TS-1

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Development of MMP-9-responsive nanodiamond-based biosensor for tumor metastasis detection

Introduction

Metastasis plays a crucial role in cancer-related mortality. Patients under different metastasis status usually have varies prognosis and survival. Hence early detection of metastasis is of vital importance in cancer diagnosis and treatment. Promisingly, several specific metastasis biomarkers have been identified as their expression in cancer sites are unique, thus providing a potential strategy in cancer diagnosis. Matrix Metalloproteinase 9 (MMP-9) is a favourable metastasis marker since its expression has been demonstrated to be correlated with metastasis level. Therefore, in this study, we utilized a MMP-9-stimuli peptide that could be cleaved by MMP-9 specifically, and developed nanodiamond (ND)-peptide complex as MMP-9 specific biosensor for metastasis detection.

Methods

The MMP-9 substrate peptide was chemically conjugated onto NDs to obtain ND-peptide complex, ND-MMP9. The peptide was also labelled with fluorescent dyes so as to better monitor the cleavage signal, which represented the activity of MMP-9. Characterization of ND-MMP9, as well as sensitivity and accuracy of MMP-9 detection, were then investigated in vitro.

Results

ND-MMP9 had favourable drug delivery properties and exhibited stronger in-tube serum stability compared to the naïve substrate peptide. ND-MMP9 could be cleaved by MMP-9 and its cleavage signal could be quantified as well as visualized using confocal microscopy. Furthermore, in human hepatocellular carcinoma cell lines, the cleavage was correlative with MMP-9 expression level.

Conclusion

This ND-peptide model could be used as a satisfactory biosensor for metastasis detection.

TS-2

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Evaluating the Effectiveness of Gold Nanoparticles as Radiosensitizer in Glioblastoma cell line using a Clinical Linear Accelerator

Objectives
Treatment of Glioblastoma (GBM) is challenging, largely due to the rapid degradation of temozolamide (TMZ), which acts as a radiosenstizer. The inability to deliver an effective dose of TMZ and lack of target specificity causes significant systemic toxicity. The novelty of gold nanoparticles (AuNP) is their selective uptake by tumour cells through enhanced permeability retention. This facilitates widening of the therapeutic window by acting as a tumour-specific radiosensitizer. The primary aim was to validate the radiosensitization effect of AuNP on GBM cell line using a clinical linear accelerator and measuring the dose enhancement factor (DEF). Secondary aim was to assess the mechanism of radiosensitization.

Methods
Human U87 GBM cell line was seeded in 96 well-plate and incubated with 40 nm AuNP in varying concentrations (control, 50µg/ml, and 100µg/ml). Dark field microscopy was used to visualize the internalization of AuNP within the cytoplasm. Well-plates were exposed to radiation doses of 2, 4, 6 and 8Gy using a 6MV photon beam. Cell viability was assessed at different time points over one week using tetrazolium assay. Finally growth curves were determined, expressed as a ratio of number of surviving cells to calculate DEF. Finally, γ-H2A.X and Tunel assays were done to assess for increased double stranded DNA breaks and apoptosis respectively.

Results
The AuNP by itself had no effect on cell viability. The average DEF for GNP 50µg/ml was 1.07. For AuNP 100µg/ml, the average DEF was 1.18. No clinically significant difference between control and AuNP 50µg/ml. However, significant enhancement of 1.24 and 1.23 was seen with AuNP 100µg/ml at 4Gy and 8Gy. Furthermore, an increase in double stranded DNA breaks and apoptosis was also observed.

Conclusion
We have demonstrated AuNP-mediated radiosensitization on a clinical platform. AuNP 100µg/ml exhibits higher radiosensitization and significantly less cytoxicity compared to similar TMZ experiments.

TS-3

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Genomic predictors of neoadjuvant chemotherapy (NACT) outcomes in breast cancer (BC)

Background
We studied NACT effects on BC mutational landscape

Methods
Baseline (BL) and post-NACT tumor/matched normal DNA from 12 chemonaïve BC patients (pts) on NACT (Doxorubicin/Cyclophosphamide+sunitinib; NCT01176799) were subject to whole exome sequencing. We correlated changes in mutant variant allele frequency (mVAF) of nonsynonymous somatic single nucleotide variants from 34 genes in known BC signaling pathways with clinical outcome. Poor outcome was defined as <50% target lesion reduction after NACT or BC relapse/progression (PD) within 2 years; significant change was defined as >0.2 difference in BL & post-NACT mVAF

Results
Mean tumor size was 6.4+2.9cm; 50% were N+; 8% were M1; 7/12 pts had poor outcomes. Mean percent change in no. of somatic mutations was +14% vs -30% in pts with poor vs good outcomes, p=0.04. Tumors had mutations in PI3K (58%), NOTCH (42%), Wnt (42%), TP53 (33%) and FOXA (17%) pathways; 11 pts had >1 of 23 putative driver mutations identified (Table 1). mVAF declined in pts with good outcomes, apart from a new NOTCH2 mutation in A2 and rise in mVAF in A4. mVAF persisted/ rose in pts with poor outcomes; emergent mutations (AKT1, PIK3CA) occurred in 2 pts

Conclusions
Chemoresistant/ emergent mutations were identified by tracking mVAF in BC pts on NACT

TS-4

Tuan Zea TAN, Jieru YE,Kuee Theng KUAY ,Valerie Heong ,Diana LIM ,Jeffrey LOW ,Mahesh CHOOLANI ,David SP TAN ,Ruby Yun-Ju HUANG

Molecular subtype of ovarian clear cell carcinoma enriched of ALDH signature correlates with advanced disease and poor disease-free survival

Background

Ovarian clear cell carcinoma (OCCC) is characterized by chemotherapy resistance and poor prognosis. The incidence rate of OCCC has been on a rising trend and it constitutes 14% of the epithelial ovarian cancer in Singapore. While recognized as a distinct histology, there is no difference in the clinical management of OCCC. In order to explore potential targeted therapeutic for OCCC, we seek to identify clinically-relevant gene expression molecular subgroup that may offer clue to personalized OCCC management.

