR-5-59

Translating Functional Tumour Volume and Biology of Peritoneal Carcinomatosis to Identify Suitable Candidate for Cytoreductive Surgery in Ovarian Carcinoma

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Introduction and Project Objectives: In ovarian carcinoma (OC), cytoreductive surgery (CRS) is the primary treatment strategy; however, peritoneal carcinomatosis (PC) may prevent complete CRS. PC burden is quantified using the surgical peritoneal cancer index (sPCI) determined by laparotomy or laparoscopy and is correlated with reduced likelihood of achieving complete CRS. One disadvantage of sPCI is the invasive nature. Diffusion-weighted imaging (DWI) is a functional magnetic resonance imaging (MRI) sequence which has been shown to improve OC tumour characterisation and PC detection [1]. DWI is quantified with apparent diffusion coefficient (ADC) which reflects tumour cellularity and aggressiveness. Previous work has shown that a semi-automated quantification method based on ADC could accurately demarcate functional tumour volumes [2]; this could aid in quantifying PC burden and thus predicting the likelihood of achieving complete CRS. The objectives of our study were: (1) to develop a non-invasive method of assessing PC burden; and (2) to evaluate its predictive value in determining incomplete tumour debulking in patients with advanced or recurrent OC.

Methods: Patients with advanced (International Federation of Gynecology and Obstetrics [FIGO] stage III/IV) or recurrent OC were recruited for DWI prior to surgery. Clinicopathological factors including age, FIGO stage, and pre-surgical serum cancer antigen (CA125) levels were also collected. PC lesions were semi-automatically segmented with a clustering algorithm on DWI which retained voxels associated with solid tumour components. Functional peritoneal cancer index (fPCI) is based on the segmentation results of tumour volume in 13 abdominopelvic regions with additional points given to the involvement of critical sites. The ADC values of the largest PC lesion of each patient were also recorded. fPCI was then correlated with sPCI and surgical complexity and was also assessed on its ability to predict incomplete CRS.

Results: Fifty-three patients (mean age: 56.1 years) were prospectively recruited. Complete CRS was achieved in 38 patients. Significant correlations were found between fPCI and sPCI ($r > 0.757$, $p < 0.001$). fPCI was found to be correlated with surgical complexity scores ($p = 0.043$), and that patients who achieved CRS had significantly lower fPCI compared to patients with incomplete debulking ($p < 0.001$). A predictive model combining fPCI, ADC, and FIGO stage achieved an AUC of 0.947 in predicting incomplete CRS.

Conclusion: fPCI, a non-invasive DWI-based scoring system, offers a semi-automated method of quantifying PC burden. Furthermore, a predictive model including fPCI, ADC, and FIGO could predict the likelihood of incomplete CRS with high accuracy.

Project No.: 03143616

AMR-6-69

A Comprehensive Study on the Effects of Antioxidant Supplements in Liver Cancer Development and Treatment

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Introduction and Project Objectives: Controversy over the benefits of antioxidants supplements in cancers persists for long. Using hepatocellular carcinoma (HCC) as a model, we investigated the effects of exogenous antioxidants N-acetylcysteine (NAC) and glutathione (GSH) on tumor initiation and growth.

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Methods: Multiple mouse models, including diethylnitrosamine (DEN)-induced and Trp53KO/C-MycOE-induced HCC models, mouse hepatoma cell and human HCC cell xenograft models with subcutaneous or orthotopic injection were used. In vitro assays including ROS assay, colony formation, sphere formation, proliferation, migration and invasion, apoptosis, cell cycle assays were conducted. Western blot was performed for protein expression and RNA-sequencing to identify potential gene targets.

Results: In these multiple different mouse and cell line models, we observed that NAC and GSH promoted HCC tumor initiation and growth, accompanied with significant reduction of intracellular reactive oxygen species (ROS) levels. Moreover, NAC and GSH promoted cancer stemness, and abrogated the tumor-suppressive effects of Sorafenib both in vitro and in vivo. Exogenous supplementation of NAC or GSH reduced the expression of NRF2 and GCLC, suggesting the NRF2/GCLC-related antioxidant production pathway might be desensitized. Using transcriptomic analysis to identify potential gene targets, we found that TMBIM1 was significantly upregulated upon NAC and GSH treatment. Both TCGA and in-house RNA-sequence databases showed that TMBIM1 was overexpressed in HCC tumors. Stable knockdown of TMBIM1 increased the intracellular ROS; it also abolished the promoting effects of the antioxidants in HCC cells. On the other hand, BSO and SSA, inhibitors targeting NAC and GSH metabolism respectively, partially abrogated the pro-oncogenic effects induced by NAC and GSH in vitro and in vivo.

Conclusion: Our data implicate that exogenous antioxidants NAC and GSH, by reducing the intracellular ROS levels and inducing TMBIM expression, promoted HCC initiation and tumor growth, and counteracted the therapeutic effect of Sorafenib. Our study provides scientific insight regarding the use of exogenous antioxidant supplements in cancers.

Project No.: 04152336

AMR-7-97

Characterization of Anti-CCL28 as a Novel Therapy to Overcome Sorafenib Resistance in HCC

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Introduction and Project Objectives: Sorafenib is the first molecular drug approved for and remains as an alternative first-line drug treatment advanced hepatocellular carcinoma (HCC). However, the efficacy of sorafenib treatment is only modest. Therefore, there is need to investigate the key mechanisms by which sorafenib resistance develops in HCC patients. Here, we aimed to provide experimental evidence that targeting chemokine (C-C motif) ligand 28 (CCL28) is an efficient therapeutic approach in sorafenib-resistant (SR) HCC.

Methods: Transcriptome sequencing was performed in 41 cases of HKU-QMH patient cohorts and TCGA database was also applied to examine the top upregulated members of cytokines family. The mRNA expression of CCL28 was further validated in human HCCs by real-time PCR. Expression changes of CCL28, HIF-1α and HIF-1α target genes upon hypoxic or sorafenib treatments were assessed by real-time PCR, ELIZA, or Western Blotting. The resistance to sorafenib in HCC cells was examined by annexin-V apoptosis assay. Regulatory T (Treg) cell infiltration was assessed using migration chambers and by flow-cytometric analysis. Orthotopic liver xenograft model in nude mice was used to investigate the efficacy of combination treatment of sorafenib and anti-CCL28 antibody in HCC.

Results: Here we report that CCL28 was the top three most upregulated cytokines in HCC in HKU-QMH cohorts and TCGA. It was overexpressed in human HCCs and its upregulation was correlated with a metastatic phenotype in HKU-QMH clinical cohort. CCL28 expression was significantly induced upon hypoxic treatment (1% O2) in HCC cells and was dramatically attenuated upon HIF-1α knockdown or digoxin treatment. Sorafenib treatment upregulated CCL28 expression in HCC cells and CCL28 overexpression was also seen in the SR HCC cells. In SR HCC cells, we observed that HIF-1α target genes, such as CA9, LOX, VEGF, and CCL28, were significantly upregulated upon sorafenib treatment as compared to the corresponding mock control. However, our results did not support that CCL28 induced Treg cell infiltration in HCC both in vitro and in vivo. Of note, combination treatment with sorafenib and anti-CCL28 neutralizing antibody showed significant tumor regression in orthotopic liver xenograft model in nude mice.

Conclusion: CCL28 may play a role in tumor progression and sorafenib resistance in HCC, likely via a non-Treg cell regulatory pathway, and HIF-1α is a major determinant of CCL28 expression in SR HCC cells. Combination treatment with sorafenib and anti-CCL28 neutralizing antibody may act a novel and efficient therapeutic approach in HCC.

Project No.: 03142966

AMR-8-131

Intestinal Organoid Cultures of Early Onset Colorectal Cancer in Hong Kong

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Abstracts for Poster Presentation: Advanced Medical Research

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Introduction and Project Objectives: The incidence of sporadic early-onset colorectal cancer (EOCRC) has been increasing worldwide, and has been especially high in Hong Kong for over three decades. However, the underlying pathogenic mechanism remains poorly understood due to the lack of a comprehensive study to delineate the molecular alterations, as well as a representative cell model for biological studies. Aims and Objectives: We aimed to generate a living biobank of CRC with paired normal, particularly for sporadic EOCRC, and to document the key drivers in this cohort and the maintenance of genomic stability over long-term culture.

Methods: We established organoids from colectomy samples, with priority given to young CRC patients (≤50). Organoid and frozen tissue genomic profiles were studied in parallel. We also continuously cultured 10 paired tumour-normal organoids in vitro for 6 months and evaluated the changes in genomic profile. Methods: DNA and RNA were extracted from organoids and frozen tissues for whole-exome sequencing (WES) and RNA sequencing. Somatic variants were identified and compared between organoid and frozen tissues, early and late passage organoids.

Results: We established an organoid biobank from 20 CRC patients, with 11 being sporadic EOCRC. This biobank captured tumours evolved from both the conventional adenoma carcinoma sequence and the serrated neoplasia pathway. Five tumour organoids derived from 2 patients carried a PTPRK-RSPO3 fusion, representing the first in vitro 3D cell model of its kind. After long-term culture, the number of variants, their mutant allelic fractions and inferred copy number alterations were well preserved in tumour organoids. However, we observed clonal dominance in normal organoids.

Conclusion: We established a CRC biobank with distinct clinical features and genomic profiles. Heterogeneous tumour organoids are genomically stable, while subclones in normal organoids evolved and dominated during long-term cultures. **Implications:** This biobank is a valuable resource for biological studies and drug sensitivity screening.

Publications: The results of this study were included in a paper published in GUT: Yan HHN, Siu HC, Ho SL, Yue SSK, Gao Y, Tsui WY, Chan D, Chan AS, Wong JWH, Man AHY, Lee BCH, Chan ASY, Chan AKW, Hui HS, Cheung AKL, Law WL, Lo OSH, Yuen ST, Clevers H, Leung SY. Organoid cultures of early-onset colorectal cancers reveal distinct and rare genetic profiles. Gut Epub ahead of print: [26 March 2020]. doi:10.1136/gutjnl-2019-320019. The publication is available at BMJ journal through http://dx.doi.org/10.1136/gutjnl-2019-320019.

Project No.: 02132886

AMR-9-141

MiR-199a-3p as a Key Target in Stemness and Chemoresistance in Ascitic Ovarian Cancer Cells

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Introduction and Project Objectives: Overcoming drug resistance is an inevitable challenge to the successful treatment of cancer. The functional role of abiotic factors in tumor progression is becoming increasingly clear.

Method and Results: Here, we show that ascitic fluid-induced shear stress in conjunction with growth factors in the tumor microenvironment endow ovarian cancer cells with stem-like and drug resistant properties. Notably, among several microenvironmental factors tested, hepatocyte growth factor (HGF), which is abundantly present in the ascites, significantly downregulated miR-199a-3p in a similar pathway as shear stress through transcriptional downregulation of primary miR-199a-1, but not miR-199a-2, through a c-Met/PI3K/Akt signaling pathway through a positive feedback loop. This is accompanied with a decrease in miR-199a-3p expression and upregulation of stemness CD44, ALDH3 and chemoresistance markers ABCG2 and P-gp. Low expression miR-199a-3p expression and Akt activation were associated with platinum resistance of ovarian cancer and confer poorer progression-free survival. miR-199a-3p mimic significantly suppressed ovarian tumor metastasis and its ectopic expression in combination with cisplatin or paclitaxel further decreased the peritoneal dissemination of ovarian cancer in vivo. Our results also confirmed successful miR-199a-3p overexpression and its downstream effectors including a reduction of Oct4, CD44 and as well as ABCG2 and P-gp expression.

Conclusion: Together, these findings unveil the regulation of miR-199a-3p under ascitic shear flow and highlight its importance in ovarian cancer chemoresistance. The novel approach using miR-199a-3p mimic may serve as an effective therapeutic option to resensitize ovarian tumor cells to standard chemotherapy.

Project No.: 05163536

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AMR-10-165

The Role of Regulatory B Cells (Bregs) in Tumor Recurrence after Liver Transplantation for Liver Cancer Patients

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Introduction: Liver transplantation (LT) is the only promising treatment option for selected patients with hepatocellular carcinoma (HCC). However, tumor recurrence after LT remains a critical issue. Regulatory B cells (Bregs) promote cancer recurrence in various human cancers. Early graft injury may recruit lymphocytes to promote tumor recurrence after liver transplantation.

Project Objective: We aimed to investigate the role of regulatory B cells (Bregs) in tumor recurrence after liver transplantation and further to explore the underlying mechanisms through a series of clinical association study, in vitro functional experiments and animal models.

Methods: The association among graft injury, Bregs and tumor recurrence was evaluated both in clinical cohort and rat LT models. The role of CXCL10/CXCR3 signaling in Breg mobilization was further studied in CXCL10-/- and CXCR3-/- mice model simulating post-transplantation liver graft injury (hepatic ischemia/ reperfusion (IR) followed by major hepatectomy).

Results: Clinically, the percentage of circulating Bregs in recipients with graft weight ratio (GWR)<60% was higher compared to the recipients with GWR≥60% after LT. Recipients with high percentage of circulating Bregs on day 7 after LT showed a higher incidence of tumor recurrence (recurrence vs. non-recurrence, 0.62±0.08 vs. 0.34±0.02% of PBMCs, $p<0.05$). The association among liver graft injury, Bregs mobilization and tumor recurrence was further confirmed in rat LT models. Functional study demonstrated that Bregs induced tumor recurrence by promoting HCC proliferation and invasion through NF-κB/MMPs signaling pathway. And mobilization and recruitment of Bregs was CXCL10/CXCR3 signaling dependent.

