BS-1

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Functional and structural roles of retinoic acid receptor alpha mutations in breast fibroepithelial tumors

Fibroepithelial tumor of the breast is a group of lesions consisting of the benign fibroadenoma, which is highly common in young women, and the relatively rare but potentially aggressive phyllodes tumors. Surgery is currently the only treatment option available. We have recently demarcated the genomic landscape of breast fibroepithelial tumors, which revealed high frequencies of mutations in RARA in all subtypes, suggesting this to be one of the driving factors in fibroepithelial tumorigenesis. While previously known RARA mutations found in acute promyelocytic leukemia (APL) are translocations resulting in the fusion with other genes such as PML, this has been the first report of tumor-associated frequent point mutations in RARA. RARA encodes RARα, a nuclear receptor for retinoic acid and certain retinoids. RARα mutations found in breast fibroepithelial tumors are located in the ligand-binding domain, which has ligand-binding, protein binding and transcriptional activity properties. This project seeks to investigate the functional effects and structural mechanisms of breast fibroepithelial tumor-associated RARα mutations, and to evaluate the feasibility of targeting at mutant RARα as therapeutic strategy. Our in vitro assay results revealed the differential binding properties to ligands and interaction partners displayed by mutant RARα compared to the wild type receptor. We have also established isogenic cell lines from breast fibroepithelial tumors and showed that RARα agonists inhibit the viability of cells expressing wild type but not mutant RARα. These data form the basis for the discovery of mutant RARα -specific agonists to be further developed as therapeutics.

BS-2

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In vivo transmission of EBV to negative NPC cells via co-transplantation of Akata cells

Introduction

Although the Epstein–Barr virus (EBV) DNA is consistently detectable in nasopharyngeal carcinoma (NPC) biopsy, EBV infection is rarely found in normal nasopharyngeal epithelial tissues. The virus source and the route of entry into tumor epithelial cells remain uncertain. The facts already known are: lytic EBV infection in oropharyngeal epithelial cells, despite an infrequent event, is believed to be a major source of infectious EBV particles for salivary transmission; To achieve long-term persistence in vivo, EBV colonizes the memory B-cell pool and hijacks their cellular machinery for establishing latent infection. Any of the two could be the sources of EBV infection.

Methods

In order to address these issues, we employ co-transplantation of EBV negative NPC line HONE1 cells with EBV-producing Akata cells or cell-free viral particles in immunodeficient mice models.

Results

In vivo data showed that none of HONE1 cells were infected by cell-free EBV particles. When Akata cells were administered via intravenous or subcutaneous injections (no matter short or long distance), no infection occurred in HONE1 cells. While the only infection seen came from co-transplantation the two types of cells together via subcutaneous.

Conclusion

Our models favor transmission of EBV infection via cell-cell contact rather than cell-free virus. The distant migration of Akata cells along chemotaxis is not likely to be involved in EBV transmission. Our study suggests that subepithelial and intraepithelial EBV positive lymphocytes could be the source of infection in NPC tumor cells, since they are in close proximity. Lytic induction does not increase infection further. Moreover, EBV transmission was unidirectional, only occurring from lymphocytes to epithelial cells, not vice versa (test in co-transplantation of C666 cells and EBV negative Ramos cells, data not shown).

BS-3

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Regenerable Altruism Drives Chemotolerance in Clonal Cancer Cells

Background

Cancer is thought to represent a breakdown of multicellular cooperation and reversion to Darwinian dynamics characterized principally by self-interested competition. However, there is increasing evidence that cancer cells can behave as communities, manifesting social behaviors that influence cancer progression.

Methods

Circulating tumour cells enrichment, microRNA extraction, qRT-PCR, MTS assays, RNA in situ hybridization, immunohistochemical staining, SILAC-based mass spectrometry, flow cytometry, ChIP-sequencing, CRISPR-Cas9 genome editing, western blotting.

Results

We report here evidences of altruistic cooperation in clonal breast cancer cells. Non-genetic heterogeneity in expression of a microRNA miR-125b leads to sectoring of clonal breast cancer cell populations into minority miR-125b-high and majority miR-125b-low subpopulations. Enhanced population-wide tolerance to taxane-based chemotherapy is mediated by the miR-125b-high minority, in part through increased secretion of extracellular public goods such as insulin-like growth factor binding protein 2 (IGFBP2) and chemokine (C-C motif) ligand 28 (CCL28). Cost-benefit analysis established this helping behavior to be altruistic, as survival benefits conferred to the miR-125b-low cells, via public goods sharing, occurred at a fitness cost to the miR-125b-high cells. Notwithstanding this fitness cost, miR-125b-high altruists regenerate readily from isolated population of miR-125b-low defectors, via Kruppel-like factor 2 (KLF2)-mediated epigenetic mechanism. As therapeutic proof-of-principle, we demonstrated that blocking KLF2's binding to miR-125b's promoter markedly hindered histone acetylation, impeding regeneration of altruists from defectors and reducing global level of IGFBP2 and CCL28, consequently blunting the tolerance response to taxane.

Conclusions

Our results indicate how positive social engagement such as altruism may be employed by clonal cancer communities to drive chemotolerance.