Methods

A discovery set of 135 OCCC were extracted from an in-house ovarian cancer microarray gene expression database, CSIOVDB, was analyzed by consensus clustering. Pathway analysis of the differentially expressed genes was performed. Clinico-pathological association of the identified subgroups was also performed. The presence of the two subgroups was validated on an independent cohort of 34 fresh frozen OCCC profiled by Affymetrix gene expression microarray.

Results

Consensus clustering revealed two distinct OCCC subgroups hallmarked by the up- or down-regulation of ALDH1A1 and ALDH1A3, and are denoted as ALDH-high and ALDH-low respectively. Projection of a published ALDH signature affirmed the enrichment of ALDH signature in ALDH-high group. ALDH-high is enriched in genes related to extracellular matrix (ECM), immunity/inflammatory response, immunoglobulin, and major histocompatibility complex (MHC). On the other hand, the ALDH-low is enriched in genes related to extracellular exosome, glycoprotein, cell adhesion, nucleosome, and DNA replication. The ALDH-high subgroup is significantly associated with advanced stage of disease (Stage III&IV, $p=3.9E-6$), more heterogeneous molecular subtype distribution (Stem-B vs non-Stem-B, $p=2.2E-26$), and poorer disease-free survival (ALDH-high vs ALDH-low, HR=4.513, $p=0.0188$).

Conclusions

The molecular subtype of OCCC might be utilized to predict clinical outcomes or therapeutic responses

TS-5

Shortlisted for Poster Award Presentation

Valerie Heong, Shane Ee Goh, Darwin Tay, Bernard Wee, Xiao Wen Lee, Brendan Pang, Diana Lim, Samuel Ow, Ross Soo, Joline Lim, Raghav Sundar, Soo Chin Lee, Cheng Ean Chee, Boon Cher Goh, Wei Peng Yong, Andrea Wong, Mohamed Feroz Mohd Omar, Richie Soong, David SP Tan

Whole exome sequencing (WES) of multiple spatially distinct biopsies from single metastatic lesions to evaluate tumour heterogeneity and identify actionable truncal mutations (ATMs) in patients (pts) with advanced solid malignancies using a radiologically-guided single-pass percutaneous technique

Background

Genomic profiling approaches of single core biopsy's are confounded by genetically diverse subclonal populations resulting in difficulties in biomarker validation. We explored the use of a novel technique to obtain multiple biopsies from a single metastatic lesion to evaluate heterogeneity

Methods

15 pts (5 NSCLC; 3 ovarian; 2 colon, 2 uterine and 1 breast, cervix and HCC) were identified. Using a single pass radiologically guided percutaneous biopsy technique, we obtained multiple spatially distinct core biopsy samples from a single progressing metastatic lesion. Each core underwent DNA extraction and WES using the NextSeq500

Results

Median of 4 cores were obtained from each lesion. Complication rate was 0%. 2 pts were omitted from analysis due to poor quality DNA with 13 pts successfully sequenced. In 1 pt, only 2 of 4 cores were successfully sequenced. The median amounts of total and non-synonymous variants were 137 (27-1286) and 66 (10-649) respectively. The median (range) filtered variants detected in 1/4, 2/4, 3/4, and 4/4 biopsy cores was 63(16-91)%, 5(1-65)%, 4(0-30)% and 26(0-63)% respectively, suggesting significant subclonal diversity within a single lesion. ATMs were identitified in 8/13 pts. 4/13 pts (31%) had no shared actionable variants across all 4 cores. 3 pts received therapy with inhibitors targeting ATMs. A uterine ca pt with AKT1_E17K ATM received an AKT inhibitor with 21% tumour shrinkage and PFS 6.1 mths. 2 NSCLC pts harbouring EGFR_T790M ATM were treated with an EGFRT790M specific TKI. 1 withdrew due to toxicity after 2mths and another had PFS >16.5 mths. Tumour mutational burden (TMB) was consistent across multiple cores. A NSCLC pt with the highest TMB received a checkpoint inhibitor with ongoing > 4 mths stable disease.

Conclusions

Using this novel technique is feasible, safe and informative. It provides insights into the mutational heterogeneity of a tumour and may help prioritise therapeutic strategies.

TS-6

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Globally optimizing therapeutic combinations against bortezomib-resistant multiple myeloma using a quantitative parabolic optimization platform (QPOP)

Multiple myeloma is an incurable hematological malignancy that relies on drug combinations as first and secondary lines of treatment. The inclusion of proteasome inhibitors, such as bortezomib, into these drug combination regimens has improved median survival. Resistance to bortezomib, however, is a common occurrence that ultimately contributes to treatment failure. Thus, there remains a need to identify improved drug combinations that may serve as later lines of treatment for improved treatment against bortezomib-resistant multiple myeloma. We have developed the quantitative parabolic optimization platform (QPOP) to optimize drug combinations against bortezomib-resistant multiple myeloma. By mapping phenotypic output data to parabolic response surfaces, QPOP is able to deterministically optimize drug combinations as well as drug dosages. By continuously optimizing in multiple systems of interest, from in vitro to in vivo, drug combinations can be globally optimized for greater efficacy in increasingly complex biological systems. While QPOP does not rely on molecular mechanism modeling or prediction, identified optimal drug combinations can reverse DNA hypermethylation and silencing of tumor suppressors that occurs following acquired bortezomib-resistance in multiple myeloma. Furthermore, this drug combination is broadly effective across a range of primary multiple myeloma patient samples. Beyond bortezomib-resistant multiple myeloma, global optimization of drug combinations by QPOP can serve to improve drug combination design across a range of other cancers and diseases through a continuous optimization process across the entire drug development pipeline.