Conclusion: CXCL10/CXCR3 signaling during liver graft injury mobilized circulatory Bregs to promote tumor recurrence after liver transplantation. Circulatory Bregs may serve as a novel marker to predict and monitor cancer recurrence in the scenario of liver transplantation for liver cancer patients.

Manuscript is under revision in "Oncoimmunology (IF:8.11)": CXCL10/CXCR3-mediated Breg mobilization promotes tumor recurrence via NF-κB/MMP2/MMP9 after liver transplantation
Authors: Yan Shao, Xiao Bing Liu, Chung Mau Lo,, Xiang Qi, Jiang Liu, Chang Xian Li, Chang Chun Ling, Wai Ho Yeung, Hui Liu, Yuen Yuen Ma, Kevin Tak-Pan Ng, Kwan Man*

Project No.: 03143336

AMR-11-201

Targeting Human Papillomavirus (HPV) Negative Cervical Cancer

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Introduction and Project Objectives: While the great majority of cervical cancer is associated with persistent infection by oncogenic human papillomavirus (HPV), a small fraction of HPV negative cervical cancer is consistently identified, and little is known about their oncogenesis mechanism. Previous genomic studies suggested most HPV negative cervical cancer carry mutations in the gene ARID1A encoding one of the components of the SWI/SNF chromatin remodeling complex. Many drugs targeting ARID1A are under development as ARID1A mutations are common in many malignancies. This study aims to examine the hypothesis that most HPV negative cervical cancer are genetically associated with mutated SWI/SNF complex components; and to explore the utility of ARID1A targeting agents in treating HPV negative cervical cancer.

Methods: DNA was extracted from paraffin fixed formalin embedded (FFPE) and frozen archival cervical cancer tissues and tested with a sensitive HPV line blot assay INNO-LiPA. Immunohistochemical staining of ARID1A was performed on the FFPE tissues. Targeted sequencing was performed on 36 samples consisting of 24 HPV positive and 12 HPV negative samples. Indels in the coding sequences of SWI/SNF complex genes and PI3K-Akt signalling genes were examined. Efficacy of GSK126 and MK2206 on suppressing growth of HPV negative cervical cancer cell model C33A and HPV positive cell model HeLa, SiHa, and CaSki was assessed in 3D culture and nude mice xenograft models.

Results: The overall survival of HPV negative cancer patients was shorter than that of HPV positive cancer patients, although statistical significance was not reached. ARID1A protein expression was not significantly different in HPV positive and HPV negative tumours. This is consistent with the finding in targeted sequencing that in our cohort, ARID1A mutations were not common. Instead, ARID1B and BCL1111B were the most commonly mutated SWI/SNF complex genes in HPV negative tumours. GSK126 could effectively suppress the growth of C33A and HeLa spheroids, but not SiHa and CaSki spheroids. Similarly, GSK126 could inhibit the growth of C33A and HeLa xenograft.

Conclusion: Our results suggest that ARID1A is not always commonly mutated in HPV negative cervical cancer.

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Nevertheless, the SWI/SNF complex is still likely dysfunctional and can be targeted using EZH2 methyltransferase inhibitors such as GSK126. GSK126 is a promising candidate for treating HPV negative cervical cancer.

Project No.: 05162176

AMR-12-202

p21-activated Kinase 4 in Ovarian Cancer Chemoresistance

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Introduction: p21-activated kinase 4 (PAK4) is an oncogenic serine/threonine kinase associated with chemoresistance in ovarian cancer. As a nucleo-cytoplasmic shuttling protein, nuclear PAK4 is known to facilitate gene transactivation in cancer cells. Concerted EGFR-PAK4 signaling is important for ovarian cancer cell growth and EGFR activation promotes nuclear localization of PAK4.

Project Objectives: This study aims to elucidate the mechanism of PAK4-mediated chemoresistance in ovarian cancer through gene transcription; and to explore the possibility of enhancing the anti-tumour efficacy of chemotherapy and EGFR inhibitors by targeting PAK4.

Methods: Ovarian cancer cell lines A2780s and SKOV3 stably expressing PAK4-WT (Wild type PAK4) or PAK4-ΔNLS1 (nuclear localization signal mutant PAK4) were established. The transcriptomes of SKOV3-PAK4-WT and SKOV3-PAK4-ΔNLS1 were compared by microarray analysis using an Affymetrix Genechip. MTT assay was performed to generate dose-response curves of a PAK4 inhibitor PF-3758309 in combination with cisplatin or gefitinib in ovarian cancer cell lines OVCAR3 and TUOS3. Expression and phosphorylation status of group II PAKs members and ErbB family members before and after drug treatment were profiled by western blotting in OVCAR3 and TUOS3. Apoptosis was evaluated by cell cycle analysis with PI staining. Anti-tumourigenic effects of the drug combinations in vivo was evaluated using cell line and patient-derived xenografts.

Results: Expression of nuclear PAK4 led to significant downregulation of genes involved in DNA damage repair. BAG2 was found to be a downstream target of nuclear PAK4 to reduce chemosensitivity in ovarian cancer cells. Inhibition of PAK4 by PF-3758309 enhanced the anti-tumoural effect of cisplatin and EGFR inhibitor gefitinib in both cell line and patient-derived xenograft models.

Conclusion: PAK4 contributes to chemoresistance through transcriptional regulation of BAG2 in ovarian cancer. Targeting PAK4 in ovarian cancer may enhance the anti-tumourigenic effect promoted by cisplatin and EGFR inhibitors.

Project No.: 03143006

AMR-13-221

Targeting Stearoyl-CoA Desaturase-1 (SCD1) in Combination with Sorafenib for the Treatment of Hepatocellular Carcinoma

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Introduction and Project Objectives: We investigated the functional role and clinical significance of Stearoyl CoA desaturase-1 (SCD1) mediated endoplasmic reticulum (ER) stress in regulation of liver tumor-initiating cells (T-ICs) and sorafenib resistance, aiming to develop a novel therapeutic strategy against hepatocellular carcinomas (HCCs).

Methods: We evaluated the clinic-pathological relevance of SCD1 and its correlation with sorafenib resistance in large cohorts of HCC clinical samples by qPCR and immunohistochemical analyses. Lentiviral-based overexpression and knockdown approaches were performed to characterize functional roles of SCD1 in regulation of liver T-ICs and sorafenib resistance. Molecular pathways mediating the phenotypic alterations was identified through RNA sequencing analysis and functional rescue experiments. The combinatorial effect of SCD1 inhibitor and sorafenib was tested using our patient-derived tumour xenograft (PDTX) model.

Results: SCD1 overexpression was found in HCC which was associated with shorter disease free survival. SCD1 was found to regulate the populations of liver T-ICs; while its suppression by SCD1 inhibitor suppressed liver T-ICs and sorafenib resistance. Interestingly, SCD1 was markedly upregulated in our established sorafenib-resistant PDTXs, and its overexpression predicts the clinical response of HCC patients to sorafenib treatment. Suppression of SCD1 forces liver T-ICs to differentiate via ER stress induced unfolded protein response (UPR), resulting in their enhanced sensitivity to sorefenib. Using a patient-derived xenograft model (PDTX#1), we found that a novel SCD1 inhibitor (SSI-4) demonstrated maximal growth suppressive effect when combined with sorafenib treatment.

Conclusion: SCD1 mediated ER stress regulates liver T-ICs and sorafenib sensitivity. Targeting SCD1 alone or in combination with sorafenib might be a novel personalized medicine against HCC.

Project No.: 03142736

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AMR-14-222

Interleukin-1 Receptor Associated Kinase 1 (IRAK1) is a Target that Drives Liver Tumor Initiating Cells and Sorafenib Resistance in Hepatocellular Carcinoma

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Introduction and Project Objectives: Frequent relapse and drug resistance can be attributed to the existence of tumor-initiating cells (T-ICs) within the tumor bulk. From transcriptome sequencing of 16 pairs of clinical HCC samples, we found that Interleukin-1 receptor-associated kinase 1 (IRAK1) in the TLR/IRAK pathway was significantly upregulated in hepatocellular carcinoma (HCC). In this study, we aim to characterize and delineate the regulatory mechanism of IRAK1 on liver T-ICs, evaluate the correlation of IRAK1 expression with liver T-IC markers and sorafenib resistance in human HCC, and ultimately evaluate the therapeutic efficacy of IRAK1/4 inhibitor in combination with sorafenib in the treatment of HCC.

Methods: In this study, we evaluated the clinic-pathological relevance of IRAK1 in large cohorts of HCC clinical samples by qPCR, western blot and immunohistochemical analyses. Lentiviral-based overexpression and knockdown approaches were performed to characterize functional roles of IRAK1 in regulation of liver T-ICs including self-renewal, tumorigenicity, chemoresistance, drug resistance to sorafenib, migration and expression of liver CSC markers and sorafenib resistance. Molecular pathways mediating the phenotypic alterations was identified through RNA sequencing analysis and functional rescue experiments. Lastly, the combinatorial effect of IRAK1/4 inhibitor and sorafenib was tested using HCC xenograft models.

Results: IRAK1 overexpression was observed in HCC at the mRNA and protein levels and correlated with advanced tumor stages and poor patents survival. Interestingly, IRAK4, an upstream regulator of IRAK1, was also found to be consistently upregulated. We demonstrated that IRAK1 regulates liver T-IC properties, including self-renewal, tumorigenicity and liver T-IC marker expression. IRAK1 inhibition sensitized the HCC cells to doxorubicin and sorafenib treatment in vitro through the suppression of the apoptotic cascade. Pharmacological inhibition of IRAK1 with a specific IRAK1/4 kinase inhibitor consistently suppressed liver T-IC populations. Through RNA sequencing analysis by comparing gene expression profiles between IRAK1-knockdown and control cells, we identified Aldo-Keto Reductase Family 1 Member 10 (AKR1B10) as a novel downstream target of IRAK1. Clinically, AKR1B10 was found to be overexpressed in HCC, which was significantly correlated with IRAK1 expression. Functional analysis demonstrated that knockdown of AKR1B10 negated the IRAK1-induced T-IC functions via modulation of the AP-1 complex. Using an HCC xenograft model, we found that an IRAK1/4 inhibitor in combination with sorafenib synergistically suppressed the tumor growth.

Conclusion: Our data suggests that targeting the IRAK4/IRAK1/AP-1/AKR1B10 signaling pathway may be a potential therapeutic strategy against HCC.

Project No.: 04150266

AMR-15-48

Early Growth Genetics and Cardiometabolic Risk in Chinese Adolescents

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Introduction and Project Objectives: Accumulating evidence suggested a genetic link between prenatal growth and cardiometabolic diseases later in life. This study aims to: 1) discover novel common genetic variants associated with birthweight (a proxy for measuring prenatal growth) in Chinese adolescents and also in multiple cohorts of Chinese population; 2) explore the long-term influence of birthweight-related variants on cardiometabolic traits measured during adolescence.

Methods: In the discovery stage, we performed genome-wide association study (GWAS) in 3,772 Chinese-ancestry individuals from four independent sources: a) 1872 adolescents from a community-based school survey for risk factor assessment; b) 915 children from the Hyperglycaemia and Adverse Pregnancy Outcomes study at the Hong Kong centre; c) 452 adults from hospital staff and a territory-wide health screening program; and d) 533 related individuals from the Hong Kong Family Diabetes Study. Within each cohort, around 4.8 million high-quality SNPs were tested for the association with birthweight using either a linear regression or a linear mixed model, with adjustments for gender, gestational age (if available) and principal components (PCs). Results of individual studies were combined by meta-analysis under a fixed-effects model. Samples for in silico replication were taken from the published trans-ancestry meta-analysis of birthweight in up to 321,223 individuals, contributed by the EGG Consortium. We tested the association between our top hits for birthweight and cardiometabolic traits (including the obesity traits, fasting glucose and insulin levels, HOMA-IR, HOMA-β, blood pressure, lipid profiles and albumin-creatinine ratio) in the cohort of adolescents using a linear regression with adjustments for PCs,

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sex, age, and/or body mass index.

Results: In the GWAS for birthweight, we have identified 15 suggestive loci for birthweight in adolescents using a standard threshold of P<10^-5; but none was previously reported and was replicated in independent cohorts. In the meta-analysis of GWAS using multiple Chinese cohorts, a total of 20 distinct genomic regions were prioritized (P<1×10^-5), including a reported locus GCK and 19 suggestive novel loci. Using the data contributed by the EGG Consortium, we observed replication of associations for two signals located at GCK (P=4.5×10^-3) and DLGAP2 (P=5.0×10^-3) loci. Moreover, the birthweight-lowering allele of the GCK variants was significantly associated with higher fasting glucose level measured during adolescence (1.1×10^-4 < P < 3.5×10^-3).

Conclusion: We have identified novel associations between the fetal glucose-raising alleles at GCK loci and reduced birthweight in Chinese population. This study demonstrated evidence of shared genetic determinants between early growth phenotype and cardiometabolic risk factors.

Project No.: 05161386

AMR-16-54
Evaluating the Clinical Utility of Genome Sequencing for Cytogenetically Balanced Chromosomal Abnormalities in Prenatal Diagnosis

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Introduction and Project Objectives: Balanced chromosomal abnormalities (BCAs) are changes in the localization or orientation of a chromosomal segment without visible gain or loss of genetic material. BCAs occur at a frequency of 1 in 500 newborns and are associated with an increased risk of multiple congenital anomalies and/or neurodevelopmental disorders, especially if it is a de novo mutation. As GS sequencing cost continues to be reduced, it is foreseeable that GS will become more affordable for clinical use in the near future. In order to judge the feasibility and clinical utility of GS in the evaluation of BCAs, the clinical implications will be examined.