BS-4

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Integrated Deep Learning and Bayesian Analysis for Prioritization of Candidate Cancer Genes from Next-Generation Sequencing Data

The advent of next-generation sequencing technology has enabled large-scale interrogation of the genome to identify variants in patient samples. The accurate identification of functional variants can provide critical insights into the disease process to guide diagnosis and treatment. However, the use of clinical genomics remains limited as (i) the accurate identification of variants remains suboptimal, and (ii) the large number of variants identified may be difficult to interpret without a systematic approach of ranking by functional importance.

Here, we describe the development of a deep learning neural network to improve the accuracy of variant-calling, and a Bayesian classification method for the probabilistic ranking of functionally relevant genes. We show that an optimized neural network can call variants more accurately than single variant callers or concordant callers, with F1 score improvements of 6.5 percent in synthetic datasets and 4.5 percent in reference datasets over the best concordant methods. Following the identification of high confidence variants, we further demonstrate that a Bayesian network system can rank relevant candidate cancer genes in a Diffuse Large B-Cell Lymphoma (DLBCL) patient sample.

We propose that the combined use of deep learning and Bayesian network analysis could be extended to build an analytical pipeline for clinical use to augment diagnosis and treatment of diseases by identifying high-confidence variants and ranking them systematically.

BS-5

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Development of an In Vivo Transposon-based Approach for Parallel Validation of Candidate Cancer Genes

Recent exome sequencing efforts have uncovered complex somatic mutations in human cancer that underlie their heterogenous biologic Understanding how these mutations cooperate and act to drive tumourigenesis is a major challenge and current experimental approaches are focused on functional testing of one or a few genes at a time.

Here, we present a transposon-based approach in mice for the parallel validation of candidate cancer genes. In this approach, candidate cancer genes are cloned into promoterless barcoded transposon constructs and introduced into a mouse embryonic stem (ES) cell line expressing an inducible form of the piggyBac transposase. The ES lines carrying the candidate genes are used to generate chimeric mice, and the transposons are mobilized to allow expression of different combinations of candidate genes. Combinations of expressed candidate genes in tumours are identified by quantitative real-time PCR (RT-PCR) using barcode-specific primers.

We demonstrate that chimeric mice carrying 8 candidate breast cancer genes (4 paired wildtype and mutant forms) developed tumours following transposase activation. By barcode-specific qRT- PCR, we found that 2 candidate mutant genes (PIK3CA and KPNA5) were highly expressed in these tumours indicating functional cooperativity. In support of this, co-expression of these candidate oncogenes in MCF10A breast cells promoted anchorage-independent growth in a soft-agar assay and tumor growth in mouse xenografts.

We propose that the extension of this transposon-based approach would provide a platform for functional validation of multiple candidate oncogenes in parallel to uncover cooperating pathways in cancer initiation and progression

BS-6

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Differential radiosensitivity between synchronous primary and metastatic patient derived head and neck cancer cell lines and the effects of hypofractionation

Purpose

Primary head and neck cancer is known to be radiosensitive however, once the disease becomes metastatic, it is unclear if the radiosensitivity remains the same. We aim to investigate the difference in intrinsic radiosensitivity and dose fractionation effect on patient derived primary and metastatic head and neck cancer cell line with a fluorescence-based viability assay.

Methods and Materials

Two different patient-derived tumour cell lines were used in this study: HN137G parental and HN137R metastatic. This was obtained simultaneously at diagnosis from a single patient with metastatic tongue squamous cell carcinoma. After single dose irradiation at 2Gy and 7Gy using a linear accelerator, we used an Alamar Blue cell viability dye which allowed us to do continuous sampling at different time points to determine surviving fraction (SF).

Results

Upon subjecting cell lines to an irradiation dosage of 2 Gy, we observed that the parental cell line experienced an expected dip in SF (SF2 = 0.5) whereas the metastatic cells SF remained stagnant (SF2 = 1). When the cells were subjected to an irradiation dosage of 7 Gy, both the parental and metastatic cells had a significant decline in SF post-irradiation to a different extent. The parental cells SF decreased by 50% (SF7 = 0.26) whereas the metastatic cells SF decreased by 25% (SF7 = 0.74).

Conclusion

While both cell lines were radioresistant, the metastatic cell line was extremely radioresistant with an SF2 of 1. This would be expected if the patient had previously received irradiation, however, these cell lines were developed from a treatment naive patient at the same time point. Hypofractionation to 7Gy managed to overcome the radioresistance. Clinically, this suggests that metastatic tumours may be more radioresistant than the primary tumour and there may be a role for hypofractionation to higher doses for better disease response when treating metastatic sites in head and neck cancer.

Shortlisted for Poster Award Presentation

BS-7

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An upstream RUNX3 enhancer regulates the development of gut-associated anti-tumorigenic CD8+ cytotoxic T lymphocytes

Background

Non-coding DNA functional elements such as transcriptional enhancers play central roles in establishing spatio-temporal patterns of gene expression for normal development. Perturbations of enhancers through mutation, rearrangement, deletion and amplification have been shown to underlie numerous human hereditary disorders. Recently, the discovery of somatic mutations in enhancers and the acquisition of super-enhancer activity that drive oncogene expression in various malignancies highlight the importance of enhancer regions in the context of cancer. Runx3 gene has been shown to be a tumor suppressor or an oncogene in a multitude of human cancers. However, the transcriptional regulation of Runx3 remains elusive due its large gene locus size (378kb) and hence the identification of candidate regulatory elements is still a challenge.