TS-7

Shortlisted for Poster Award Presentation

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Activation of the ATM–Chk2 and ATR–Chk1 DNA Pathways by MLN8237 Sensitizes Nasopharyngeal Carcinoma Cells to Cisplatin-induced Cytotoxicity

Introduction

Majority of nasopharyngeal carcinoma (NPC) cases are locally advanced or metastatic with poor prognosis despite treatments with first-line therapies. An urgent need for second-line therapies attracts development of novel therapeutic entities. Therefore, this study aims to investigate anti-proliferative and anti-cancer effects of MLN8237, an investigational Aurora Kinase A (AURKA) inhibitor, alone and in combination with Cisplatin in NPC.

Methods

Anti-proliferative effects of MLN8237 alone and in combination with Cisplatin were studied using MTS assay. siRNA interference was conducted to evaluate functions of Survivin and XIAP in MLN8237-induced apoptosis. Effects of combination treatment on cell cycle distribution and anti-cancer mechanisms involved were investigated using flow cytometry and western blot.

Results

MLN8237 inhibited cell proliferation (IC50 of 7.66 ± 1.48 µM and 11.81± 2.23 µM in HONE-1 and HK-1 cells respectively) through AURKA deactivation. siRNA interference revealed that XIAP, but not Survivin, modulated MLN8237's sensitivity. MLN8237 augmented Cisplatin-induced cytotoxicity with synergistic combination indices of 0.70 ± 0.06 and 0.75 ± 0.08 in HONE-1 and HK-1 cells respectively. Further studies in HONE-1 cells showed that combination therapy activated DNA damage response pathway via upregulation of γH2AX, ATR, Chk1 and Chk2. This in turn led to S-phase arrest with upregulation of Cdk2, Cyclin E, Cyclin A and pRb. Combination therapy also potentiated apoptosis as shown by increased cleavage of PARP, caspase-3 and caspase-9.

Conclusion

MLN8237 alone and in combination with Cisplatin are promising therapeutic strategies against NPC. In-vivo studies are warranted to further verify anti-cancer effects of MLN8237 alone or in combination with Cisplatin against NPC.

TS-8

Jin-fen Xiao[1], Qiao-yang Sun[1], Ling-wen Ding[1], Wenwen Chien[2], Anand Mayakanda[1], Yan-Yi Jiang[1], Xin-Yi Loh[1], Ngan B. Doan[3], Brandon Castor[3], Jonathan W. Said[3], Henry Yang[1], H. Phillip Koeffler [1,2]

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c-Myc-BMI1 axis is critical for SETDB1 to enhance breast tumorigenesis

Characterizing over-activated oncogenic signaling leading to advanced breast cancer is of clinical importance. In this study, we showed that SETDB1, a histone H3 lysine 9 methyltransferase is aberrantly expressed and behaves as an oncogenic diver in breast cancer. SETDB1 enhances c-Myc and Cyclin D1 protein expression via promoting the internal ribosome entry site (IRES) mediated translation of c-Myc/Cyclin D1 mRNA, resulting in over-activation of c-Myc signaling to promote cell cycle progression and confers a growth/self-renewal advantage to breast cancer cells. Activated c-Myc-BMI1 axis is essential for the SETDB1-mediated breast tumorigenesis, as silencing either c-Myc or BMI1 profoundly abolishes the growth/colony formation advantage conferred by SETDB1. On the other hand, c-Myc protein directly binds to the SETDB1 promoter region and enhances the transcription of SETDB1, suggesting a positive regulatory interplay occurs between SETDB1 and c-Myc. This study identified SETDB1 as a prominent oncogene and characterized the underlying mechanism of SETDB1 in driving breast cancer, and therefore provides a therapeutic rationale for targeting SETDB1/BMI1 signaling in breast cancer.

TS-9

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Correlation of Mitotic Kinase Overexpression with DNA repair defects in lymphoid malignancies

Background
Overexpression of the mitotic kinase Polo-like kinase-1 (Plk1), has been described in various tumours, including lymphoma, breast, gastric and lung cancers. Plk1 has been shown to modulate the recovery from the G2M DNA damage checkpoint. However, the relationship between mitotic kinase overexpression and the status of DNA repair pathway activation in lymphoid malignancies is still undetermined.

Method
In this study, we use an automated multispectral system (Vectra 2) on 30 cases of diffuse large B cell lymphoma (DLBCL) to quantitatively determine Plk1 expression level in formalin-fixed parafin-embedded (FFPE) lymphoma samples. The mitotic kinases expression level were correlated with markers of DNA damage response pathway such as gH2Ax, pATR, pChk1 and proliferation markers such as Ki67, Myc and Mini Chromosome Maintainance protein-2 (MCM2). We setup a platform to validate antibodies in FFPE setting by using FFPE lymphoma cell blocks treated with specific DNA damage agents or siRNA.

Result And Conclusion
In this poster, we describe our preliminary findings using validated antibodies for multiplex immunohistochemistry on a set of 30 DLBCL cases, including a novel correlation between Plk1 expression level and Ki67/ Myc, two markers of aggressive behaviour and proliferation in DLBCL.

TS-10

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Discovery of Novel Plasma Exosome Biomarkers that Predicts NSCLC Risk and Discriminates Early and Advanced NSCLC Using Quantitative Proteomics Approach

The prevalence of non-small cell lung cancer (NSCLC) will rise with global longevity. Due to the lack of validated screening system, NSCLC cases are often diagnosed at advanced stages. The influence of exosomes in a myriad of pathophysiological processes and their ability to elicit functional response via the transfer of carried within cargos, have made them highly desirable clinical biomarkers. To date, proteomics studies on circulatory exosomes in lung cancer research are under-explored. The inability to distinguish benign from aggressive NSCLC remains one of the greatest challenges in the management of this disease. Here, we aim to identify and develop an exosome-derived protein diagnostic panel which facilitates the reliable diagnosis of stage-specific NSCLC.

Plasma exosomes were isolated from early-, late- stage NSCLC patients and from cancer-free control subjects using ultracentrifugation. Exosomal proteins were tryptic digested and labelled with 6-plex TMT reagents. TMT-labeled peptides were mixed and fractionated to reduce sample complexity before being analyzed in LC-MS/MS. Comparative proteomics profiling of perturbed exosomal proteins were performed using bioinformatics tools to shortlist candidate biomarkers of NSCLC.