Methods: In this pilot project, we used short read genome sequencing (GS) to retrospectively re-sequence ten prenatal subjects with de novo BCAs and compared the performance of GS with the original karyotyping. To detect all chromosomal abnormalities, including cryptic genomic imbalances, GS data were analyzed by in-house bioinformatics pipeline customized for structural variants (SV) and copy number variants (CNV) detection.

Results: GS characterized all BCAs found by conventional karyotyping with the added benefit of precise sub-band delineation. In nine out of ten cases in this study (90%), the conventional karyotype results were revised by at least one sub-band. By identifying BCA breakpoints at the nucleotide level using GS, we found disruption of OMIM genes in three cases and identified cryptic gain/loss at the breakpoints in two cases. Of these five cases, four cases reached a definitive genetic diagnosis and were classified as pathogenic under the ACMG pathogenicity framework while the other one case had a BCA interpreted as unknown clinical significance. The additional information gained from GS can change the interpretation of the BCAs and has the potential to improve the genetic counselling and perinatal management by providing a more specific genetic diagnosis.

Conclusion: To conclude, the findings from this study demonstrated the advantages of GS over conventional karyotyping on the detection of BCAs. GS allows the precise detection of BCA breakpoints and cryptic genomic imbalances surrounding the regions of BCAs. This results in better evaluation on the risk of congenital anomalies in BCA on a case-by-case basis.

Project No.: 05162986

AMR-17-93
Genetic Study of the SNARE Gene Family in Hong Kong Chinese with Bipolar Disorder

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Introduction and Project Objectives: Bipolar disorder is a mood disorder with extreme mood swings previously known as "manic-depression". It was estimated to be 2.5% among Hong Kong's general population. The hereditability is estimated to be 80%–85%.3 Genetic research has the power to explain significant aspects of mental illness by identifying genetic factors that can explain substantial components in the variation of human behavior. SNARE complex proteins are necessary for

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vesicular neurotransmission and could be an important class of proteins associated with susceptibility to bipolar disorder. This project aims to study the association of genetic variants that associate with the susceptibility of bipolar disorder in Hong Kong Chinese patients with a special focus on SNARE genes and their interaction partners.

Methods: We used firstly a candidate gene targeted deep resequencing approach (N=30) to examine potential damaging mutations in SNARE complex and associated proteins. Then we use a bigger sample with the rest of our subjects for genotyping analyses. Our target is a group of BPD patients of Han Chinese ethnicity in Hong Kong (a total of 628 subjects, including BPD, some other psychiatric patients and healthy controls).

Results: We found a risk allele of LONRF1 (LON peptidase N-terminal domain and ring finger 1) that associated with BPD. Regarding dominant model, two SNPs in LONRF1 gene rs12678448 and rs3802268 were found to be associated with psychiatric disorders. Besides LONRF1, several genes with potentially damaging risk alleles are — USO1, UNC13B, STX2 and SNAP29. Two genes were found to have potentially damaging Insertion/Deletion mutations NAPA and PI4KA. SCFD1 gene has a potential splicing mutation.

Conclusion: In summary these SNARE related genes that associated with BPD could be targets for novel drug therapy. The finding of circadian rhythm gene polymorphism in LONRF1 may imply that some subjects having a particular variant may benefit more than others by light therapy which has been shown to be effective in some cases of bipolar disorder.

Project No.: 03144526

AMR-18-147

Whole Genome Sequencing Analysis of Genetically Undiagnosed Euploid Fetuses with Increased Nuchal Translucency

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Introduction: Increased nuchal translucency (NT) detected during the first trimester is a well-known and widely accepted marker for chromosomal aneuploidies or genomic disorders. The risk of having major fetal anomalies, miscarriage, and fetal death increases markedly in relation to the thickness. Chromosomal microarray studies revealed that ~8% of these fetuses carry pathogenic copy number variants (CNVs). However, the genetic etiologies yet to be discovered may be diverse in terms of genes and variant types. A holistic and comprehensive approach to uncover CNVs, single nucleotide variants (SNVs), structural variants (SVs) is imperative for the investigation of this patient group.

Project Objectives: In this study we propose to (i) study the genome wide spectrum and frequency of genetic variants and spatial genomic organization during early fetal development, (ii) investigate the variability of genomic variants associating with the increased NT related birth defects, (iii) discuss the feasibility of application of WGS in the prenatal setting.

Methods: Fetuses with increased nuchal translucency (>3.5mm) with structural abnormalities previously undiagnosed by karyotype and chromosomal microarray were recruited for whole genome sequencing at >100X depth to detect SNVs, indels, structural variations (including inversions, translocations), noncoding variants, and mosaicisms. Results were integrated for interpretation in accordance with the guidelines of the American College of Medical Genetics and Genomics. Pathogenic or likely pathogenic (P/LP) variants were selected for molecular validation.

Results: Overall, 15 trios were enrolled in this study, including ten cases with isolated increased NT and five cases with additional structural malformations. Whole Genome sequencing detected additional diagnostic findings in 5 trios (33%), including four cases with P/LP single-nucleotide variants in the genes COL2A1, ANKRD11, ARMC4, GATA4, and one mosaic (40%) turner syndrome. In one fetus, a cryptic complex structural rearrangement was detected which involved a 150.1kb insertion seq[hg19]ins(2;12)(q33.2;q24.31). The insertion segment was divided into 11 sub segments, of which five were inserted to chromosome 2q33.2. This complex rearrangement disrupted the BMPR2 gene and could be associated with Primary Pulmonary Hypertension and/or Venoocclusive Disease 1.

Conclusion: Our study demonstrates high read-depth genome sequencing can facilitate diagnosis of euploid fetuses with increased nuchal translucency. The diagnostic findings across different mutation types detected by genome sequencing were important for clinical management and decision making. GS could be suggested to be a more comprehensive prenatal genetic test. Recently, we have implemented genome sequencing in prenatal care in Hong Kong to bridge the gap between clinical phenotypes and underlying undiagnosed genetic etiologies.

Project No.: 04152666

Abstracts for Poster Presentation: Advanced Medical Research

AMR-19-166

Oligodendrocyte Pathology in Ataxia-Telangiectasia - The Cellular Basis of Myelin Abnormality in a Rare Genetic Disease

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Introduction and Project Objectives: The early loss of myelination in the cerebral cortex is a common pathology in the aging brain. In rare diseases with distinct genetic deficits in DNA double strand breaks repairs, Ataxia Telangiectasia (A-T), premature myelin loss was commonly observed. We hypothesized that the compromised DNA repair in the oligodendrocyte (OL) lineage may be associated with age-related myelin loss. To investigate if DSB repair mediated by A-T mutated protein (ATM) is critical to myelination, we compared the histopathology of OLs in the human A-T brain tissues.

Methods: The post-mortem human frontal cortex and cerebellar cortex specimens of normal control (n = 18, mean age =18.5 years), A-T (n = 10, mean age = 26.2 years) were obtained from NeuroBioBank, National Institute of Health. The genomic mutations in each A-T case were confirmed by TruSight Inherited Disease Sequencing Panel on an Illumina platform. The histological changes of OL lineage and their DNA damage burden were examined by immunohistochemistry with the corresponding gene expression profiled by an array of real-time polymerase chain reaction analysis. The pathological data were collated with the targeted genomic sequencing analysis followed by in silico modelling.

Results: Consistent with clinical ataxia, immunohistochemistry revealed a significant reduction of OLs in the cerebellar cortex, but not frontal cortex, of A-T tissues. Such cerebellar OL degeneration was strongly associated with frameshift ATM mutations but appeared to be independent of neuronal loss. Importantly, our structural analysis, three-dimensional modelling and docking experiments of the mutant ATM proteins in silico indicated that key OL-specific proteins MBP and MyRF are putative substrate of ATM kinase activity. Particularly, when the ATM structure is prematurely terminated at the spiral/pincer regions (1-1892 aa), these putative interactions are compromised and likely to related to the different severity of OL loss in A-T patients.

Conclusion: Our findings suggested that the cerebellar but not cortical OL population in the A-T brain. While such regional specificity warrants further investigations, this study suggested that the vulnerability of OL to ATM dysfunction may be the cellular basis of myelin loss in A-T and the aging brain.

Project No.: 04151436

AMR-20-174

Diagnostic Value of Whole Exome Sequencing in Chinese Patients with Rare Pediatric Onset Neuromuscular Diseases Having Diagnostic Challenges

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Introduction and Project Objectives: Neuromuscular disorders (NMDs) comprise a group of rare heterogeneous genetic diseases with a broad spectrum of overlapping clinical presentations that makes diagnosis challenging. Notably, the recent introduction of whole-exome sequencing (WES) is introducing rapid changes on the genetic diagnosis of NMDs. We aimed to investigate the diagnostic value of WES for rare pediatric-onset NMDs.

Methods: We applied integrated diagnostic approach and performed WES in 50 Chinese subjects (30 males, 20 females) with undiagnosed pediatric-onset NMDs despite previous comprehensive diagnostic tests. The patients were categorized in four subgroups according to phenotyping and investigation findings. Variants on NMDs gene list and open exome analysis for those with initial negative findings were identified.

Results: WES identified causative variants in ACTA1 (n = 2), POMT1, COL6A1 (n = 2), MTMR2, LMNA, SELENON, DNM2, TGFB1, MPZ, IGHMBP2, and LAMA2 in 13 patients. We identified the first reported case of MTMR2 in Chinese. Two subjects have variants of uncertain significance (VUSs) in TTN and SCN11A, unlikely to be pathogenic due to incompatible phenotypes. The mean interval time from symptom onset to genetic diagnosis was 10.4 years (range from 1 month to 33 years). The overall diagnostic yield of WES in our cohort was 26% with findings comparable to overseas studies. Open exome analysis was necessary to identify the pathogenic variant in TGFB1 that caused skeletal dysplasia with neuromuscular presentation.

Conclusion: Our study shows a clear role of WES in the pathway of integrated diagnostic approach to shorten the diagnostic odyssey in patients with rare NMDs. WES is recommended as the first-tier genetic testing for rare NMDs.

Project No.: 03142176

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AMR-21-196

Parental Expectations of Raising a Child with Disability in the Decision-Making for Prenatal Testing and Termination of Pregnancy: The Local Needs for Genetic Counselling and Public Education

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Introduction and Project Objectives: Prenatal testing is widely available in clinical practice in Hong Kong. The study aimed to examine attitudes toward prenatal testing and termination of pregnancy (TOP) among parents and obstetric providers in relation to their views on raising a child with disability.

Methods: An explanatory sequential mixed-methods study was conducted. A survey among 274 parents and 141 providers was followed by interviews with 26 parents and 10 providers. Using multivariate analysis, the relationships between attitudes were examined. Thematic analysis was used to identify the reasons behind the attitudes.

Results: Parents and providers reported different expectations of a child with disability, of which affecting their attitudes of termination of pregnancy. Parents reported more positive attitudes toward raising a child with disability and more moral views about TOP. In contrast, providers reported more variations in attitudes toward offering prenatal testing and TOP. Significant associations were found between attitudes toward prenatal testing, raising a child with disability, reproductive autonomy, and TOP. Three major themes were identified: (1) meanings of parenthood from genetic tests; (2) views toward TOP and parental responsibility; and (3) implications of advanced extended prenatal genetic testing.

Conclusion: Perceived social-cultural norms of disabilities and parental expectations of raising a child with disability influence decision-making regarding TOP. Providers need to explore parental values in disability and TOP rather than assume parents share their views. As more conditions of the fetus are able to be detected, the implications of the technology and disabilities need to be addressed in antenatal care.

Project No.: 03144536

AMR-22-230

Risk Assessment of Hereditary Breast and Ovarian Cancer Syndrome in Chinese Population by Multiple-Gene Sequencing

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Introduction: Differences in the mutation spectrum across ethnicities. suggest that it is important to identify genes in addition to common high penetrant genes to estimate the associated breast cancer risk.

Project Objective: To assess the prevalence of other breast cancer associated genes in Chinese high-risk breast and/or ovarian cancer patients.

Methods: A total of 1,338 high-risk breast cancer patients who tested negative for germline BRCA1, BRCA2, TP53 and PTEN mutations between 2007-2017 were selected from the Hong Kong Hereditary Breast Cancer Family Registry. Patient samples were subjected to next-generation DNA sequencing using a multigene panel. All detected pathogenic variants were validated by bi-directional DNA sequencing. The sequencing data was co-analyzed by our in-house developed bioinformatics pipeline.

Results: Sixty-one pathogenic variants (4.6%) were identified in 11 cancer predisposition genes. The majority of the carriers (77.1%) had early-onset of breast cancer (age <45), 32.8% had family members with breast cancer and 11.5% had triple-negative breast cancer (TNBC). The most common mutated genes were PALB2 (1.4%), RAD51D (0.8%) and ATM (0.8%). A total of 612 variants of unknown significance (VUS) were identified in 494 patients, and 87.4% of the VUS were missense mutations.

Conclusion: An additional 4.6% of the patients were identified in patients who tested negative for germline BRCA1, BRCA2, TP53 and PTEN mutations using the multigene test panel.

Project No.: 03143406

AMR-23-23

Behavioural Dysexecutive Syndrome after Stroke

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Introduction and Project Objectives: Behavioural dysexecutive syndrome (BDES), a common phenomenon in stroke patients, usually manifests as agitation/aggression, euphoria and apathy. This study aimed to evaluate the clinical course, prevalence

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and clinical and brain imaging correlates of BDES in a cohort of Hong Kong stroke survivors.