Methods

A combinatorial in silico approach comprising comparative genomics and retroviral integration site (RIS) mapping was employed to identify and prioritize candidate regulatory elements of Runx3 for subsequent experimental validation in vivo in zebrafish and mouse models.

Results

We report the identification and characterization of an upstream Runx3 element, eR3, with enhancer activity in hematopoietic cells. eR3 activity is critical in mediating Runx3 expression for the development and tumor antigen-specific lytic function of CD8+CD103 (integrin αE)+ cytotoxic T lymphocytes (CTLs). Loss of eR3 specifically in CD8 T cells compromised the suppression of tumorigenesis in murine cancer models. In human, single nucleotide polymorphisms (SNPs) in eR3 were overrepresented and associated with weakened CTL activity in colorectal cancer patients.

Discussion

Our study provides strong evidence that eR3 plays a role in immune surveillance against gut-associated tumors by upregulating Runx3 expression in specific CTLs. Runx3 therefore protects cells from tumorigenesis by both cell-autonomous and non-cell-autonomous mechanisms

BS-8

Shortlisted for Oral Presentation

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Identification of Super-enhancer-associated Cancer Genes Provides Novel Therapeutic Targets in Adult T-cell Leukemia/Lymphoma

Background

Adult T-cell leukemia/lymphoma (ATL) is a lymphoproliferative disorder arises from mature T-lymphocytes. Due to a high genetic heterogeneity across ATL samples, there is a lack of functional evidence of these genetic abnormalities in ATL pathogenesis.

Super-enhancers (SE), marked by a high level of acetylation of histone H3 lysine 27 (H3K27Ac), are often enriched at cancer genes in various malignancies. Identification of such SE would pinpoint critical factors that directly contribute to pathogenesis.

Methods

We analyzed H3K27Ac ChIP-seq dataset in 10 primary ATL samples, 1 ATL cell line, 1 T-cell acute lymphoblastic leukemia cell line (T-ALL) and 4 normal T-cell samples (Th1, Th2, Th17 and thymus). We performed RNA Pol II ChIP-seq and gene expression analysis after THZ1 small-molecule CDK7 inhibitor treatment in 1 ATL cell line. A targeted loss-of-function analysis of cancer gene candidates was then performed in ATL cell lines.

Results

The unbiased hierarchical clustering based on the genomic locations of SE demonstrated that ATL samples were classified into the same cluster which was distinct from normal T-cells or T-ALL. We found that SE are frequently enriched at the genes involved in T-cell activation and T-cell signaling pathway, including CD2, IL2RA/CD25 and TNFRSF8/CD30, both in ATL and normal mature T-cells, reflecting the origin of ATL cells. SE at the CCR4 gene, a known ATL cancer gene, were observed in all ATL samples, showing the feasibility of our approach. Additionally, we identified previously-uncharacterized genes that were highly activated in ATL cells and required for cell growth. THZ1 downregulated the expression of these genes and inhibited ATL cell growth.

Conclusion

Using a combinatorial approach of SE profiling, gene expression analysis after THZ1 treatment and functional analysis to identify genes that are aberrantly activated and required in cancer, our study provides a novel strategy to identify critical cancer genes.

BS-9

Shortlisted for Oral Presentation

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Role of G9a and Myc in Liver Cancer

Liver cancer is the second most common cause of cancer-related death in the world with the highest incidence in Asia. Current treatments have limited efficacy in patients with advanced HCC, and the only clinically-approved neoplastic agent for the treatment of advanced HCC is Sorafenib, which marginally improve HCC patient survival by 2 to 3 months. Therefore, there is a need to develop better therapeutic options for the treatment of HCC. Utilizing our oncogene-specific mouse models of cancer, we have previously demonstrated that oncogenes such as Myc, determine specific cancer properties. Myc mediates a number of biological functions through epigenetic regulation, yet the mechanisms of this regulation as well as therapeutic implications in cancers are unclear. We decided to use our Myc liver tumor model to investigate the epigenetic regulation of HCC. We showed that Myc-driven liver tumors have a unique H3K9 methylation pattern with corresponding upregulation of G9a. This phenomenon of increased H3K9 methylation and G9a was further observed in our Myc-positive HCC patient-derived xenografts (PDXs), demonstrating the clinical relevance of our tumor model. In addition, using both potent and specific pharmacological inhibitors of G9a (UNC0646 and UNC0642) and genetic knockdown of G9a by lentiviral means, we were able to reduce the global H3K9me2 and Myc levels significantly, with concomitant increased in tumor suppressor genes important for metastasis. More importantly, we showed that HCC patients with higher Myc and G9a expression levels portend a poorer survival with lower mean survival months. Our work suggests that targeting G9a could prove to be a potential therapeutic avenue for Myc-driven liver cancer. This will increase our understanding of the underlying epigenetic mechanisms of aggressive tumor initiation as well as lead to improved therapeutic and diagnostic options for Myc-driven hepatic tumors.