Differentially expressed candidates were selected based on the following criteria: (a) protein must be identified based on ≥ 2 peptides with 95% confidence and quantified in at least two of the triplicates; (b) Proteins have to display at least 1.2-fold change; (c) and proteins must display significant ($p < 0.05$) differential expression. 69 proteins fulfilled the following the stringent criteria, and intensive literature review resulted in the final selection of 17 novel proteins, for verification in individual patient samples.

The interrogation of exosome proteome is a promising approach to uncover the wealth of biomarker information, and aberrant changes in the protein compositions may reflects the pathological process of NSCLC.

TS-11

Shortlisted for Poster Award Presentation

Michelle Meng Huang Mok[1], Kelly Ooi Kee Ong[1], Chelsia Qiuxia Wang[1,2], Takayoshi Matsumura[1], Desmond Wai Loon Chin[1], Cai Ping Koh[1], King Pan Ng[1], Masatoshi Yanagida[2], Namiko Yamashita[2], Bindya Jacob[1,2], Seiichi Mori[1], Ritsuko Shimizu[4], Masayuki Yamamoto[4], Yoshiaki Ito[1,2], Shirley Kow Yin Kham[3], Allen Eng-Juh Yeoh[1,3], Wee Joo Chng[1], Motomi Osato[1,2,3,5,6]

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Co-targeting Notch and JAK-STAT signaling pathways in acute megakaryoblastic leukemia

Background
Children of Down syndrome (DS), the most common congenital disease, are at high risk of developing a specific subtype of acute myeloid leukemia, acute megakaryoblastic leukemia (AMKL). According to Malaysia-Singapore multicenter leukemia studies for childhood acute leukemia, the prevalence of AMKL in Singapore and Malaysia is strikingly high as compared to those in the worldwide populations, and its prognosis is unfavorable. Despite such widespread and local relevance, the mechanistic basis for AMKL remains elusive.

Methods
Retroviral insertional mutagenesis (RIM) was employed on Runx1 transgenic (Runx1-Tg) and Gata-1 knockdown (Gata-1.05) AMKL mouse model. Targeted deep sequencing strategy was utilized in 34 AMKL patient samples and 8 AMKL cell lines. Gene expression profiling was performed on 47 AML (including 9 AMKL) cell lines. In vitro cell based drug testing assays were also performed on the AMKL cell lines.

Results
RIM analysis on the mouse model identified hits in JAK-STAT and Notch signalling pathways. Further targeted deep sequencing identified loss-of-function mutations in Notch pathway genes in 7 out of 34 (20.5%) AMKL patients. Compared with 39 non-AMKL AML cell lines, gene expression analysis on a panel of 8 AMKL cell lines revealed striking downregulation of Notch mediator RBPJ, together with SGPL1 and S1PR3, genes involved in sphingolipid (S1P) signaling. Notch agonist phenethyl isothiocyanate (PEITC) was shown to rescue S1PR3 expression, triggering ligand-independent Notch activation, and induce apoptosis in AMKL cells. Additionally, PEITC worked synergistically with JAK inhibitor 1 to significantly suppressed AMKL cell proliferation.

Conclusions
Here we report that JAK-STAT activation and Notch inactivation are key driver mechanisms for AMKL leukemogenesis. Additionally, this study demonstrates that ligand-independent Notch activation via S1P signaling can be harnessed to create novel targeted therapies for AMKL.

TS-12

Yi BAO[1], Soo Chin LEE[1], Qiang Yu[2]

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Role of PPP2R2B in Resistance to Targeted Therapies in Breast Cancer

HER2 amplification and PIK3CA mutations are predominant oncogenic lesions observed in breast cancer, and thus HER2 targeted therapies and PI3K inhibitors have been being developed to prolong remission for the disease. However clinical benefit of these drugs has been limited by intrinsic or acquired resistance. Here we hypothesize that PPP2R2B, a regulatory subunit, in PP2A complex plays a role in regulating resistance to HER2 or PI3K inhibitor. Our data have shown that PPP2R2B expression is low in breast cancer cells that are resistant to these inhibitors and overexpression of the gene sensitizes breast cancer cells to the targeted treatment. Moreover, epigenetic drugs are able to restore the expression of PPP2R2B in the resistant cells suggesting that the drug resistance might be achieved through epigenetic regulation and combination of epigenetic drugs and targeted therapies might be a solution to overcome the resistance.

TS-13

Xinyi WANG[1], Dawn Sijin NIN[1,4], Zheng Wei LEE[1], Wei Zhao[1], Xin Mei SHI[2], Joseph Soon Yau NG[3], Jeffrey Jen Hui LOW[3], Bok Ai CHOO[2], Lih-Wen DENG[1]

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GAGE Family of Proteins: Potential Biomarker For Irradiation-Resistance In HPVAssociated Cervical Cancers

Background
Cervical cancer is one of the most common gynecological cancers afflicting women today. Chemo-irradiation is the non-surgical treatment option most often prescribed. However resistance to this line of treatment in certain subsets of patients have been a major challenge that has yet to resolved. This study was conceived with the aim to identify potential biomarkers to predict patient response to irradiation (IR) therapy.

Methods
Utilizing in-house generated IR-resistant cervical cancer cell lines, we created Parental and IR-resistant tumor xenograft models to better recapitulate the tumor environment. Xenograft tumors were harvested for RNA that was analyzed using the microarray platform. Identified target genes were validated in vitro and with patient samples.

Results and Conclusions
Microarray analysis identified 10 genes and 1 gene family that were up regulated in the IR-resistant group. In vitro validation identified the up-regulation of GAGE family of proteins as the potential gene signature associated with IR-resistance. GAGE-family of proteins especially the GAGE12 family was shown to be selectively up regulated in IR-resistant xenograft tumors. Immuno-histological staining of patient samples showed a group of patients with increased GAGE12 staining post-irradiation therapy and a group of patients with high-GAGE12 levels before therapy. Clinical follow-up of these patients are on going, to track their response to IR to better correlate GAGE12 levels to IR response.