Methods: This longitudinal study enrolled a cohort of 369 stroke survivors and 237 healthy controls. For stroke survivors, baseline measurements were obtained at 3 months after the index stroke and follow-up measurements were obtained at an average of 38 months post-stroke. Healthy controls were assessed once only upon recruitment. BDES was assessed using the Chinese version of the Dysexecutive Questionnaire (self-version). The stroke severity, disability in daily activities, global cognitive and executive function, anxiety and depressive symptoms and other clinical information were obtained. The presence and location of infarcts were evaluated via magnetic resonance imaging (MRI).

Results: At 3 months post-stroke, the prevalence of BDES was 18.7%. The Hospital Anxiety Depression Scale anxiety subscale score (odds ratio [OR]=1.184, 95% confidence interval [CI]=1.083-1.295, p<0.001), presence of current depression (OR=4.055, 95%CI=2.060-7.983, p<0.001) and Mini-Mental State Examination score (OR=0.805, 95%CI=0.705-0.906, p<0.001) were identified as significant predictors of the presence of post-stroke BDES in a multivariate logistic regression. No significant MRI correlate was identified. The BDES group exhibited poorer performances on the Chinese version of Frontal Assessment Battery (compared with the non-BDES and healthy control groups, respectively: 10.1±2.5 vs 12.8±2.4 and 12.5±2.8, p=0.001 and 0.016), Colour Trails Test (error: 4.9±4.9 vs 0.6±1.7 and 0.3±0.7, p<0.001 for both; nearly-missed response: 6.2±7.3 vs 0.6±2.5 and 0.1±0.4, p<0.001 for both; prompts: 7.2±7.4 vs 1.1±2.8 and 0.4±1.3, p<0.001 for both; and time required to complete CTT2: 215.0±80.4 vs 162.5±76.8 and 127.6±87.2 seconds, p=0.040 and 0.001), Category Fluency Test (intrusion response: 1.4±1.8 vs 0.4±1.0 and 0.3±0.6, p<0.001, for both and total correct response: 33.0±11.3 vs 41.3±10.6 and 46.6±11.8, p=0.025 and <0.001) and Arrow Test (response time: 47.5±27.1 vs 24.6±10.5 and 20.4±5.4 seconds, p<0.001 for both and interference score: 104.6±101.0 vs 24.8±36.2 and 13.2±7.3, p<0.001 for both).

Conclusion: Many stroke survivors develop BDES within 3 months post-stroke. The study results indicate that anxiety symptoms, current depression and poor cognitive functioning predict BDES at 3 months after the index stroke. BDES was related to poor performance during executive functioning tasks such as conceptualisation, category fluency and motor programming.

Project No.: 02130726

AMR-24-25

Development of 3D DNA Nanocages as Safe and Cost-effective Nanocarriers for BBB Penetration and Potential Use in Targeted Drug Delivery in the Brain System

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Introduction and Project Objectives: Millions of people worldwide are being affected by neurodegenerative diseases including brain tumor, stroke, Parkinson's disease and Alzheimer's disease, etc. The clinical applications of chemotherapy in brain have been severely limited due to the restricted transport of sufficient amount of active therapeutic agents across the blood-brain barrier (BBB) for disease treatment in the central nervous system (CNS). So far, typical two strategies including invasive and noninvasive drug delivery have been investigated to enhance therapeutic efficacy in the brain system. However, they still suffers from several drawbacks including high cost, high risk and high level of discomfort in patients, loss of drug activity after chemical modification, and complicated preparation steps. To ameliorate this problem, scientists have been gradually explored the use of nanocarriers including liposomes, albumin nanoparticles, polymeric nanoparticles, metallic nanoparticles or synthetic dendrimers for drug delivery to brain systems. They have been functionalized with a variety of targeting moieties in order to facilitate the penetration across the BBB. However, some studies indicated that drug loading efficiency of polymeric nanoparticles is not very high (~ 10%) and some drugs such as paclitaxel could dissociate from albumin nanoparticles very shortly after administration in the blood stream. In addition, it is well-known that polymeric or metallic nanoparticles induce cytotoxicity under a high concentration of accumulation in a living system. Thus, development of alternative nanocarrier systems which would exhibit efficient drug loading, substantial cellular uptake, low cytotoxicity and BBB penetration is still of great research and clinical interest for targeted drug delivery to the brain tumor.

Methods: In this work, we have successfully assembled 3D DNA nanocages and functionalized them with BBB penetration peptides.

Results: We showed that self-assembled DNA nanocages are promising tools for delivery applications because of their substantial cellular uptake, low cytotoxicity, high bio-stability and biocompatibility.

Conclusion: The fully double-stranded feature of nanocages is favorable for different cargo loading mechanisms such

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as intercalation and minor groove binding. Therapeutic agents such as anticancer drugs could be efficiently loaded onto functionalized 3D DNA nanocages and then carried to specific sites of interests in cellular environment. Importantly, self-assembled DNA nanocages with/without peptide functionalization are able to pass through the BBB and get into the brain system in vivo.

Project No.: 03141076

AMR-25-34

Massively Parallel Discovery of Combination Therapies for Parkinson's Disease

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Introduction and Project Objectives: Multigenic perturbations lead to many human diseases including Parkinson's disease (PD), which is the second most common neurodegenerative disease. Discovering synergistic drug combinations that target the perturbed genes could improve motor symptoms and suppress neurodegeneration in PD. However, conventional methods in identifying promising drug combinations are labor intensive and cost-ineffective. Our newly developed screening platform, combinatorial genetics en masse (CombiGEM)-CRISPR enables rapid assembly of barcoded combinatorial genetic libraries for high-throughput functional characterization of genetic perturbations. To facilitate the discovery of effective drug combinations, this project aims to perform massively parallel studies with CombiGEM-CRISPR to isolate druggable gene combinations that suppress PD-associated cytotoxicity in rotenone- and MPP+-induced models, and to validate genetic hits and the matching drug combination effects in in vitro and in vivo models of PD.

Methods: Combinatorial CRISPR-Cas9 screens were performed using pairwise guide RNA library comprising 7,569 combinations, targeting 28 druggable genes.

Results: Specific druggable gene knockouts and the matching drugs that rescue cells from rotenone- and MPP+-induced toxicities were identified. We validated the effect of the top hit HSP90B1 + HDAC2 identified from the screens on suppressing PD-associated cytotoxicity. To translate the genetic combinations to therapeutic candidates, we applied drug combinations that correspond to the druggable targets and measure their effects on rotenone- and MPP+-induced cell death in SK-N-MC cells using MTT activity assays. (17-(Dimethylaminoethylamino)-17-demethoxygeldanamycin (17-DMAG) and vorinostat were used as the drugs to target HSP90B1 and HDAC2, respectively. Our results indicated that 17-DMAG and vorinostat act synergistically to enhance cell survival against rotenone- and MPP+- induced toxicity when compared to single-drug treatments in SK-N-MC cells. In addition, we observed this drug combination reduced toxicity induced by alpha-synuclein expression in transgenic flies, another well-characterized model of PD.

Conclusion: We have successfully carried out large-scale profiling studies to evaluate the effect of druggable gene combination en masse, and identified the simultaneously knockout of HSP90B1 and HDAC2 protects cells from rotenone- and MPP+-induced toxicities. We further validated the protective effect of the matching drug combination regimen (17-DMAG + vorinostat) using multiple in vitro and in vivo models of PD. Our work paves the way for further exploring the efficacy of the identified combination regimen for therapeutic use.

Project No.: 04151416

AMR-26-56

Activation of Hedgehog Signaling Promotes Development of Mouse and Human Enteric Neural Crest Cells, Based on Single-cell Transcriptome Analyses

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Introduction and Project Objectives: It has been a challenge to develop fully functioning cells from human pluripotent stem cells (hPSCs). We investigated how activation of hedgehog signaling regulates derivation of enteric neural crest (NC) cells from hPSCs.

Methods: We analyzed transcriptomes of mouse and hPSC-derived enteric NCs using single-cell RNA sequencing (scRNA-seq) to identify changes in expression associated with lineage differentiation. Intestine tissues were collected from Tg(GBS-GFP), Sufuf/f; Wnt1-cre, Ptch1+/- and Gli3Δ699/Δ699 mice and analyzed by flow cytometry and immunofluorescence for levels of mRNAs encoding factors in the hedgehog signaling pathway during differentiation of enteric NCs. Human NC cells (HNK-1+ p75NTR+) were derived from IMR90 and UE02302 hPSC lines. hPSC were incubated with hedgehog agonists (SAG) and antagonists (cyclopamine) and analyzed for differentiation. hPSC-based innervated colonic organoids were derived from these hPSC lines and analyzed by immunofluorescence and neuromuscular coupling assay for expression of neuronal subtype markers and for assessing the functional maturity of the hPSC-derived neurons, respectively.

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Results: scRNA-seq analysis revealed that neural fate acquisition by human and mouse enteric NCs requires reduced expression of NC- and cell cycle- specific genes and upregulation of neuronal- or glial-lineage specific genes. Activation of the hedgehog pathway was associated with progression of mouse enteric NCs to the more mature state along the neuronal and glial lineage differentiation trajectories. Activation of the hedgehog pathway promoted development of cultured hPSC into NCs of greater neurogenic potential by activating expression of genes in the neurogenic lineage. The hedgehog agonist increased differentiation of hPSCs into cells of the neuronal lineage by upregulating expression of GLI2 target genes, including INSM1, NHLH1, and various bHLH family members. The hedgehog agonist increased expression of late neuronal markers and neuronal activities in hPSC-derived neurons.

Conclusion: In enteric NCs from humans and mice, activation of hedgehog signaling promotes differentiation into neurons by promoting cell-state transition, expression of genes in the neurogenic lineage, and functional maturity of enteric neurons.

Project No.: 03143236

AMR-27-75

Ketamine Inhibits Stress-Induced Dendritic Spine Elimination through Activation of Parvalbumin Interneurons

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Introduction and Project Objectives: Ketamine is a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist that is commonly used for anaesthesia. Recent studies show that a single subanaesthetic dose of ketamine exerts robust antidepressant effects on treatment-resistant depressive disorder patients. Animal studies also show that ketamine exerts antidepressant-like effects and increases dendritic spine density. Nevertheless, it is unclear how ketamine affects dendritic spine dynamics under stressed condition in vivo.

Methods: To investigate the underlying mechanism of ketamine antidepressant effects, we used in vivo two-photon transcranial imaging microscopy to examine the effects of ketamine on dendritic spine plasticity in the frontal association cortex (FrA) in 1-month-old chronic restraint stressed mice.

Results: We found that restraint stress induced dendritic spine loss by decreasing the rate of spine formation and increasing the rate of spine elimination. Ketamine inhibited stress-induced spine loss mainly by protecting mushroom spines from elimination. Ketamine also induced re-formation of spines in close proximity to previously stress-eliminated spines. Electrophysiological and in vivo imaging experiments showed that ketamine enhanced activity of parvalbumin (PV) interneurons under stress condition and counterbalanced the stress-induced net loss of PV axonal boutons. In addition, selective chemogenetic excitation of PV interneurons mimicked the protective effects of ketamine on dendritic spines against stress.

Conclusion: Chronic stress exposure has been reported to induce dendritic spine loss and reduce dendritic arborization in prefrontal cortex and hippocampus. Previous studies reported that ketamine increased dendritic spine density in naive or stressed animals using fixed brain tissues. However, it is unclear whether the increase in dendritic spine density is owing to the promotion of spinogensis or inhibition of spine elimination. By using in vivo two-photon imaging, we traced how repeated stress affected synaptic structure dynamics in the FrA and how ketamine counteracted stress effects in 1-month-old mice. We showed that stress-induced loss of dendritic spines was the result of reduced spine formation and enhanced spine elimination. We also found that ketamine counteracted the loss of dendritic spines by preventing stress-induced spine elimination, while having minimal effect on spine formation. In addition, ketamine increased the activity of genetically labelled PV interneurons in the FrA of mice under acute stress in vivo. Furthermore, data from chemogenetic experiments showed that selective activation of PV interneuron prevented stress-induced spine elimination, whereas inhibition of PV interneuron abolished the protective effect of ketamine against stress-induced spine elimination, suggesting the involvement of PV interneuron activity in the modulation of dendritic spines by ketamine. Taken together, our data provide new insights on the effects of ketamine on synaptic circuitry under stress and a possible mechanism to counteract stress-induced synaptic impairments through PV interneurons activation.

Project No.: 03143096

AMR-28-76

Human Pluripotent Stem Cell-derived Ectomesenchymal Stromal Cells Promote More Robust Functional Recovery than Umbilical Cord-derived Mesenchymal Stromal Cells after Hypoxic-ischaemic Brain Damage

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Introduction and Project Objectives: Hypoxic-ischaemic encephalopathy (HIE) is one of the most serious complications in neonates and infants. Mesenchymal stromal cell (MSC)-based therapy is emerging as a promising treatment avenue for HIE. However, despite its enormous potential, the clinical application

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of MSCs is limited by cell heterogeneity, low isolation efficiency and unpredictable effectiveness. In this study, we aimed to examine the therapeutic effects and underlying mechanisms of human pluripotent stem cell-derived ectomesenchymal stromal cells (hPSC-EMSCs) in a rat model of HIE.

Methods: hPSC-EMSCs were induced from either human embryonic stem cells or induced pluripotent stem cells. Stem cells or the conditioned medium (CM) derived from stem cells were delivered intracranially or intranasally to neonatal rats with HIE. Human umbilical cord-derived MSCs (hUC-MSCs) were used as the therapeutic comparison control and phosphate-buffered saline (PBS) was used as a negative control. Lesion size, apoptosis, neurogenesis, astrogliosis and microgliosis were evaluated. The rotarod test and Morris water maze were used to determine brain functional recovery. RNA-seq and ELISA assays were used to determine the secretory factors that were differentially expressed between hPSC-EMSCs and hUC-MSCs.