BS-10

Shortlisted for Poster Award Presentation

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Crosstalk between PPARγ and Annexin A1 in Tumor-Associated Macrophages in Breast Cancer

Background
Tumor-associated macrophages (TAMs) choreograph various aspects of the tumor microenvironment. Macrophages exhibit cellular plasticity and can be polarized to M1/M2 subtypes in presence of different microenvironment "signals". Peroxisome proliferator-activated receptor gamma (PPARγ) is a ligand-activated transcription factor expressed in macrophages, which has been shown to polarize macrophages towards the activated M2 phenotype in metabolic diseases. Annexin A1 (ANXA1), an anti-inflammatory protein is highly expressed in metastatic breast cancer.

Methods
TAMs expression was evaluated in the breast cancer patient samples. Percentage of TAMs was evaluated using flow cytometry in the breast tumors from MMTV mice along with the gene expression of PPARγ and ANXA1.Macrophage education by breast cancer cells was assessed by ex vivo differentiation of bone marrow derived macrophages (BMDMs) by flow cytometry, western blotting and mRNA expression.

Results
Clinically, we found that M2 TAMs were highly enriched in Claudin-low breast cancer subtype and was strongly associated with PPARγ and ANXA1 expression. In the MMTV mouse model, TAMs were higher in the breast tumors compared to the normal mammary tissues. Additionally, the BMDMs were skewed to a more M2 TAM-like phenotype upon co-culture with breast cancer cells as well as upon treatment with PPARγ agonist. Interestingly, upon treatment with the breast cancer conditioned media, expression of PPARγ along with its downstream targets was reduced in ANXAI-knockout BMDMs as compared to its wild type counterparts, suggesting a role of ANXA1 in regulating PPARγ activation.

Conclusions
This study demonstrates a novel role ANXA1 in regulating expression of PPARγ in the TAMs of breast tumor microenvironment. Further studies are underway to explore the signaling mechanism involved in governing this dynamic process.

BS-11

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Use of Quadratic Parabolic Optimisation Platform in Combinatorial Drug Therapy for Akt/β-catenin-driven Hepatocellular Carcinomas

Background
Hepatocellular carcinoma (HCC) is the most common primary liver cancer, accounting for more than 700,000 deaths worldwide each year. Hepatic resection and liver transplantation with adjuvant chemo- and radiotherapy are the mainstay of HCC treatment, but the 5-year survival rate remains poor because of frequent recurrence and intrahepatic metastasis. Currently, Sorafenib is the only FDA-approved antineoplastic drug for HCC, but its survival benefit is modest. Thus, there is a need to identify new therapeutic targets to improve current HCC treatment modality.

Methods
In our study, we used a HCC mouse model driven by Akt and β-catenin oncogenes to investigate potential drug combinations that are effective against this subgroup of liver cancer, via a phenotype-based quadratic parabolic optimisation platform (QPOP).

Results
Co-activation of both Wnt/β-catenin and Akt/mTOR pathways was found in 17% of our HCC patient cohort. More importantly, these patients showed poorer survival than those with either Wnt/β-catenin or Akt/mTOR pathway activation alone, demonstrating the clinical relevance of our study. In addition, we discovered that Akt/β-catenin tumors contained a subpopulation of cells with stem/progenitor-like characteristics (identified through side population analysis and expression of the cancer stem cell marker CD44) which may contribute to tumor sustenance and drug resistance. Consequently, using QPOP, we identified optimal combinations of small molecule inhibitors that could work synergistically in mitigating tumor proliferation and formation.

Conclusion
Our study supports the use of a clinically relevant tumor model and drug optimisation platform in the development of new targeted drug therapies against Akt/β-catenin-driven HCC.

BS-12

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Roles of Tumor Microenvironment in Pancreatic Cancer

Pancreatic cancer is a deadly disease- it ranks as the 12th most common cancer, and is the 5th most common cancer-related death in the world. A current lack of understanding of this disease is reflected by the lack of biomarker for early detection and effective therapeutic drugs to stop disease progression that give rise to high mortality. Recent studies reported that pancreatic progenitors are protected by stellate cells in the microenvironment that contributes to drug resistance. To better elucidate the progenitor-stellate relationship, we obtained human cancer specimens from clinicians which were digested and cultured in vitro. Progenitor and stellate cells were characterised by immunocytochemistry and tumour formation assays in mice. Our mouse work demonstrated that progenitor-derived xenografts indeed formed bigger tumour when grown in the presence of stellate. Subsequent RNA sequencing profiled that progenitor cells were linked to a greater extent of activated metabolic pathways than stellate cells which was validated by metabolomic assays with success. Further in vitro high throughput niche-based drug screening experiments performed with both co-mix and individual cell types revealed several interesting drugs that may target the metabolic pathways. These drugs are believed to inhibit the protective effects of stellate cells, which in turn makes progenitors susceptible to the killing effects of drugs. In all, these work demonstrated exciting results that uncovers tumour-stromal interactions potentially translatable into novel therapeutic strategies in the clinics.

BS-13

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Inactivation of STAT3 phosphorylation by KPT-330 Potentiates DAN Damage Induced by Gemcitabine in Nasopharyngeal Carcinoma Cells

Introduction

Chemoresistance limits the effectiveness of conventional therapeutics used in NPC. XPO1 over-expression has been associated with poor clinical outcomes in other cancers. This study aims to evaluate the anti-cancer potential of KPT-330, an XPO1 inhibitor, as monotherapy, and in combination with gemcitabine in NPC.