TS-14

Shortlisted for Oral Presentation

Jianbiao Zhou[1,2], Chonglei Bi, Ying Qing Ching,Jing-Yuan Chooi, Xiao Lu, Jessie Yiying Quah,Sabrina Hui-Min Toh,Zit-Liang Chan, Tuan Zea Tan, Phyllis SY Chong, Wee-Joo Chng

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Inhibition of LIN28B Impairs Leukemia Cell Growth and Metabolism in Acute Myeloid Leukemia

Background
Current conventional chemotherapy for acute myeloid leukemia (AML) can achieve remission in over 70% of patients, but a majority of them will relapse within 5 years despite continued treatment. The relapse is postulated to be due to leukemia stem cells (LSCs), which are different from normal hematopoietic stem cells (HSCs). LIN28B is microRNA regulator and stem cell reprogramming factor. Overexpression of LIN28B has been associated with advance human malignancies and cancer stem cells (CSCs), including AML. However, the molecular mechanism by which LIN28B contributes to the development of AML remains largely elusive.

Methods
We modulated LIN28B expression in AML and non-leukemic cells and investigated functional consequences in cell proliferation, cell cycle and colony forming assays. We performed a microarray-based analysis for LIN28B-silencing cells and interrogated gene expression data with different bioinformatic tools. AML mouse xenograft model was used to examine the in vivo function of LIN28B.

Results
We first showed that increased LIN28B expression was associated with worse survival in AML patients. We demonstrated that targeting LIN28B in AML cells resulted in cell cycle arrest, inhibition of cell proliferation and colony formation, which was induced by de-repression of let-7a miRNA. On the other hand, overexpression of LIN28B promoted cell proliferation. Data point to a mechanism where that inhibition of LIN28B induces metabolic changes in AML cells. IGF2BP1 was confirmed to be a novel downstream target of LIN28B via let-7 miRNA in AML. Notably, silencing LIN28B led to slow tumor growth in vivo.

Conclusions
In sum, these results uncover a novel mechanism of an important regulatory signaling, LIN28B/let-7/IGF2BP1, in leukemogenesis and provide a rationale to target this pathway as effective therapeutic strategy

TS-15

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The chicken chorioallantoic membrane as a robust model for the ovarian clear cell carcinomas

Ovarian cancer is one of the deadliest gynaecological cancers around. In Singapore, the ovarian clear cell carcinoma (OCCC) has doubled in incidence in the last two decades. However, the difficulty in prediction of this cancer type using the Risk of Malignancy Index as well as the lack of local data has made the diagnosis, knowledge of disease progression and treatment methods challenging for clinicians. The intratumoural heterogeneity, which may not be represented in a single biopsy further complicates treatment methods. To elucidate this cancer subtype, we have inoculated both epithelial (RMG-2, JHOC-9 and TAYA) and mesenchymal (OVTOKO, KOC7C and RMG-5) OCCC cell lines onto the chicken chorioallantoic membrane (CAM). The sizes of the tumours for each cell line formed (n=7) were then measured on embryonic day 15. Interestingly, the tumour size corresponded with their ALDH statuses, known to be correlated with tumourigeneicity. KOC7C, which formed the largest average tumour size, also displayed the highest level of ALDH amongst these 6 cell lines. OVTOKO and RMG-5, which had the smallest average tumour sizes, displayed negligible ALDH levels. The epithelial cell lines displayed average ALDH levels, corresponding to their tumour sizes. Although preliminary, we propose the CAM model as a robust model to study the OCCC subtype, as well as other ovarian and non-ovarian cancer subtypes. Compared to the mouse model, the CAM model is relatively inexpensive, quick, and a larger sample size using different bites of the biopsies could be performed to account for the intratumoural heterogeneity. Due to a lack of immune system before embryonic day 15, almost any type of cancer cells or patient biopsies could be inoculated or grafted onto the CAM for expansion and in-real-time testing of drug treatments while patients undergo standard chemotherapy. The standard line of treatment compared to proposed treatment options could then be presented to them using a CAM REMATCH database

TS-16

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Role of Metaplasia in Gastric Cancer Pathogenesis

Aim
Intestinal metaplasia (IM) is a preneoplastic condition for gastric cancer (GC). The phenotypic pattern of mucin protein expression is altered in IM. Our aims were to identify subtypes of IM according to their pattern of mucin expression, their distribution in at risk patients of GC in Singapore and to assess the IM subtypes which were associated with progression to GC.

Methods
Endoscopic biopsies were obtained from Singapore Chinese subjects aged 50 years and above (n=2987), from 2004 till 2012, systematically reviewed by expert pathologists. Biopsies of 546 cases with moderate to marked IM were stained with Haematoxylin and Eosin, HID/AB and immunohistochemistry was performed for MUC1, 2 and 5AC followed by quantitative staining analysis. Patients were followed up for a mean period of 46.6 months (range 0-88 months). Statistical analysis was performed and p value<0.05 was considered significant.

Results
24 cases progressed to dysplasia/carcinoma (LGD, n=9; EGN, n=15) from 546 patients. MUC1 expression was observed in 24.4%, MUC5AC in 43.4% and MUC2 in 100% cases. Based on HID/AB and mucin expression, type I IM was seen in 55.3%, type II in 43.3% and type III in 1.4%. Type II IM was further subdivided into subtype IIA (MUC1, 2, 5AC positive), IIB (MUC1, 2 positive) and IIC (MUC2, 5AC positive). MUC5AC and multifocal IM were significantly associated with progression to gastric dysplasia (p<0.05). Type III IM was associated with the highest rate of progression to dysplasia. Predictive power of combined MUC5AC expression and multifocal IM were higher than individual markers and clinicopathological features.

Conclusion
Incomplete IM (except type IIB) were significantly associated with the risk of progression to dysplasia/adenocarcinoma. Multiple biopsies should be evaluated in patients with IM to identify multifocal IM; and MUC5AC expression should be routinely evaluated to help assess the progression to gastric dysplasia or adenocarcinoma.