Results: hPSC-EMSCs showed a higher neuroprotective potential than hUC-MSCs, as demonstrated by a more significant reduction in lesion size and apoptosis in the rat brain following hypoxia-ischaemia (HI). Compared with PBS treatment, hPSC-EMSCs promoted endogenous neurogenesis and alleviated astrogliosis and microgliosis. hPSC-EMSCs were more effective than hUC-MSCs. hPSC-EMSCs achieved a greater recovery of brain function than hUC-MSCs and PBS in rats with HIE. CM derived from hPSC-EMSCs had neuroprotective and neurorestorative effects in vitro through anti-apoptotic and neurite outgrowth- and neurogenesis-promoting effects. Direct comparisons between hPSC-EMSCs and hUC-MSCs revealed the significant enrichment of a group of secretory factors in hPSC-EMSCs, including nerve growth factor (NGF), platelet-derived growth factor-AA and transforming growth factor-β2, which are involved in neurogenesis, synaptic transmission and neurotransmitter transport, respectively. Mechanistically, the CM derived from hPSC-EMSCs was found to potentiate NGF-induced neurite outgrowth and the neuronal differentiation of NPCs via the ERK/CREB pathway. Suppression of ERK or CREB abolished CM-potentiated neuritogenesis and neuronal differentiation. Finally, intranasal delivery of the CM derived from hPSC-EMSCs significantly reduced brain lesion size, promoted endogenous neurogenesis, mitigated inflammatory responses and improved functional recovery in rats with HIE.

Conclusion: hPSC-EMSCs promote functional recovery after HI through multifaceted neuromodulatory activities via paracrine/trophic mechanisms. We propose the use of hPSC-EMSCs for the treatment of HIE, as they offer an excellent unlimited cellular source of MSCs.

Project No.: 03140496

AMR-29-79

FGF21 Mediates the Anti-Depressant Effects of Exercise by Coordinating the Crosstalk between Brain and Peripheral Organs

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Introduction and Project Objectives: Major depression is a common psychiatric disease affecting a large percentage of people worldwide. Increasing evidence suggests that diabetes and obesity are major risk factors for depression and that lifestyle intervention, especially physical exercise, is one of the most effective strategies to prevent depression. Fibroblast growth factor (FGF) 21 is a metabolic hormone critically involved in energy metabolism, and it is also a potential drug for treating obesity-related metabolic disorders. Circulating FGF21 level is markedly elevated in both rodents and humans with physical exercise. This study aims to investigate the role of FGF21 in mediating the antidepressant effects of exercise, and to test our hypothesis that FGF21 exerts its anti-depressant effects by mediating the multi-organ crosstalk between liver, muscle and brain.

Methods: Both global and conditional genetic knockout (KO) mice were employed to explore the role of FGF21 in exercise-induced alleviation in depression-like behaviors and the underlying mechanisms. Furthermore, the modulatory effects of FGF21 on the tryptophan-kynurenines pathway were evaluated using both gain- and loss-of-functional experiments.

Results: Exercise training significantly alleviated depressive symptoms in wild-type (WT) mice, however, these anti-depressive effects of exercise were largely diminished in FGF21 KO mice. Replenishment with recombinant mouse FGF21 alone was sufficient to reverse obesity-induced depression through alleviation in neuroinflammation and improvement in neurogenesis and plasticity. Muscle-specific depletion of the FGF21 co-receptor β-Klotho further demonstrated that the anti-depressant effects of FGF21 were attributed in part to its direct actions in skeletal muscle by controlling the tryptophan-kynurenine axis. Furthermore, adiponectin served as a downstream mediator of FGF21 to confer the anti-depression effects of physical exercise.

Conclusion: Collectively, these data identify FGF21 as an important mediator for the anti-depressant effects of exercise through suppressing hippocampal neuroinflammation and promoting neurogenesis and plasticity. The anti-depressant effect of FGF21 was partially dependent on its ability to induce

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adiponectin secretion in adipocyte and to modulate the tryptophan-kynurenine axis in skeletal muscle. The findings shed new light on how physical exercise prevents major depression and protect neuronal functions by modulating hormone-mediated multi-organ crosstalk and also raise the possibility that FGF21 and its agonist may represent a promising therapeutic agent for depression.

Project No.: 03144516

AMR-30-104
Local Infusion of Cholecystokinin in the Auditory Cortex and Successive Sound Stimuli Lead to Epileptic Seizures

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Introduction and Project Objectives: Epileptic seizures represent an imbalance between neural excitation and inhibition that result from an abnormal synaptic plasticity. Cholecystokinin (CCK), an extremely abundant neuropeptide in central nervous system, has indispensable role in regulating neural excitatory and inhibitory balance. Our previous results proved that CCK-positive neurons from entorhinal cortex (EC) determine neocortical plasticity. In this project, we sought to develop an epilepsy model through electrical activation of the EC, develop an audiogenic model of epilepsy through the administration of CCK-4, and also to test CCK-B receptor (CCK-BR) antagonists as potential anti-epileptic drugs so as to extend our understanding of the role of CCK in the development of epilepsy.

Methods: In order to evoke seizure activities through electrical kindling, a chronic microelectrode was implanted in the EC followed by daily application of a high-frequency electrical stimulus in CCK-A and –B receptor knockout (CCK-ABR-KO) and wildtype mice. Audiogenic epilepsy model was developed by intraperitoneally injecting mice with CCK-4 or long-acting CCK-4 analogue followed by pairing with low-intensity noise (~50 dB), myoclonic jerks of the head and neck in response to noise or light (control) stimulus after pairing was analysed using MATLAB custom algorithm. A fast-screening of CCK-BR antagonists based on whether they can block long term potentiation in cortical brain slices was carried out by applying theta burst stimulation (TBS) in the presence of selected CCK-BR antagonists and kainic acid (KA) model of temporal lobe epilepsy was adopted to test the efficacy of these antagonists against epileptic seizures.

Results: Local electrographic seizure known as afterdischarge was evident in EC kindled mice. CCK-ABR-KO mice showed a higher afterdischarge threshold and prolonged period of post-ictal depression compared with wildtype. After pairing with noise, CCK-4 injected mice showed myoclonic jerks of the head and neck with brief twitching movement which rhymed in oscillation of noise. Different from classic audiogenic seizure model that uses high-intensity noise (~100 dB) to induce seizure, our mouse model shows synchronized movement in the presence of low intensity noise (~50 dB) that the mouse showed no response previously. Also, administration of CCK-BR antagonists (YF476 or YM022) reduced the frequency of spontaneous convulsive seizures in our KA epileptic mice model.

Conclusion: Our results reveal that CCK-ABR-KO mice are resistant to induction of convulsive seizure, CCK-4 administration can evoke audiogenic seizure and blocking CCKB-receptor can alleviate epilepsy in KA model. Our findings therefore suggest the role of CCK in epilepsy and the anti-epileptic effects of CCKB-receptor antagonists.

Project No.: 03141196

AMR-31-138
High-throughput Brain Activity Mapping and Machine Learning as a Foundation for Systems Neuropharmacology

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Introduction and Project Objectives: The development of next-generation technologies for mapping dynamic patterns of neural activity has the potential to revolutionize understanding of brain function in both health and disease. One critical application of these technologies is the systematic characterization of existing pharmacological agents used to treat brain disorders along with novel experimental therapeutics in order to advance the development of next-generation therapies.

Methods: In this project, we describe a high-throughput, in vivo drug screening strategy that combines automated whole-brain activity mapping with computational bioinformatics analysis. Our strategy utilizes functional brain physiology phenotypes derived from live, non-anesthetized zebrafish that have been treated with compounds of interest as an input for predicting the therapeutic potential of neuroactive compounds. This technology relies on an autonomous robotic system capable of manipulating awake zebrafish larvae for rapid microscopic imaging of their brains at the level of cellular resolution, which allows for rapid assessment of action potential firing across a whole zebrafish brain; as a result, a large number of whole-brain activity maps (BAMs) can be acquired for a compound library.

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Results: Our study was performed in two phases. The first phase utilized a 179 compound "training set" of clinical used approved drugs, which were used to generate information-rich BAMs. Next, the intrinsic coherence among the BAMs for drugs in the training set was determined by a consensus clustering algorithm. This analysis revealed that certain BAM drug clusters were associated statistically with the drugs' therapeutic categories as determined by the World Health Organization (WHO) Anatomical Therapeutic Chemical (ATC) classification system. Using the test case of anti-epileptic drugs, in the second phase of the study the clustering results were used to build a functional classifier along with a ranking mechanism, which successfully predicted anti-epileptic candidates from a library of 121 non-clinical compounds. Excitingly, this analysis provided novel insights in the form of specific compounds and processes, for example epigenetic mechanisms, that have the potential to help guide development of next-generation anti-epileptic agents with novel mechanisms of action.

Conclusion: Take as a whole, the HT-BAMing technology, computational approaches, and foundational dataset for systems neuropharmacology we describe has the potential to provide insight into mechanisms of action of poorly understood pharmacological agents and novel compounds. Future applications of this strategy in conjunction with genetically engineered zebrafish models of CNS disorders has tremendous potential to assist in the discovery of novel disease-modifying pharmacological agents to expand the treatment options available to patients with CNS disorders.

Project No.: 03141146

AMR-32-157
Striatal Dopamine Transmission in Individuals with Isolated Rapid Eye Movement Sleep without Atonia: A Search for Precursor Biomarker for Neurodegeneration

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Introduction and Project Objectives: REM sleep behaviour disorder (RBD) is a specific precursor of synucleinopathic neurodegeneration. However, neurodegenerative implication of isolated REM sleep without atonia (RSWA) in the absence of dream enactment behavior is unclear. The current study aimed to examine the neurodegenerative implication of isolated RSWA among first-degree relatives (FDRs) of RBD patients, as reflected by their loading of neurodegenerative risk factors and prodromal markers and stratal dopamine transmission function.

Methods: This case-control study recruited a total of 50 age and sex-matched subjects (Mean age = 58.6±9.1 years. 34% female) into three arms: FDRs of RBD patients with isolated RSWA (n=16), FDRs of RBD patients without isolated RSWA (n=18) and controls who did not have any RWSA and family history of RBD (n=15). Subjects underwent comprehensive clinical and polysomnographic assessment. Striatal dopaminergic transmission function of the subjects was assessed by triple-tracer (18F-DOPA, 11C-Raclopride and 18F-FDG) PET/CT scan.

Results: The three groups did not differ in their striatal dopaminergic transmission function as measured by triple-tracer PET/CT scan. While they did not differ in their Prodromal Parkinson's Disease likelihood ratio by MDS Research Criteria, they differed significantly in their prevalence of a family history of clinically diagnosed synucleinopathies (Parkinson's disease (PD) or dementia of Lewy bodies (DLB)) among their FDRs (Fischer exact test: FDRs with RSWA vs FDRs without RSWA vs non-RBD FDRs controls = 58.8% vs 22.2% vs 0%, p = 0.001).

Conclusion: Using GEE analysis, RSWA is a significant predictor of having a family history of clinically diagnosed synucleinopathies (B=1.61, Wald 95% CI= 0.14 – 3.08, Wald chi-square = 4.59, d.f. = 1, P = 0.032).

Project No.: 04153036

AMR-33-192
Alleviation of Early High Mortality and Aggravated Brain Pathology by Lutein in a Genetic Type I Diabetic Mouse Model after Experimental Stroke

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Introduction: Type 1 diabetic patients experienced a higher mortality and shortened median survival after stroke.

Project Objectives: to understand the mechanisms of earlier mortality and aggravated brain damage in type I diabetic patients after ischemic stroke using a genetic mouse model of type I diabetes (Ins2Akita/+ mice) and to identify the

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therapeutic potential of lutein treatment.

Study Design: male Ins2Akita/+ mice as a type I diabetic animal model with their age-matched littermates (11-12 weeks old) will be challenged with experimental stroke induced by middle cerebral artery occlusion with or without lutein treatment.

Methods: Hyperglycemic Ins2Akita/+ mice were challenged with transient middle cerebral artery occlusion to induce experimental stroke

Results: After 2h of long ischemia, hyperglycemic Ins2Akita/+ mice exhibited aggravated neurological deficits, increased infarct size and hemorrhagic transformation as early as 2h after reperfusion. Earlier death and higher mortality rate were observed in Ins2Akita/+ mice with a longer duration of reperfusion and hemorrhagic transformation further exaggerated at 22h of reperfusion in those survived. Since 2h after reperfusion, decreased ZO-1 and increased MMP-9 immunoreactivities in the infarct cores, down-regulation of ZO-1 but up-regulation of VEGF, p-Erk1/2 and p-p38 at protein level, and elevated mRNA expression of ER stress-related CHOP were seen in Ins2Akita/+ ipsilateral brains. After 0.5h of ischemia, infarcts similar to those induced by 2h long ischemia were observed in Ins2Akita/+ mice at 23.5h after reperfusion but with milder neurological deficits. Administration of lutein, an anti-inflammatory and anti-oxidative agent, was successful in reducing neurological deficits in Ins2Akita/+ mice subjected to 0.5h of ischemia, although the suppression of infarct aggravation was not statically significant.

Conclusion: Results of 2h long ischemia suggested that hyperglycemia plays an important role in the exacerbation of stroke at an early stage by compromising blood vessel integrity and exerting inflammatory response, while results of 0.5h short ischemia showed the neuroprotective effect of lutein against ischemia/reperfusion injury.

Implications (for health care services, health care delivery, health policy in Hong Kong): Lutein is neuroprotective and may act as a safe potential treatment after stroke attack in diabetic patients.