Methods

Anti-proliferative effects of KPT-330 and gemcitabine in NPC cells were examined using MTS assay. Drug effects on cell cycle phases and cellular apoptosis were studied using flow cytometry. Western blot was performed to elucidate anti-cancer mechanisms of combinatorial treatment.

Results

XPO1 was found to be highly expressed in untreated primary and immortalized NPC cells by an average of 6.23 fold and 3.97 fold respectively, compared to normal nasopharyngeal cells, ($p<0.05$). KPT-330 reduced XPO1 expression and inhibited NPC cell growth in a dose-dependent manner. KPT-330 treatment increased the fraction of CNE2 cells in G2/M phase (16.45 ± 0.92%) in comparison to control (8.29 ± 0.95%) ($p<0.05$), highlighting G2/M phase arrest induction. Combinatorial treatment reduced NPC cell viability, with a 13.26 fold reduction in gemcitabine IC50 value ($p<0.05$), yielding a Combination Index of 0.59 ± 0.0253, thereby indicating drug synergism. We have found for the first time a novel pathway where KPT-330 can synergize with gemcitabine to suppress phospho-STAT3, in turn reducing its protection against DNA damage induced by gemcitabine, as indicated by a marked elevation of phospho-H2A.X levels in NPC cells treated with the combination. Enhanced caspase-8, caspase-9, caspase-3, and PARP cleavage confirmed a greater increase in apoptosis induction after combinatorial treatment.

Conclusion

KPT-330 is a novel agent capable of exerting anti-cancer effects in NPC as monotherapy and in synergism with gemcitabine.

BS-14

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BCL6 modulates the TP53 and STAT pathways in glioma

Glioblastoma multiforme (GBM) remains the most aggressive brain malignancy with little improvement in prognosis or therapy for decades. Recently, we identified BCL6, also known as ZBTB27, to be a novel oncogene in GBM. In this study, we performed IHC analysis of 153 primary human glioma specimens and 8 normal brain samples. BCL6 expression is robustly elevated in tumor samples and positively correlated with glioma pathological grade. High BCL6 expression strongly predicts a worse prognosis of GBM patients. Depletion of BCL6 in human GBM cells reduced the incorporation of BrdU, promoted the cellular senescence and inhibited the growth of human GBM cells in vivo. Next, genome-wide occupancy of BCL6 in GBM cells was characterized by ChIP-seq assay. Genomic regions centered on BCL6 peaks are co-enriched with RNA-Pol II and flanked with strong H3K27ac and H3K4me3 modifications. Moreover, pathway enrichment analysis of BCL6 peak associated genes reveals a significant enrichment of JAK-STAT, TP53, ERBB and MAPK pathways. We demonstrated further that BCL6 represses the TP53 pathway and promotes the JAK-STAT pathway activation in GBM cells. Together, our findings uncover potential downstream targets and provide a better understanding of BCL6 function in GBM.

BS-15

Shortlisted for Poster Award Presentation

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BCL6 promotes glioma and serves as a novel therapeutic target

ZBTB transcription factors orchestrate gene transcription during tissue development. However, their roles in glioblastoma multiforme (GBM) remain unexplored. Here, through a functional screening of ZBTB genes, we identify that BCL6 is required for GBM cell proliferation and is overexpressed in GBM samples. Using a somatic transgenic mouse model, Bcl6 confers the proliferative and invasive stimuli to glioma cells in vivo. Moreover, we discover AXL as a novel downstream target of BCL6. Through AXL, BCL6 enhances both MEK-ERK and S6K-RPS6 axes. Pharmacological inhibition of BCL6 activity effectively blocks GBM growth and inhibits AXL expression. Together, these findings uncover a novel glioma-promoting role of BCL6, and provide the rationale of targeting BCL6 as a potential therapeutic approach

BS-16

Shortlisted for Oral Presentation

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TIP60 represses telomerase expression by inhibiting Sp1 function

Background
HIV1-TAT interacting protein (TIP60) is a haploinsufficient tumor suppressor. The potential mechanisms endowing its tumor suppressor ability remain incompletely understood. TIP60 plays a vital role in virus-induced cancers. In human papillomavirus (HPV) induced cervical cancer, it down-regulates the expression of HPV oncoprotein E6, which in turn destabilizes TIP60. This intrigued us to identify the role of TIP60, in the context of a viral infection, where it is targeted by oncoproteins.

Methods
We used an array of molecular biology techniques such as chromatin immunoprecipitation, expression analysis and SILAC-mass spectrometry. To study telomerase activity, we performed quantitative telomere repeat amplification protocol (q-TRAP). We used luciferase reporter assays to study promoter regulation.

Results
We have established the hitherto unknown role of TIP60 in repressing the catalytic subunit of the human telomerase complex, TERT, a key driver for immortalization. Mechanistically, we identified that TIP60 acetylates Sp1, a known positive regulator of TERT. The acetylation of Sp1 at a lysine residue in the DNA binding domain of Sp1 by TIP60, inhibits Sp1 binding to the TERT promoter. We identified that TIP60-mediated growth suppression of HPV-induced cervical cancer is mediated in part due to TERT repression.

Conclusion
In summary, our study has identified a novel substrate for TIP60 catalytic activity and a unique repressive mechanism acting at the TERT promoter in virus-induced malignancies.