TS-17

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Natural resistance-associated macrophage protein 1 (NRAMP1) gene promoter polymorphism is associated to BCG response in non-muscle invasive bladder cancer (NMIBC) patients

Background
Bacillus Calmette-Guerin (BCG) immunotherapy is the standard treatment for patients with NMIBC after tumor resection to reduce cancer recurrence and progression. NRAMP1 modulates macrophage function and has been implicated in the response to BCG therapy in a murine orthotopic model of bladder cancer.

Methods
In this study, we evaluated several Single Nucleotide Polymorphisms (SNPs) in NRAMP1 and immune response genes for their impact on recurrence and progression of bladder cancer in NMIBC patient treated with BCG immunotherapy. Genomic DNA were isolated from blood specimens obtained from The Chinese University of Hong Kong and National University Hospital Singapore. The study cohort consisted of 200 normal controls and 177 evaluable EORTC intermediate to high risk NMIBC patients. Patients underwent post-transurethral resection and intravesical treatment with BCG or BCG with interferon alpha. The SNP genotype were identified by High Resolution Melt (HRM) analysis and Sequence analysis. Kaplan-Meier together with Log-Rank test and Cox regression methods were used to analyse the data.

Results
The genotype frequencies were similar between controls and patients irrespective of country of recruitment. NRAMP1 rs34448891 Allele 3/3 and Allele 3/2 were the most common genotype, in the patient group 70.6% and 20.9% respectively. Patient with homozygous Allele 3 had increased recurrence risk (p=0.047) and increased progression risk (p=0.018). A significant risk of cancer recurrence were also observed in patients carrying the IL17A rs2275913 GG (p=0.019) and IL18R1 GG was associated with increased progression risk (p=0.033). Patients with both rs34448891 allele 3/3 and rs2275913 GG genotype were more likely to have cancer recurrence (p=0.022).

Conclusions
Rs34448891 Allele 3 genotype is associated with higher NRAMP1 expression and increased macrophage activity and this may be the reason patient with this genotype may be less responsive to BCG immunotherapy.

TS-18

Shortlisted for Poster Award Presentation

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FAM3C promotes lung cancer cell metastasis by regulating the Src-STAT3 pathway through activation of RalGDS/RalA signalling

Background
High mortality rate of lung cancer is due to increased metastasis initiated by EMT. FAM3C (ILEI), Interleukin-like EMT inducer, promotes EMT in cancer cells but its underlying mechanism remains unknown. This study aimed to investigate the roles of FAM3C in lung cancer progression.

Methods
In this study, shRNA transfection, western blot, immunohistochemistry staining, wound healing assay, matrigel invasion assay, colony forming cell assay, MTS and Co-IP assay were used for functional studies of FAM3C in human cell lines.

Results
The IHC staining indicated that FAM3C expression in lung cancer specimens was significantly increased compared to those in tumour adjacent and normal lung tissues. In functional studies, shRNA-mediated FAM3C knockdown in SKMES-1 cells resulted in significant reduction of cancer cell invasion, migration and epithelial-mesenchymal transition. In addition, the FAM3C knockdown cells showed reduced cell proliferation, colony formation and enhanced chemosensitivity to cisplatin. Contrastingly, overexpressed FAM3C in H838 cells induced EMT, cell proliferation, invasion, migration and metastasis. Through bioinformatic analysis, the overexpression of FAM3C was strongly associated with the upregulation of phosphorylated RalA (Ser194) which is involved in the progression of several cancers. RalA GTPase, a Ras downstream signaling molecule, activates RalA binding protein 1 which subsequently activates the phosphorylation of Src and STAT3 (Tyr705) in the downstream signaling pathway. Co-IP assay confirmed the protein-protein interaction between FAM3C and RalA. Furthermore, FAM3C expression is highly correlated with the phosphorylation of RalA expression in NSCLC cell lines. Additionally, pro-metastatic PITPNC1 acts as upstream mediator of secreted FAM3C-mediated metastasis.

Conclusions
These findings demonstrate that FAM3C induces EMT to promote lung cancer metastasis by regulating the Src-STAT3 pathway through RalGDS/RalA signaling.

TS-19

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Oncogenic roles of Maternal Embryonic Leucine Zipper Kinase (MELK) in gastric cancer and its therapeutic targeting using small molecule inhibitor OTS 167

Maternal Embryonic Leucine Zipper Kinase (MELK) belongs to the subfamily of AMP-activated Ser/Thr protein kinases. It's expression is up regulated in various cancers and is known to be associated with cancer progression, maintenance of stemness and poor prognosis. The present study investigates the tumorogenic role and biological functions of MELK in gastric cancer using OTS167, a novel and selective small molecule inhibitor. MELK was found to be up regulated in clinical samples of gastric cancer that correlated with distant metastasis and poor survival outcomes. Strikingly, MELK inhibition caused a senescence like phenotype in gastric cancer cells with an increase in replication stress, DNA double stranded breaks and reduced expression of stem cell markers. Absence of MELK expression led to the arrest of cell cycle at G2M phase with an increase in P53 mediated cellular apoptosis. On the other hand, a decrease in cellular invasion and prolifearion was also observed. Moreover, OTS167 treatment of mouse models bearing gastric cancer PDX's resulted in a significant inhibition of tumor growth. Taken together, our study highlights the potential of MELK as a therapeutic target in gastric cancer and underscores the clinical benefits of its pharmacological inhibition using OTS167.

TS-20

Shortlisted for Oral Presentation

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Characterization of MET-N375S as an Activating Mutation in Squamous Cell Carcinoma of the Lung

Background
Significant progress has been made in the treatment of lung carcinoma. EGFR kinase mutations, EML4-ALK rearrangements and exon 14 skipping alterations are amongst actionable biomarkers to guide therapy in lung adenocarcinoma. However, the rarity of genetic aberration in squamous cell carcinoma (SCC) has restricted the use of molecular-targeting agents. While MET amplification is uncommon in NSCLC, missense mutation N375S has been reported in Asian population and associated with poorer survival in tongue SCC patients.