Project No.: 03142256

AMR-34-220

Investigating the Impact of Periodontitis on Neuroinflammation, Neuropathology and Neurodegeneration in Alzheimer's Disease

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Introduction: Periodontitis, a source of chronic systemic inflammation, is a pathological inflammatory condition of the gum that leads to progressive destruction of the periodontium. Given that inflammation plays a pivotal role linking periodontal infections and various systemic diseases, increasing lines of evidence also indicated that periodontitis might participate in the progression of neurodegenerative diseases such as Alzheimer's disease (AD).

Project Objectives: As inflammation within the brain is a prominent feature of AD, chronic, low-grade inflammatory conditions such as that seen in periodontitis may thus exacerbate neuroimmune responses and AD progression. Our present study aimed to apply two different experimental models of periodontitis (bacterial-induced periodontitis and ligature-induced periodontitis) in 3xTg-AD mice and examine cognitive dysfunctions and neuropathology

Methods: For bacterial-induced periodontitis, female 3xTg mice at 6 months of age were injected with heat-killed P. gingivalis bacteria into their buccal mucosa 3 times per week every other week for a total of 5 weeks. For ligature-induced periodontitis, another group of mice had silk sutures tied around the maxillary second molars for the same duration of time. Effects of periodontitis on sickness behavior and cognitive functions were assessed by open field, spontaneous Y-maze, and puzzle box test. Following behavioral testing, the jaws and gums were harvested for the evaluation of periodontal status. Different brain regions were harvested for further biochemical and immunohistochemical analysis.

Results: Both models of periodontitis led to a significant loss of periodontal bone level, which was accompanied by increased gene expression levels of IL-1β and TNF-α in the gums. Results from the behavioral tests revealed that bacterial injection exacerbated both short- and long-term memory function, while ligature placement reduced exploratory motivation and exacerbated long-term memory function in AD mice. When assessing for tau pathology, significantly higher levels of phosphorylated tau proteins were detected in the brains of AD mice following induction of periodontitis. Concomitant with the increase in phosphorylated tau proteins, our findings also showed that AD mice injected with heat-killed bacteria were presented with elevated microglial and astrocytes immunoreactivity in the brain.

Conclusion: Findings from the present study confirmed that experimental periodontitis could enhance the brain inflammatory response and subsequently exacerbate AD tau

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pathology and cognitive functions in 3xTg mice.

Project No.: 04151216

AMR-35-27

Effects of RANKL Inhibition on Promoting Healing of Bone Erosion in Rheumatoid Arthritis Using HR-pQCT: A 2-year, Randomised, Double-blind, Placebo-controlled Trial

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Introduction and Project Objectives: Partial repair of bone erosions in rheumatoid arthritis (RA) is known from high-resolution peripheral quantitative CT (HR-pQCT) studies in patients with moderate to high disease activity using anti-tumour necrosis factors and anti-IL-6 biological therapies. Whether receptor activator of nuclear factor kappa B ligand (RANKL) inhibition by denosumab is superior to placebo in healing of existing erosions in patients with RA with low disease activity or in remission on conventional synthetic disease-modifying antirheumatic drugs is uncertain. The aim of the study was to evaluate the effects of denosumab on erosion healing at 2–4 metacarpophalangeal (MCP) head as determined by HR-pQCT in patients with RA with stable disease.

Methods: This was a randomised, placebo-controlled, double-blind study. Patients with RA with disease activity score 28 joints (DAS28) ≤5.1 were randomised (1:1) to subcutaneous denosumab 60 mg or placebo once every 6 months for 24 months. Patients were treated to the target of DAS28 remission or LDA throughout the study period according to a standard protocol. The primary outcome was erosion healing at MCP 2–4 on HR-pQCT at 12 months. The effects of denosumab on erosion and joint space parameters on HR-pQCT and radiographs, disease activity and health assessment questionnaire-disability index (HAQ-DI) were also examined.

Results: At 24 months, HR-pQCT images were analysed in 98 patients. One-third of the patients achieved sustained low disease activity throughout the study. At 12 months, changes in erosion parameters on HR-pQCT were similar between the two groups. At 24 months, new erosions (19% vs 9%, p=0.009) and erosion progression (18% vs 8%, p=0.019) were more common in the placebo group than the denosumab group. Erosion healing was seen in a significantly higher proportion of patients in the denosumab group (20% vs 6%, p=0.045) at 24 months. Logistic regression analysis revealed that the use of denosumab was associated with erosion healing (OR 3.39, 95% CI 1.08 to 10.63) after adjustment for covariates. No significant changes in joint space parameters on HR-pQCT, van der Heijde-Sharp erosion score, DAS28 and HAQ-DI were observed in the two groups at 12 and 24 months. The treatments were well tolerated.

Conclusion: Although no differences in erosion parameters were observed at 12 months, denosumab was more efficacious than placebo in erosion repair on HR-pQCT after 24 months. It could be considered a treatment option for retarding bone damage progression independent of the disease activity control.

Project No.: 04152616

AMR-36-45

The Efficacy of Buscopan® in Reducing Pain during Ultrasound-guided Manual Vacuum Aspiration (MVA): A Randomized Controlled Trial

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Introduction and Project Objectives: Patients undergoing ultrasound-guided manual vacuum aspiration (USG-MVA) for early pregnancy loss complains of moderate pain from uterine spasms. The current pain control regimen includes the use of oral non-steroidal anti-inflammatory drugs (NSAIDs) taken an hour before the procedure, paracervical block (PCB) and topical lidocaine gel applied just before the insertion of the catheter. We hypothesized that the addition of Buscopan®, an anti-spasmodic drug, may improve the pain control during USG-MVA.

Methods: This was a prospective, double-blinded, randomized controlled trial conducted between February 2018 and January 2020 in Prince of Wales Hospital. 111 women were assigned to receive a 1ml intravenous injection containing either a 20mg Buscopan® (n = 55) or saline (n = 56) as placebo immediately before the USG-MVA procedure. Primary outcome was the pain scores immediately and 2 hours after the USG-MVA. Secondary outcomes were complications, side effect profiles, psychological states, physiological stress (saliva alpha-amylase, sAA) and client satisfaction. Two-way mixed ANOVA was used to evaluate for main effects and interactions.

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Results: For the 'Buscopan®' group, median abdominal pain scores were 15% lower immediately post-procedure and 21% lower 2 hours post-procedure, though not statistically significant. Repeated measures ANOVA indicated that the both vaginal and abdominal pain scores improved significantly with the time (Vaginal: $F(1,108) = 180.10$, $p < 0.0001$; Abdominal: $F(1,108) = 83.41$, $p < 0.001$) but were independent of randomization group (Vaginal: $F = 0.32$, $p = 0.320$; Abdominal: $F = 1.41$, $p = 0.650$). No difference was noted for the complications and side effect profiles between the two groups. Measured Log10 sAA levels reduced significantly with time ($F(2.8, 286.1) = 6.30$, $p < 0.001$) but not with group ($F = 0.10$, $p = 0.960$). However, the 'Buscopan®' group reported significantly higher ($p = 0.032$) mean VAS satisfaction scores compared to the placebo group (79.0 ± 17.3 vs 73.4 ± 24.1).

Conclusion: Ultrasound-guided manual vacuum aspiration is associated with a moderate amount of uterine contraction pain. Women receiving Buscopan® for pain relief were, in general, more satisfied with the procedure than those who received a placebo. Higher satisfaction scores in those receiving Buscopan® may be related to the slight reduction in immediate post-procedure abdominal cramping pain, rather than vaginal pain. Anti-spasmodics can be helpful in the reduction of USG-MVA associated abdominal cramping pain. Further studies with larger doses or alternative anti-spasmodics are warranted.

Project No.: 05160406

AMR-37-50

Commissioned Programme on the CUHK Phase 1 Clinical Trial Centre at the Prince of Wales Hospital

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Introduction and Project Objectives: Phase 1 clinical trial is the foundation of translational research, bridging advances in basic science to clinical application. The objectives of the CUHK Phase 1 Clinical Trial Centre (P1CTC) are to: 1) develop an internationally recognized centre for conducting phase 1 clinical trials, 2) build a specialized team and infrastructure to support phase 1 clinical trials, 3) promote knowledge transfer among stakeholders in drug development and 4) provide pharmacokinetic (PK)/ pharmacodynamic platform to support the development of investigational medicinal products (IMP), Traditional Chinese Medicine (TCM) and other complementary products

Methods: The entire 11/F (EF block) of the Prince of Wales Hospital was converted into a P1CTC that comprises of 24 beds equipped with facilities for resuscitation and safety monitoring devices, 2 consultation rooms, recreational space, drug storage room, specimen-processing area, nurses stations and offices. Two liquid chromatography mass spectrometry machines were acquired to support PK work.

The Centre was supported by a clinical team (physicians, nurses, pharmacist, research assistant and workman) and an operation/ business development team. The Chief Director, 2 Medical Directors and 2 Deputy Medical Directors supervised the operation, research activities and strategic direction.
The Centre was governed by the Faculty of Medicine, Clinical Research Management Office and Clinical Research Ethics Committee for adherence to Standard Operating Procedures and other practice policies. This safeguards accuracy and integrity of scientific output and ensured subject safety.

Results: From 2014 to 2019, the Centre completed 46 phase 1 clinical trials and recruited over 600 subjects. Thirty percent of these studies involved IMPs in oncology and 70% in hepatology, endocrinology , TCM and other disciplines. In August 2016, the Centre was recognised by China Food and Drug Administration (CFDA) for conducting clinical testing of pharmaceutical compound and 7 CFDA studies were completed. Other notable achievements included the establishment of a precision oncology program to molecularly match patients to anti-cancer drug trials, formation of the Asia-One Phase 1 Research Consortium in oncology and the launch of a webpage and e-platform for facilitating recruitment of healthy volunteers. The Centre hosted educational observerships for overseas visitors and masterclasses in Methods of Cancer Research.

Conclusion: The Centre was established in accordance to the objectives of the commissioned programme to support early phase evaluation of novel compounds. In our commitment to promote biotechnology development in Hong Kong, the Centre will continue to expand capacity and facilitate support to key stakeholders in drug development.

Project No.: CTC-CUHK

AMR-38-55

Using Ultrasound for Screening Scoliosis to Reduce Unnecessary Radiographic Radiation - A Prospective Diagnostic Accuracy Study on 442 Schoolchildren from the Scoliosis Screening Program in Hong Kong

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Introduction and Project Objectives: Angle of Trunk Rotation (ATR) and Moiré Topography are used in screening scoliosis in Hong Kong. Those screened positive for suspected scoliosis will have x-ray assessment. Subjects with Cobb angle ≥ 20° are referred for specialist care. There were cases with Cobb angle < referral threshold of 20° thus being subjected to unnecessary x-ray exposure. Our objective is to determine if ultrasound can identify subjects "not for specialist referral" to reduce unnecessary x-ray exposure.

Methods: Schoolchildren screened positive for suspected scoliosis were prospectively recruited from the scoliosis screening program. In addition to whole spine radiography, ultrasound of the spine was independently performed on the same day. X-ray-based referral status, i.e. "Cobb≥20°-for specialist referral" or "Cobb<20°-not for specialist referral", was the gold standard. The ultrasound-based referral status was determined with the ultrasound spinous process angle (SPA). ATR was also measured.

Results: 442 subjects (243 females and 199 males, mean age 13.2±1.8 years) with various degrees of coronal curvatures (mean Cobb angle of major curve 14.0°±6.6°, range 0-39.0°) were studied. 78 subjects (17.6%) had Cobb angles ≥ 20°. Patient-based analysis showed that area under the ROC curve was 0.735 ($p<0.001$) with ultrasound-derived SPA alone for predicting the referral status, and improved to 0.832 ($p<0.001$) when ATR was incorporated into the prediction model. The sensitivity and specificity were 92.3% and 51.6% respectively at a probability cut-off of 0.11. The positive and negative predictive values were 29.0% and 96.9% respectively.

Conclusion: This study provided strong evidence that ultrasound together with ATR measurement was useful for identifying schoolchildren who did not require specialist referral with Cobb angle < 20°. This helped to reduce unnecessary X-ray exposure in the referral workflow of the scoliosis screening program. In the present study, 42.5% of subjects who would have been subjected to whole spine radiography could avoid taking x-ray with incorporation of ultrasound into the screening program. On the other hand, 1.4 % of subjects had false negative results among whom only 3 (0.7%) had major Cobb > 25°. Ultrasound could therefore be considered for incorporation into the scoliosis screening program to minimize radiographic exposure in line with the "As Low As Reasonably Achievable" (ALARA) principle of radiation safety especially for immature subjects.

Project No.: 04152896

AMR-39-74

Deciphering the Molecular Mechanism of Protein Arginine Methyltransferase (PRMT) 1 in the Regulation of Hepatic Glucose and Lipid Metabolism

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Introduction and Project Objectives: Non-alcoholic fatty liver disease (NAFLD) is strongly associated with obesity and type 2 diabetes and it is an increasingly prevalent liver disease characterized by high intrahepatic triglyceride accumulation due to various causes other than excessive alcohol consumption. Despite of an accumulating number of studies have revealed the complex mechanism behind NAFLD, there is still no pharmacological therapy available to treat this disease in the clinic. In this study, we investigated a novel role of protein arginine methyltransferases (PRMT) 1 in the activation of fatty acids β-oxidation in NAFLD.