BS-17

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Post-translational Modification Regulates ADAR1 in Breast Cancer

Adenosine deaminase acting on RNA 1 (ADAR1) is the primary enzyme which catalyzes adenosine-to-inosine (A-to-I) changes on the RNA sequence and generates diversity in the transcriptome. Aberrant editing levels that are associated with deregulated ADAR1 levels have been described in numerous types of cancer and is believed to contribute to the aggressiveness of the cancer. Here, we found that higher median levels of ADAR1 expression in estrogen receptor (ER)-positive breast cancer cases are associated with worse patient prognosis, whereas the inverse is true for ER-negative breast cancer cases. We identified a differentially phosphorylated site between ER-positive and ER-negative breast cancer cell lines by mass spectrometric analysis. This phosphorylated site lies within the ADAR1 protein in the double stranded RNA binding domains (dsRBDs). We show that there is a higher basal endogenous editing levels of antizyme inhibitor 1 (AZIN1) transcript, a verified ADAR1-specific editing substrate, within the ER-positive group compared to the ER-negative group in clinical breast cancer samples. Collectively, we hypothesize that phosphorylation within the dsRBD may serve as a molecular mechanism for the modulation of ADAR1 editing function, which in turn attributes to the edited RNA landscape in ER-positive breast cancer

BS-19

Lim Shen Kiat[1], Hossein Tabatabaeian[1], Ssu Yi Lu[1], Shin-Ae Kang[1], Sundaram Gopinath Meenakshi[1], Sampath Prabha[1], Siew Wee Chan[1], Wan Jin Hong[1], Lim Yoon Pin[1]

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Hippo Kinase Suppresses Breast Cancer by Silencing WBP2 via The Dicer-MicroRNA Pathway

WBP2 transcription coactivator is emerging as a new breast cancer oncogene and a node of convergence between EGF, Wnt and Hippo signaling pathways through its interaction with YAP and TAZ proteins. Its expression is tightly controlled. WBP2 is regulated by Wnt, Estrogen and EGF signaling at the post-translational level, including phosphorylation, subcellular translocation, protein-protein interaction, ubiquitination and proteasomal degradation. However, the direct effect of Hippo pathway on WBP2 has not been reported before. Here, we show that MST negatively regulated WBP2 expression in a kinase-dependent but LATS-independent manner.

MST-mediated downregulation of WBP2 was observed in a considerable number of triple negative breast cancer (TNBC) cell lines and inhibited the transcription co-activation, in vitro transformation and in vivo tumorigenesis activities of WBP2. The negative regulation of WBP2 by MST was demonstrated to involve miRNA but not proteasomal and lysosomal degradation.Our data support the existence of a novel MST-Dicer signaling axis, which in turn regulates miR-23a expression. MiR-23a targeted WBP2, its functional network of genes (e.g. YAP, TEAD) and suppressed proliferation of MDA-MB-231 and BT549 TNBC cells. Significant inverse relationships were observed between the expression levels of WBP2 and MST or miR23a in clinical specimens.

In conclusion, WBP2 is discovered to be a target of the Hippo/MST kinase; miRNA is identified as yet another rheostat in the complex regulation of the WBP2 and its oncogenic function. A better understanding of the intricate regulatory network could be exploited to target WBP2 for cancer therapy especially TNBC against which there is no standard treatment

BS-20

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Detection of *CEBPA* Mutations in Acute Myeloid Leukemia by Next-generation sequencing

Background

Mutations in the CEBPA gene play an important role in refining the prognosis of patients with AML. AML patients harboring CEBPA or NPM1 mutations without FLT3-ITD has been identified as having a prognostic risk similar to that of AML with favorable cytogenetic changes. In addition, biallelic CEBPA mutations have also been reported to confer favorable prognostic impact in patients with AML. However, the high GC content and sequence complexity, broad spectrum of mutations make the analysis of this gene challenging. The study aims at Sanger sequencing (detection limit of 15% mutated CEBPA) and claim more data collection on the use of highly sensitive next-generation sequencing (NGS).

Methods

Using the Nextera XT library preparation kit and the PCR Amplicon Workflow, we presented a robust CEBPA-specific NGS assay to retrospectively analyzed 137 bone marrow or peripheral blood samples. To compare sequencing methods, we performed both Sanger sequencing and NGS to test all 137 samples in our cohort on the MiSeq instrument (illumine, San Diego, CA): A one-amplicon CEBPA long-range PCR was performed with a minimal NGS depth of 500X.

Results

As technical comparison, all mutations previously detected by Sanger sequencing were analyzed by NGS. Using NGS, we identified 31 CEBPA mutations in 16 patients, with 27 mutations were detected by both methods. Three mutations were detected only using NGS whereas one mutation was missed by NGS. Bioinformatics analysis showed that the CEBPA library generated a minimum read depth per base across the CEBPA gene ranging from 3631x to 28184x across 137 samples. The variant allele frequencies (VAF) which was detected by NGS range from 5.61% to 53.65%.

Conclusion

Here, we validated a more sensitive and robust method in the detection of mutations in CEBPA gene. High sequencing coverage (3631x to 28184x) improved the accuracy of the analysis as NGS can detect mutant allele burden as low as 5.61%. In addition, our NGS results also corroborate with Sanger results with a positive predictive value of 96.4%, sensitivity of 97.6% and specificity of 90.0%. We found overall a good concordance between the two methods (97.4 %), but better sensitivity of NGS when it comes to identification of low frequency mutations (<10 %).