Methods
Using isogenic cell lines expressing either wild-type or mutant MET, the functional mechanisms of MET-N375S in lung SCC cells were characterized by defining the downstream effectors (Proteome profiling), biological functions (in vitro 3D growth, invasion and migration assays; in vivo tumour growth), and protein-protein interaction (SILAC). Binding partners of MET-N375S was verified using co-immunoprecipitation (co-IP) and proximity ligation assay (PLA). High content screening was performed to identify effective anti-tumour compounds.

Results
Ectopic expression of MET-N375S in lung SCC cells led to dysregulation of the MAP and SRC kinases in concurrence with enhanced migratory, invasive and colony forming ability in vitro. Tumourigenicity was observed upon injection of MET-N375S in SCID mice. Mechanistically, interactomic analyses demonstrated an increase in kinase-dependent binding affinity of N375S towards HER2. PLA was performed to validate the HER2-MET interaction. High content screening on a panel of kinase-specific compounds revealed potential druggable hits specifically targeting N375S-expressing tumours.

Conclusion
We describe a role of MET-N375S as an activating mutation which may drive malignancy and tumourigenicity through its heterodimerization with HER2. Clinically, MET-N375S could be utilized as a potential predictive biomarker for patients with advanced SCC to be treated with selective MET and/or HER2 inhibitors.

TS-21

Wanpei Cai[1], Maurice Chan[1], Seow Ching Wan[1], Siang Wei Tue[1], Mei Ling Chong[1], Jasper Ng[1], Chris Tan[1]

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Clinical Validation of a Novel RNA-based NGS Panel for Detection of Gene Fusions in Solid Tumors Using FFPE Samples

Background

Gene fusions are gaining significance as biomarkers for targeted cancer therapy. For example, inhibitors of the anaplastic lymphoma kinase (ALK) protein have greatly improved prospects for patients with EML4-ALK fusion positive non-small cell lung tumors. A favourable property of an RNA-based method is that only expressed, potentially oncogenic gene-fusions are detected. A proprietary kit built for gene-fusions detection, including novel fusions, using next-generation sequencing (NGS) was used. As the kit is designed for research use only, the aim of this study is to validate the assay for clinical application.

Methods

RNA was extracted from FFPE slides using the ReliaPrep FFPE Total RNA MiniPrep System (Promega) and quantitated using Qubit HS RNA Assay (ThermoFisher Scientific). NGS libraries were constructed using the proprietary kit according to manufacturer's instructions. Eleven indexed libraries were pooled and run on a MiSeq (Illumina). Sequenced data was analysed online using a designated website. Samples included an ALK-RET-ROS1 Fusion RNA FFPE Reference Standard (Horizon), FFPE cell lines with known gene fusions (Ignyta, Inc.), 10% ROS1 RNA, and archival clinical FFPE samples. Analytical samples comprising ROS1-positive FFPE-derived RNA, spiked into negative clinical FFPE samples at 1-30%, were also employed.

Results

A total of 66 samples were processed in 6 runs. All runs were successful with <2% PhiX error rate, 700-1000 K/mm2 cluster density, >80% of cluster passing filter rate, and the percentage of bases ≥Q30 was >80%. Overall, 63/65 (97%) samples passed QC at the sample level. The limit of detection was 50ng (RNA input) and 1% (mutation frequency for gene-fusions). For samples with known gene-fusion data, all expected gene-fusions were correctly detected and no false-positive call was observed.

Conclusions

The performance of the assay was considered acceptable for clinical trial testing.

TS-22

Shortlisted for Oral Presentation

Jing Yuan Chooi[1], Zhigang Xie[2], Hannah Lee Foon Swa[3],Jayantha Gunaratne[3], Dennis Kappei[2], Wee Joo Chng[1,2,4]

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Identification of Functional Proteins Interacting with MMSET in Multiple Myeloma

Multiple myeloma (MM), characterize by the uncontrolled proliferation of malignant plasma cells occurs mainly in the elderly population. The t(4;14) translocation is the second most common translocation in MM and is associated with poor prognosis. NSD2 is overexpressed in MM due to t(4;14) translocation. NSD2 contains a catalytic SET domain for its histone methylation activity. Recent studies demonstrates the importance of NSD2 in the proliferation, clonogenicity and tumorigenicity of t(4;14) myeloma cells in vitro and in vivo. These data suggest that NSD2 is an important oncogenic factor in t(4;14) MM. However, the detailed molecular mechanism remains poorly understood. To address the relevant pathways that contributed to myelomagenesis, a SILAC-based mass spectrometry analysis was selected to determine NSD2 interacting proteins. NSD2 interacting proteins are enriched in processes related to translation, RNA processing and chromatin organization. Interaction between NSD2 and several representative proteins including ADAR1, DDX5, SMARCA2, Topoisomerase IIα, RPS6 and RPL4, was observed. ShRNA-mediated knockdown of NSD2 resulted in a reduction in nascent protein synthesis. In addition, the loss of NSD2 reduces global H3K36me2 level and caused chromatin to transit from an open to closed structure. NSD2 also binds and mediates the expression of a subset of genes involved in apoptosis and cell cycle. Our study highlights novel functions of NSD2 in multiple myeloma. The detailed mechanistic studies will be useful for identification of novel therapeutic targets in cancer overexpressing NSD2.

TS-23

Shortlisted for Poster Award Presentation

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Identifying The Oncogenic Role Of Usp10 As The Regulator Of Pten Function In Breast Cancer

Background

The PI3K pathway is the most commonly activated signaling pathway in human cancer. The lipid phosphatase PTEN is the major negative regulator of the PI3K pathway which de-phosphorylates PIP3 and inhibits AKT activation thus "switching off" the pathway. Loss of PTEN functionality has been reported in several cancer types which further contributes to tumorigenic impact of PI3K pathway. This study aims to investigate the role of a deubiquitinating enzyme, USP10 in regulation of PTEN functionality and PI3K pathway in breast cancer.