Methods and **Results:** In the mice with adeno-associated virus (AAV)-facilitated over-expression of PRMT1, it dramatically attenuated hepatic steatosis upon 4-month high-fat diet (HFD) feeding with improved liver function. Meanwhile, we observed an accelerated fatty acids β-oxidation rate in the liver lysate with PRMT1 over-expression upon HFD when compared to HFD control group. Along with the activation of fatty acid β-oxidation, we found that the expression of PGC-1α was increased at both mRNA and protein levels in PRMT1 over-expression mice. Thus, we hypothesised that, despite of the methylation of PGC-1α at protein level, PRMT1 regulated it at transcriptionally level as well. By using methyltransferase inactive PRMT1 mutant (G80R), we identified the binding site of HNF-4α, which is a transcription factor regulated by PRMT1 and served as co-activator of PGC-1α with PRMT1 using luciferase reporter assay.

Conclusion: Taken together, these data suggested a novel role of PRMT1 in the regulation of fatty acid β-oxidation upon long-term HFD feeding through the activation PGC-1α at transcriptional level.

Project No.: 03143966

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AMR-40-121

Cardiovascular Disease Risk Factors in Late Adolescence of Late Preterm and Early Term Births: A Prospective Observation from the "Children of 1997" Birth Cohort

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Introduction: Evidence from Western settings suggests that individuals born preterm have higher blood pressure. The association of late preterm and early term birth with cardiovascular disease risk factors, and whether infant growth mediates these associations, is however less clear.

Project Objectives: To assess the associations of late preterm and early term birth with cardiovascular disease risk factors in young adulthood and to assess whether any of the associations are mediated by accelerated infant weight growth.

Methods: In the prospective Hong Kong birth cohort "Children of 1997" (n=4857, 60% follow-up), we used multivariable linear regression, with multiple imputation and inverse probability weighting, to examine the associations of gestational age, i.e. late preterm (34 0/7 to 36 6/7 weeks), early term (37 0/7 to 38 6/7 weeks) and term birth (39 0/7 to 42 6/7 weeks), with cardiovascular disease risk factors at 17.5 years. We tested whether any association was mediated by accelerated weight gain from birth to 12 months, defined as an increase in weight z-score ≥0.67, using the Pearl's mediation formula.

Results: Among the included cohort participants born at ≥34 week gestation, 183 (3.8%) were late preterm births, 1419 (29%) early term births and 3198 term births (67%). Compared to term births, late preterm births had higher BMI z-score (0.27, 95% CI 0.01,0.44), waist-to-hip ratio z-score (0.29, 95% CI 0.11, 0.47), waist-to-height ratio z-score (0.31, 95% CI 0.12, 0.49), fat mass index (0.47, 95% CI 0.05, 0.90), lean mass index (0.38 95% CI 0.10, 0.65) and systolic blood pressure (2.92 mmHg, 95% CI 1.27, 4.57), but not % body fat (0.88, 95% CI -0.23, 2.02) at 17.5 years. The associations with higher weight to height indices but not with higher waist ratios or blood pressure were partially mediated by accelerated weight gain from birth to 12 months. Fasting lipid profile and HbA1c levels did not differ by gestational age.

Conclusion: Young adults born late preterm without in-utero growth restriction or postnatal growth faltering had elevated systolic blood pressure, suggesting a full-term gestation is beneficial to cardiovascular health and should be encouraged. Accelerated infant growth partially mediated the higher fat mass and lean mass but not higher waist ratios or blood pressure in adults born late preterm, implicating different pathways to different markers of cardiovascular health and avoiding rapid infant growth in such late preterm births cannot prevent the adverse impact of shorter gestational age on subsequent blood pressure.

Project No.: 03143766

AMR-41-122

Serum Interleukin 6 and Insulin Resistance – A Mendelian Randomization Study

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Introduction: Low grade inflammation, as characterised by a higher interleukin 6 (IL-6) level, is associated with insulin resistance and type II diabetes. However it is unclear whether the previous observed association is causal or due to confounding or reverse cauality.

Project Objectives: To assess whether higher IL-6 level causally associates with insulin resistance using Mendelian randomisation.

Methods: In a prospective Hong Kong birth cohort "Children of 1997" (3498 participants), we used multivariate linear regression with multiple imputation to examine associations of IL-6 concentrations with insulin resistance assessed from homeostasis model assessment-insulin resistance (HOMA-IR). To examine the causal relations of IL-6 with HOMA-IR using Mendelian randomisation, we used genetic predictors of IL-6 from the Magnetic Consortium to assess effects on HOMA-IR in "Children of 1997" and in the Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC). We combined the genetic variant specific Wald estimates using inverse-variance weighted (IVW). We evaluated the heterogeneity in the IVW estimate using the MR-Egger regression.

Results: Genetically predicted IL-6 was significantly associated with HOMA-IR in MAGIC consortium, however the association was weakened when a SNP associated with body mass index was removed. The same direction of the associations between IL-6 and HOMA-IR was seen in the Mendelian randomisation analysis in the "Children of 1997" birth cohort, however some associations did not reach statistical significance, partly attributed to the weaker genetic instruments and smaller sample size.

Conclusion: Some of our findings support a potential causal role of IL-6 in the development of insulin resistance. However, given the limitation of the present Mendelian randomisation analysis, including weak instrument and potential pleiotropic effects, further studies using stronger genetic instruments are warranted to confirm whether IL-6 is a therapeutic target to

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improve insulin sensitivity and avert the progression to type II diabetes.

Project No.: 03143776

AMR-42-130
Selective Overexpression of SIRT1 in Adipose Tissue Protects Adiponectin Deficiency Induced Liver Injury

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Introduction and Project Objectives: SIRT1 is a metabolic sensor regulating energy homeostasis in mammals. Previous results demonstrate that overexpression of human SIRT1 selectively in adipose tissue of mice (Adipo-SIRT1) enhances insulin sensitivity and prevents metabolic ageing. Preliminary results indicated that clusterin, a molecular chaperone, was up-regulated by SIRT1 in adipose tissue. The present study investigated the role of adipose SIRT1 in the prevention of obesity-induced NAFLD.

Methods: Mice including those with adipose overexpression of human SIRT1 (Adipo-SIRT1) or their wild type (WT) littermates, and those lacking clusterin expression without (CKO) or with adipose overexpression of human SIRT1 (CKOAdipo-SIRT1) were fed with a high fat diet (HFD) for 12-weeks, starting from the age of five-weeks. Body weight and fat mass, circulating glucose, lipid and insulin levels, systemic insulin sensitivity and energy expenditure were monitored on a regular basis. At the end of treatment, serum samples were collected for analyzing the lipid contents, fatty acid composition and lipoprotein particles. Liver and adipose tissues were subjected to histological staining, biochemical and molecular analyses. Mitochondria and mitochondria-endoplasmic reticulum contacting sites (MERCs) were isolated from adipose tissue for structural and functional comparisons.

Results: The present study revealed that overexpression of adipose SIRT1 prevented dietary obesity-induced metabolic abnormalities [insulin resistance, glucose intolerance and dyslipidemia] and obesity-induced NAFLD. SIRT1 was enriched at MERCs to trigger mitohormesis and unfolded protein response, in turn preventing HFD-induced ER stress. Clusterin was significantly upregulated and acted together with SIRT1 to regulate the protein and lipid compositions at MERCs in adipose tissue of Adipo-SIRT1. The results demonstrated that adipose SIRT1 enhanced the clusterin presence and omega-3 polyunsaturated fatty acids (PUFA) during HDL biogenesis in adipose tissue. The latter facilitated the transportation of omega-3 PUFA from adipose to liver.

Conclusion: The present study demonstrated that adipose SIRT1 enhanced the transportation of omega-3 PUFA via clusterin-containing HDL to liver, in turn eliciting the protective functions against NAFLD.

Project No.: 04151796

AMR-43-136
Dragon Protein Ameliorates Acute Kidney Injury by Inhibiting Necroptosis in Proximal Tubular Cells

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Introduction and Project Objectives: Acute kidney injury (AKI) is increasingly prevalent in Hong Kong and worldwide, and it associates with high morbidity, mortality and cost. AKI also increases the risk of developing chronic kidney disease (CKD) and exacerbates the ongoing CKD. Unfortunately, there are no effective treatment strategies available. It is well accepted that acute tubular necrosis is a key feature of AKI. Recent studies have found that programmed necrosis (necroptosis) contributes significantly to AKI. The necroptotic pathway has become a novel target for AKI treatment. Dragon is a membrane-associated protein. Our previous studies demonstrated that Dragon was highly expressed in tubular epithelial cells of the kidney, and tubular cell-specific deletion of Dragon increased necroptosis and AKI induced by ischemia/reperfusion (I/R) or oxalate crystals. We therefore hypothesize that exogenous Dragon protein also inhibits necroptosis in proximal tubular cells and prevents AKI. Our specific objectives included examination of the effects of Dragon.Flag and Dragon.Fc on kidney injury and functions in ischemia/reperfusion-induced AKI (IRI); and determination of the effects of Dragon.Flag on kidney injury and functions in cisplatin-induced AKI and renal lithiasis.

Methods: We induced acute kidney injury in mice by bilateral ischemic reperfusion (I/R), cisplatin and sodium oxalate. Mice were treated with and without Dragon.Flag.

Results: We found that Dragon.Flag protein inhibited necroptosis in proximal tubular cells in culture. Administration of Dragon.Flag protein into mice attenuated tubular epithelial cell necroptosis and ameliorated AKI induced by I/R, cisplatin or oxalate crystals.

Conclusion: Our results suggest that exogenous Dragon inhibits necroptosis and improves AKI in mice. Our previous study showed that Dragon induced apoptosis in renal tubular epithelial cells in the mouse model of unilateral ureteral obstruction. Therefore, the role of Dragon in kidney repair

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remains to be further investigated.

Project No.: 05161376

AMR-44-150

Endoscopic Submucosal Dissection (ESD) versus Transanal Minimally Invasive Surgery (TAMIS) for Early Rectal Neoplasms: A Prospective Randomized Controlled Trial

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Introduction and Project Objectives: Transanal minimally invasive surgery (TAMIS) is an effective surgical treatment for early rectal neoplasms not amenable to en bloc resection by conventional colonoscopic techniques. Endoscopic submucosal dissection (ESD) is a revolutionary endoscopic procedure that enables en bloc resection of large rectal neoplasms with low morbidity. This prospective, randomized, controlled superiority trial aimed to compare the short-term clinical outcomes, functional outcomes, quality of life, and costs between ESD and TAMIS for early rectal neoplasms.

Methods: Patients diagnosed with early rectal neoplasms (those without endoscopic signs of massive submucosal invasion or unfavorable histology on biopsy) ≥2 cm in size that were not amenable to en bloc resection by conventional colonoscopic techniques were randomly assigned (1:1) to receive either ESD or TAMIS performed by the same group of experienced colorectal surgeons. The primary outcome was 30-day morbidity/mortality. Secondary outcomes were en bloc resection rate, R0 resection rate, hospital stay, functional outcomes and quality of life, and costs. Planned enrollment was 114, but the trial was halted prematurely due to slow accrual as well as safety/efficacy data demonstrated by an unplanned interim analysis.

Results: From 6/2017 to 6/2019, 95 patients were screened for participation, and 53 eligible patients were randomly assigned to ESD (n=27) or TAMIS (n=26). The demographic data and tumor characteristics of the two groups were comparable. Two patients (7.4%) in the ESD group and 8 patients (30.8%) in the TAMIS group developed 30-day morbidity (P=0.039). One patient in the ESD group developed rectal bleeding on postoperative day 1 and required endoscopic clipping for hemostasis. No patients in the TAMIS group required reintervention for morbidity. All patients could achieve an en bloc resection, and R0 resection rate was similar between the ESD and TAMIS groups (88.9% vs. 92.3%; P=1.000). Length of hospital stay was significantly shorter in the ESD group (1.9±1.5 vs. 3.2±2.9 days; P=0.042). The total direct cost was also lower in the ESD group than in the TAMIS group (HK$80,133±15,569 vs. HK$99,782±31,416; P=0.005). Functional outcomes and quality of life did not differ between the two groups.

Conclusion: This trial was prematurely stopped because of slow accrual and a significantly lower 30-day morbidity rate demonstrated in the ESD group. The ESD group was also associated with shorter hospital stay and lower direct cost. However, given the premature termination of the trial, the results should be interpreted with caution.

Project No.: 04153006

AMR-45-152

Investigation into the Fungal Microbiome (Mycobiome) in the Cervices of Cervical Insufficiency Patients Receiving Cerclage Treatment and Resulting in Term or Preterm Birth

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Introduction and Project Objectives: Cervical insufficiency (CI), defined as premature cervical dilation/shortening, is a risk factor for spontaneous preterm birth <37 weeks (sPTB), a major contributor to neonatal mortality and morbidity. Surgical cerclage, which stitches up the weakened cervix, may prevent sPTB in CI patients, but about one-third of them still undergo sPTB after intervention. The latter group who do not benefit from cerclage could have been spared from surgical risks, but there is no good way to identify these patients. In this study, we investigated whether there were differentially abundant fungi between patients ending in sPTB and those ending in term birth (TB, birth on or >37 weeks) after cerclage intervention.

Methods: Before intervention, swab samples were collected from CI patients. Samples from women ending in sPTB and TB were sequenced for the fungal internal transcribed spacer 2 genomic region.

Results: Pre-cerclage cervical swab samples from 40 patients were sequenced. These patients ended in 14 sPTB and 26 TB after intervention. Phyla Basidiomycota and Ascomycota accounted for >90% of fungal taxa in these cervices. Fifty-three taxa including Candida parapsilosis were more abundant, and 14 taxa including Cladosporium cladosporioides were less abundant, in patients ending in sPTB after intervention than those ending in TB ($\log_2$ fold change, 1.1-8.4; adjusted p-value, 9.8E-20-0.05). A mycobiome score was calculated from the abundances of these differentially abundant taxa for each sample. Using the first quartile of the score of the sPTB group

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to define a positive test, 10 (true positive rate, 71%) of the 14 sPTB and 1 TB pregnancies (false positive rate, 4%) could be identified. Patients with a high score delivered sooner after sampling than their counterparts with a low score (Logrank test, p <1.0E-4). Notably, 95% high-score patients underwent sPTB, whereas 85% low-score patients underwent TB after intervention.