BS-21

Melissa G Ooi, Jana Jakubikova, Constantine Mitsiades, Chng Wee Joo

AMPK activators have demonstrable anticancer activity

Background

Use of metformin, an AMP-activated protein kinase (AMPK) activator, is associated with decreased cancer incidence and cancer-related mortality and may have direct anticancer activity. The anticancer effect of AMPK activators is thought to be due to direct AMPK activation and reduction of mTOR pathway with subsequent reduction in inhibition of translation initiation.

Methods

Cell viability was assessed using MTT assay. The mechanism of action of the compounds was assessed by western blot and flow cytometric analysis of apoptosis and mitochondrial membrane depolarization.

Results

We investigated the direct effects of 2 AMPK activators, metformin and acadesine (5-Aminoimidazole-4-carboxamide-1-β-D-ribofuranoside, AICAR), on a panel of multiple myeloma cell lines and solid tumour cell line. Both AMPK activators exerted on all cell lines tested in vitro a dose-dependent growth-suppressive effect that was preserved or even enhanced in the presence of bone marrow stromal cells or osteoclasts. Mitochondrial membrane depolarization and apoptosis were induced by acadesine, but not metformin. Acadesine-induced apoptosis was attenuated by Bcl-2 and enhanced by activated Akt. Both AMPK activators increased phospho-AMPK and suppressed mTOR signaling. Two glycolysis inhibitors, 3-bromopyruvate and 2-deoxyglucose, enhanced the pro-apoptotic activity of acadesine.

Discussion

Our data suggest that AMPK activators exert a direct growth-suppressive effect in various malignancies, involving inhibition of mTOR, that can be potentiated by interactions with the local microenvironment, the Akt pathway and glycolysis inhibitors. These findings strengthen the preclinical evidence supporting AMPK as a therapeutic target in cancer patients, especially for malignancies with an active Akt/mTOR pathway.

BS-22

Amit Kumar Pandey[1], Jean Kim Bee Leong[1,2], Guo Liang Low[1], Mick Guanshen Chen[1,2], Yanzhou Zhang[1], Vanitha Krishna Subbaiah[1], Deepa Rajagopalan[1,2], Kwok Kin Lee[1], Yimin Lin[1], Hwee Tong Tan[1], Maxey Ching Ming Chung[2,3], Sudhakar Jha[1,2]

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Acetylated tubulins as a diagnostic marker for treatment with microtubule polymerization inhibitors

Background
HIV-TAT1 interacting protein 60kDa (TIP60) is a lysine acetyltransferase (KAT) that is involved in transcriptional regulation and DNA damage response. TIP60 is a tumor suppressor and is down-regulated in several cancers including human papillomavirus (HPV)-induced cervical cancers. In this conference, I am going to present data where we have identified non-transcriptional targets of TIP60.

Methods
For this study, we generated stable cell line expressing Flag-tagged TIP60. Using cell lysates from this cell line we performed immunoprecipitation and identified TIP60 interacting partners. In order to identify the biological significance of TIP60 mediated acetylation on α-tubulin, we depleted TIP60 using siRNA and investigated turnover of tubulins.

Results
Among several interacting partners, we focused on α- and β-tubulin. By using various cell biology approaches we show that TIP60 interacts with α-and β-tubulin and acetylates α-tubulin both in vitro and in vivo. Depletion of TIP60 resulted in decreased tubulin levels and this is through proteasome. Furthermore, we identified the degradation of tubulin is by an E3 ubiquitin ligase, Parkin. In pathological scenarios where TIP60 levels are low, such as in HPV-positive cells, the cells are more sensitive to microtubule polymerisation inhibitor.

Conclusions
The data suggest that levels of acetylated tubulins can be used as a potential diagnostic marker to determine individuals who would be more responsive to microtubule inhibitor treatment.

BS-23

Shortlisted for Poster Award Presentation

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A Distinct Role of RhoB in Simvastatin-Induced Cytotoxicity in Breast Cancer Cells

Objective/ Background

Statins, a class of HMG-CoA reductase inhibitors, initially developed as cholesterol-lowering drugs by inhibiting the mevalonate pathway, also exhibit potential anticancer properties, but the mechanisms remain elusive. This study aims to investigate the antiproliferative effects of statins in breast cancer cell lines.

Methods

We screened a panel of breast cancer cell lines (Estrogen receptor positive (ER+):CAMA1, MCF7, T47D, ZR75-1 and Hcc1428; triple negative (TNBC):MDAMB231, MDAMB468, BT549, Hs578T and Hcc1806) and assessed the sensitivity of these cells to simvastatin. Next, we evaluated the expression of key enzymes in the mevalonate pathway and role of sterol biosynthesis metabolites in simvastatin-mediated cytotoxicity. Lastly, we performed functional assays following siRNA knockout to identify the small GTPase involved.