Methods

A genome wide shRNA screen targeting all known deubiquitinating enzymes was performed and level of phosphorylated AKT was assessed by western blotting. Significant hits of the screen were validated and mechanism of action in regulation of PTEN functionality of selected target was investigated.

Results

Screen results showed consistent decrease in AKT activity upon depletion of USP10. Recently USP10 was demonstrated to bind to PTEN (zhang et al., 2013). We discovered that USP10 stabilizes PTEN and interestingly activates AKT. However, stabilization of PTEN by USP10 was shown to be independent of USP10 de-ubiquitination function. We demonstrated USP10 degrades MEK1, which has been shown to form a complex with PTEN and MAGI1 and recruit PTEN to plasma membrane. We also identified ITCH as an E3 ligase for MEK1 and we showed stabilization of ITCH by USP10. We showed less colony formation in the USP10 depleted cells as compared to control. Expression analysis of USP10 revealed significantly higher expression of USP10 in breast cancer patients and its expression is correlated with the progression of tumor and the poorer overall survival in the breast cancer patients.

Conclusions

Our study identified USP10 as the critical regulator of PI3K pathway possibly through regulation of PTEN activity. Understanding the detailed mechanism of action of USP10 in regulation of PI3K pathway is important for discovery of better therapeutic options in breast cancer.

TS-24

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CSI Web Portal: An online platform for automated NGS analysis and data sharing

Background

Next-generation sequencing (NGS) has been a widely-used technology for biological research to understand the molecular genetics of cells in health and disease. A variety of computational tools have been developed to analyze the NGS data, which require bioinformatics skills and tedious work to handle.

Methods

We have developed CSI Web Portal which brings established bioinformatics pipelines together in a user-friendly website designed for non-bioinformaticians to provide fully automated NGS analysis and data sharing.

Results

The portal currently provides standard pipelines for analyzing samples from DNA, RNA, small-RNA, ChIP, RIP, 4C and SHAPE sequencing, and is flexible to expand with new and customized pipelines. The users can upload samples raw data and submit jobs for the desired analyses, which will be automatically processed and the results will be self-accessible via the portal.

Conclusions

CSI Web Portal helps researchers rapidly analyze their NGS data and keep it organized without a bioinformatician. The website is currently available online for the CSI labs and will be made public in the future.

TS-25

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Development of patient derived xenograft model in nasopharyngeal carcinoma (NPC)

Background
Nasopharyngeal carcinoma (NPC) is highly endemic in Southeast Asia with most tumours harbouring the Epstein-Barr virus (EBV). Cell line model in NPC may not fully recapitulate the tumour biology and reports have shown these cell lines are prone to contamination issue and may also lose the EBV expression after repeated passages. Therefore, our aim is to develop a patient derived xenograft (PDX) model in NPC and validate its continued expression of EBV in this model.

Methods
Tumours from patients with newly diagnosed or recurrent NPC were used to create a PDX model of NPC. Tissue was implanted into NSG mice and then amplified in the PDX system until passage 6 (P6). $1X10^6$ C666-1 cells were injected subcutaneously into NSG mice. Expression of EBER and cytokeratin were performed through immunohistochemistry staining to NPC. Further validation performed using flow cytometry analysis. C3/CD8/CD19/CD45-negative population was gated out and then analysed for HLA-A11/cytokeratin/EPCAM-positive population. A chi-square test was performed with $p<0.05$ considered significant.

Results
PDX required a longer time to grow (3-6 months) than the C666-1 xenograft (2-3 weeks). PDX model retain EBV infection after repeated passages for six cycle. Recurrent tumours demonstrated a higher success than primary tumours (p=0.04). The PDX-P6 showed epithelial cell origin through cytokeratin immunohistochemistry. Further validation with flow cytometry showed 90% of the cells were CD3/CD8/CD19/CD45 negative and 50% of this population positive for EPCAM/cytokeratin. Besides, the EPCAM/cytokeratin-positive population showed HLA-A11 (93%), concordance with the HLA-typing result.

Conclusions
PDX model development in NPC is technically feasible and continues to retain EBV after repeated passages. This model is invaluable for clinical trial drug or cell therapy, analyse molecular mutations, carcinogenesis pathway and microenvironment-cancer cell interactions in cancer cell progression.

TS-26

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Monoclonal antibodies against intracellular targets: The next frontier for cancer therapy.

Background
A pertinent challenge in the development of anti-cancer targeted therapy is the identification of "tumor-specific" antigens that are exclusively expressed in tumors, but not in normal tissues, so as to avoid undesirable off-target effects. However, majority of these antigens are located intracellularly and/or lack specific tools to target them, and thus traditionally regarded as undruggable.

Method
Over the past decade, we have extensively demonstrated the unconventional concept of monoclonal antibody (mAb) therapy against various intracellular antigens as a new frontier for cancer therapy, disrupting the dogma on the undruggability of such intracellular tumor-specific antigens. Experimental data from xenograft, orthotopic, and metastasis studies of tumors in more than 4,000 mice across distinct genetic backgrounds has shed insight into the mechanisms of action involved in this novel immunotherapy approach.

Results
Here, we present our "bench-to-bedside" case study of a monoclonal antibody against an oncogenic, tumor-associated intracellular phosphatase, PRL-3. Follow our maturation of this antibody technology from early murine PRL-3 monoclonal antibodies to our latest clinical-grade humanized antibody, PRL3-zumab. Notably, PRL3-zumab has an excellent safety profile in nonhuman primates, with a no-observed-adverse-effect-level (NOAEL) of 36 mg/kg.

Conclusions
Immunotherapy against intracellular targets is the next frontier in cancer therapy, and it is expected to vastly expand the repertoire of tumor-associated antigens amenable to antibody targeting. Following safety and proof-of-concept (POC) clinical trials with PRL3-zumab against PRL-3-expressing tumors, this strategy is expected to prompt the re-exploration of intracellular antigens as druggable antibody targets in cancers, particularly those with unmet medical needs.