Conclusion: Patients with high loads of differentially abundant fungal taxa identified above in the cervix were more susceptible to sPTB after cerclage intervention. If further studies can ascertain that these fungi are useful for prediction of sPTB after cerclage, then certain patients can be spared from the surgery and counselled for other intervention for preventing sPTB.

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Project No.: 03141466

AMR-46-164
Investigation of Fibrosis Development in Marginal Graft after Liver Transplantation

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Introduction: Shortage of donor organs in liver transplantation results in expansion of donor pool to marginal (small-for-size and/or fatty) grafts. However, using expanded donor criteria poses increased risk for late phase complications such as fibrosis that may lead to graft dysfunction.

Project Objective: To explore the association between atypical biliary proliferation and fibrosis in marginal liver grafts and investigate the underlying regulatory mechanisms.

Methods: Using an orthotopic rat liver transplantation model and human post-transplant liver biopsy tissues, the dynamics of oval cells in marginal liver grafts was evaluated by the platform integrating immuno-labeling techniques and ultrastructure examination. Underlying mechanisms were further explored in oval cells and an Aldose reductase (AR) knockout mouse model simulating marginal graft injury.

Results: We demonstrated that activation of aldose reductase initiated oval cell proliferation in small-for-size fatty grafts during ductular reaction at the early phase after transplantation. These proliferative oval cells subsequently showed prevailing biliary differentiation and exhibited features of mesenchymal transition including dynamically co-expressing epithelial and mesenchymal markers, developing microstructures for extracellular matrix degradation (podosomes) or cell migration (filopodia and blebs), and acquiring the capacity in collagen production. Mechanistic studies further indicated that transition of oval cell-derived biliary cells toward mesenchymal phenotype by notch 2 signaling activation ensued fibrogenic development in marginal grafts after liver transplantation.

Conclusion: Oval cells contributed to fibrogenesis in small-for-size fatty liver grafts under dynamic regulation of aldose reductase and notch signaling. Aldose reductase-driven proliferation of hepatic oval cells dominated liver regeneration in marginal liver grafts. The subsequent biliary differentiation and mesenchymal transition of these oval cells contributed to chronic graft fibrosis under the regulation of notch signaling pathway. Intervention targeting oval cell dynamics may serve as potential strategies to refine current clinical management.

Publication: Oval Cells Contribute to Fibrogenesis of Marginal Liver Grafts under Stepwise Regulation of Aldose Reductase and Notch Signaling.
Liu XB, Lo CM, Cheng Q, Ng KT, Shao Y, Li CX, Chung SK, Ng IOL, Yu J, Man K*. Theranostics (impact factor: 11.556). 2017 Oct 24;7(19):4879-4893. doi: 10.7150/thno.20085. eCollection 2017.

Project No.: 02132366

AMR-47-187
Newly Developed Biodegradable Kirschner Wires in Upper-limb Fracture Fixations

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Introduction and Project Objectives: Upper-limb fractures are commonly seen among all types of bone fractures. Sports associated fractures have been mainly found in the paediatric and young adult population, while broken bones recorded in the elderly population are mainly caused by falls. Due to its ease of application, non-degradable Kirschner wires (K-wires) are commonly used by trauma and orthopaedic surgeons for fracture fixation either in open or percutaneous method (i.e. minimally invasive approach). The K-wire fixation technique is frequently used in hand and wrist fractures of adults, or most upper limb fractures in paediatric patients. However, some of the drawbacks for the usage of K-wires include (1) the requirement of additional surgery to remove the K-wires after bone healing; and (2) the risk of pin tract infection and skin impingement. Hence, we propose the use of a newly developed degradable K-wire, which is made of magnesium alloy as an alternative implant for fracture fixation.

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Methods: Degradable Mg K-wires will be tested on bone fracture models and non-degradable conventional K-wires serving the control. Eighty rats and sixteen goats will be used to study the biological responses and structural mechanical properties of newly formed bone. The surgical procedures and post-operation care were approved by the IRB of The University of Hong Kong.

Results: The binary fixation system made of degradable K-wire and conventional titanium wire effectively promoted the bone regeneration within the defect site as compared with the conventional group. This effect may attribute to the release of Mg2+ ions upon the degradation of the Mg K-wire. In the three-point-bending test, the bone tissue from the binary K-wire fixation group demonstrates significantly higher modulus than the conventional group after implantation for 36 weeks and indicates a better recovery. The osseointegration and bone mineral density (BMD) induced by Mg K-wire was significantly increased at 36-weeks post-implantation.

Conclusion: The degradable K-wire together with titanium wire can effectively fix fractured bone mechanically. Also, the modified system can stimulate local bone formation in which high quality of bone within a short period of time.

Project No.: 03142446

AMR-48-193

Lutein and Candesartan Co-Treatment Delays the Progression of Non-Proliferative Diabetic Retinopathy in the Diabetic Ins2Akita Mouse Model

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Introduction: there is an urgent need for novel interventions to delay the progression diabetic retinopathy, the most common microvascular complication of diabetes, in the early stage. Since long-term use is expected, an acceptable safety profile is of particular concern.

Project Objectives: to identify the therapeutic potential of long term co-treatment of lutein and candesartan in non-proliferative diabetic retinopathy

Study Design: male Ins2Akita/+ mice as an animal model of non-proliferative diabetic retinopathy received lutein and candesartan supplementation for up to 7.5 months.

Methods: lutein (4.2 mg/kg/day) and/or candesartan (0.01mg/kg/day or 0.1 mg/kg/day) was administered to mice in drinking water starting 6 weeks old daily until analysis at 6.5 or 9 months of age. Plain water served as non-treatment control.

Results: In animals treated with candesartan only or co-treated with lutein and candesartan, both co-treatment and single treatment resulted in a thicker nuclear layer. Best morphological improvement was observed with 0.1mg/kg/day candesartan single treatment, but with thicker inner plexiform layer and possibly retinal edema. Both co-treatment and single treatment reduced retinal cell loss in GCL. Co-treatment increased the immunoreactivity of PKCa and calbindin while single candesartan treatment increased that of calretinin. Glial reactivity in the retina was generally lowered in co-treated animals. Co-treatment resulted in better ERG a- and b-wave amplitudes up to 6.5 months.

Conclusion: long-term lutein administration is beneficial in preserving retinal function the Ins2Akita/+ mouse retina. Long term administration of lutein together with candesartan can provide better protection to the retinal cells and preservation of retinal function than single candesartan treatment. These results point to the potential of both lutein and lutein-candesartan as long-term therapeutic intervention for retinal degeneration in patients with early diabetic retinopathy.

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AMR-49-200

Deep Expression and Functional Proteomics of Erythroid Cell Differentiation: A Molecular Resource for Understanding Red Cell Biology, Iron Metabolism and Related Diseases

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Introduction and Project Objectives: The remarkable transformation of red blood cell precursors into professional hemoglobin synthesizing cells and eventually mature erythrocytes is a fundamental model for understanding the complex molecular control of cell growth and differentiation, tissue specific gene regulation and red blood cell disorders. Understanding erythroid cell differentiation is also particularly important for elucidating the metabolism of heme and iron, which exhibit robust biosynthesis and utilization, respectively, during the red cell development. We are interested in mining the erythroid active proteome with an aim to understand the molecular mechanisms of erythroid differentiation as well as heme and iron handling. So far, proteomic studies in erythroid differentiation that provide comprehensive coverage, wide dynamic range, high accuracy in protein quantitation and functional information have yet to be established.

Methods: In the present study we have devised unprecedented strategies to obtain deep expression and functional proteomes of erythroid cell differentiation. Sub-proteomic analyses of isolated cytosolic and mitochondrial fractions and peptide pre-fractionation have been employed to obtain the proteomes of pro-erythroid cells and differentiating erythroid cells.

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Results: Our comprehensive proteomic analysis of an established model erythroid cell differentiation revealed a wide range of prototypical or previously unrecognized proteins related to hemoglobin synthesis, erythroid cytoskeleton, carbohydrate and lipid metabolism, redox regulation, vesicular transport and chaperon functions, establishing the basis for functional study of novel proteins involved in erythroid differentiation. We have also devised strategies to identify heme –associated proteins by integrative methodologies of two dimensional non-denaturing gel electrophoresis, heme staining and biological mass spectrometry to study the functional proteome of heme associated proteins.

Conclusion: Selected erythroid active proteins have been studied for their functional roles by CRISPR Cas9 knockout. In particular, functional studies making use of knockout of transferrin receptor 1 and erythroid aminolevulinc acid synthase showed not only their constitutive roles in hemoglobin production in red cells but also new iron or heme regulated proteins and mechanisms related to adaptive mechanisms toward iron or heme deficiency.

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AMR-50-210

Investigating the Effect of Intracellular Sigma Protein in the Treatment of Amyotrophic Lateral Sclerosis and Multiple Sclerosis

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Introduction and Project Objectives: Following damage to the central nervous system, an inhibitory scar is formed around the lesion site to contain the spread of damaging factors. This scar persists over time and represents a formidable barrier to regeneration and recovery. CSPGs are a major component of this glial scar and inhibit axonal outgrowth and myelination of existing axons by binding to receptors such as PTPσ and LAR.

Methods: In the present study, we investigated the effects of a small peptide named Intracellular Sigma Peptide (ISP) that targets PTPσ on recovery in mouse models of two neurodegenerative diseases that have been associated with CSPG accumulation at lesion sites, Multiple Sclerosis (MS) and Amyotrophic Lateral Sclerosis (ALS). We modelled MS-like pathology using experimental autoimmune encephalomyelitis (EAE) and chemically induced demyelination via cuprizone feeding and administered daily doses of ISP while monitoring the animals for functional changes. Transgenic mice expressing the ALS-associated SOD1-G93A gene received ISP and were monitored for motor improvements over time. Histological analysis of the tissues via immunohistochemistry, immunoblotting and electron microscope was performed at the study endpoints. To explore the cell type specific effects of ISP on major cellular players in both MS and ALS pathology, we established primary cultures of oligodendrocytes, microglia, and astrocytes. We exposed these different cell types to CSPGs in cultured and used functional assays to explore responses to the addition to ISP.

Results: Results of the present study demonstrate that ISP treatment led to a significant improvement in MS models with regards to axonal sparing, remyelination, decreased neuroinflammation and ultimately translating to functional recovery. In both models of MS, ISP treatment led to significant improvements in the assessed behaviour. In the ALS mouse model, ISP treatment accelerated pathology and decreased survival. In a model of intracerebral haemorrhage, ISP increased myelination and functional recovery while preserving the ultrastructure of the lesioned corticospinal tracts. Cell culture experiments revealed an inhibitory effect of CSPGs on microglial phagocytosis oligodendrocyte and astrocyte outgrowth, however this effect was partially counteracted by ISP.

Conclusion: The present study is of potential clinical relevance for the use of ISP in demyelinating disorders and stroke but highlights concerns on the potential use in ALS.

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AMR-51-211

Dysregulation of miR-223 and miR-431 Expression in Intestinal Tissues of Preterm Infants with Necrotising Enterocolitis: Roles in Altering Enterocyte Gene Expressions and Functions

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Introduction and Project Objectives: Necrotizing enterocolitis (NEC) is a severe inflammatory disease of the gastrointestinal tract that results in high morbidity and mortality in preterm infants. Gut dysbiosis and dysregulation of transcriptome have critical implications in the pathophysiology of the disease. Previously, we had demonstrated overexpression of tens of miRNAs in NEC tissues and provided evidence on the interactions with their target genes. Among all, miR-223 and miR-431 were in the top list of the highly expressed miRNAs. In this project, we aimed to identify the target genes of the two miRNAs and their roles in the pathophysiology of NEC.

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Methods: In silico prediction of target genes and luciferase reporter assays were employed to identify the direct miRNA/target genes. mRNA levels of downstream targets were measured by qRT-PCR in Caco-2 cells over-expressing miR-223 or miR-431 experiments and/or upon LPS or LTA challenge. Enterocyte functions were determined by cell proliferation and apoptosis assays using flow cytometry.

Results: Based on the collective evidences of bowel mRNA expressions in NEC patients and overexpression experiments in in-vitro cell models, we revealed that miR-223/NFIA and miR-431/FOXA1 could be involved in TLR4 downstream inflammatory and cellular functions, including apoptosis, cell proliferation, inflammation, muscle contraction and intestinal epithelium barrier integrity.

Conclusion: This study demonstrated dysregulation of miR-223 and miR-431 in NEC tissues, and identified the interaction between these two miRNAs and their target genes NFIA and FOXA1. The dysregulation of miR-223 and miR-431 in human intestinal tissue might play a role in NEC pathophysiology, including promoting apoptosis, suppressing proliferation, and increasing proinflammatory signals. Further, we have compared expression levels of miR-223 in enterocyte cells and purified cord blood neutrophils. Abundant expression of miR-223 have been observed in neutrophils, we speculated the increase of miR-223 bowel tissues would be contributed by the infiltrating neutrophils in the disease sites. In this regard, stool signifies a valuable sample medium for evaluation of bowel condition during the inflammatory process. Also, detection of miRNAs by quantitative assay is robust and ready to be developed into a point of care test. Thus, these miRNA targets represent potential diagnostic or prognostic biomarker for NEC and also other inflammatory bowel conditions.

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