Results

We found TNBC cells to be more susceptible to simvastatin compared to ER+ cells (mean IC50 of 7.98µM [95%CI 2.75-13.22] versus 41.74µM [95%CI 6.71-76.78]). Simvastatin treatment induced robust apoptosis in TNBC but not ER+ cells. There was no difference in mRNA expression of HMGCR, HMGCS, MVK, MVD, IDI1 enzymes involved in the mevalonate pathway between TNBC and ER+ cells. Supplementation with geranylgeranyl pyrophosphate but not farnesyl pyrophosphate, inhibited simvastatin-mediated cell death, suggesting the involvement of geranylgeranylated proteins. Here, we identified RhoB to be a key player in simvastatin-mediated cytotoxicity. RhoB showed an increase in mRNA and protein levels with simvastatin treatment in the TNBC cells but the increased expression does not correlate with increased activity. Finally, silencing RhoB was able to abrogate the cytotoxic effects mediated by simvastatin in TNBC cells.

Conclusion

Together, our data suggest a critical role of RhoB in the anticancer activity of simvastatin, which appears to be exclusive to TNBC cells suggesting that HMG-CoA reductase inhibitors may provide new therapeutic strategies for TNBC treatment.

BS-24

Shortlisted for Poster Award Presentation

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The interplay between transcription factor GRHL2 and epigenetics in the regulation of EMT in ovarian cancer

Grainyhead-like 2 (GRHL2) is a transcription factor that regulates a repertoire of epithelial genes during Epithelial-mesenchymal transition (EMT). Here, we describe the transcriptional and epigenetic landscapes regulated by GRHL2. Using Illumina HumanMethylation450K array, we identified differentially methylated CpG sites of ovarian cancer (OC) cells in correlation with EMT. These differentially methylated sites were found significantly enriched in the DNA binding motif of GRHL2, suggesting that its target genes could be regulated by DNA methylation. The DNA methylation of GRHL2 target genes were also analysed in a GRHL2 knockdown model. Overall, there was a negative correlation between the changes in CpG methylation and the alterations of gene expression after GRHL2 knockdown. Besides DNA methylation, we explored the genome-wide histone modifications involving five marks (H3K4me1, H3K4me3, H3K27Ac, H3K27me3 and H3K9me3) in an OC cell line panel and the isogenic knockdown model by ChIP-sequencing. The loss of GRHL2 resulted in a general decrease in the active marks (H3K4me1, H3K4me3, H3K27Ac) and a gain in the repressive mark H3K27me3 at GRHL2 binding sites. These changes reflected the transcriptional states of GRHL2 target genes, which showed transitions from active to low activity (47%), active to PRC2-repressed (9.75%), active to poised (2.1%), and active to heterochromatin (1.52%). Finally, we demonstrated that the EZH2 inhibitor GSK126, which suppressed the H3K27me3 level, could enhance the GRHL2 activation of its target gene E-cadherin. Therefore, the function of GRHL2, which depends on the accessibility to its target genes, could be affected by the histone modifications at the promoters or the GRHL2-binding sites.

BS-25

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Targeting cysteine addiction in ovarian clear cell carcinoma

Background

Cysteine is regarded as a semi-essential amino acid, such that it can be synthesized de novo and needs to be supplied from the diet under certain pathophysiological conditions. Excess of cysteine supply by the tumor microenvironment has been shown to promote tumor progression, not only in solid tumors, but also in leukemia. This hints for cancer cell reliance on cysteine residue.

Methods

Cystathionase (CTH) is an enzyme responsible for de novo cysteine synthesis along the trans-sulfuration pathway. Preliminary assessment of CTH using a transcriptomic database of human ovarian cancer, CSIOVDB, shows CTH to be highly expressed particularly in ovarian clear cell carcinoma (OCCC) histotype, known to be resistant to conventional platinum-based therapy. Western blot for CTH protein using seventy human ovarian cancer cell lines of different histotypes agrees with the database, suggesting the importance of CTH in OCCC. Following this finding, nine human OCCC cell lines are utilized to dissect the molecular significance of CTH in this histotype.

Results

Inhibition of CSE enzymatic activity by PAG shows dose-dependent cytotoxicity. Treatment with PAG in low cysteine media sensitizes the cells more to PAG inhibition. Furthermore, cells that remain insensitive to PAG inhibition in low cysteine media shows greater sensitivity to cisplatin when both agents are administered sequentially; PAG first, followed by cisplatin. Importantly, the observed cytotoxicity can be rescued by pre-treatment of cells with excess N-acetyl cysteine. PAG-mediated cytotoxicity are found to be associated with decrease in cysteine and glutathione levels as well as accumulation of reactive oxygen species (ROS) level.

Conclusions

Altogether the data suggest the importance of CTH activity in OCCC survival and CTH overexpression may potentially serve as a therapeutic target for OCCC.

BS-26

Vivien Koh[1], Ramnarayanan Kalpana[2], Huipeng Li[3], Tannistha Nandi[3], Angie Tan[2], Mai Chan Lau[2], Jinmiao Chen[4], Shing Leng Chan[5], Asim Shabbir[6], Jimmy So[1,6], Shyam Prabhakar[3]Patrick Tan[2,5], Wei Peng Yong[1,5]

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Single-cell transcriptomics reveals clonal diversity and tumor heterogeneity in gastric cancer

The conventional belief that cell populations were homogeneous was superseded by latest evidence showing that heterogeneity exists even within small populations of cells. Current developments in single-cell sequencing methodologies have greatly enhanced the comprehensive analysis of the cancer genome at the single-cell level. By studying one cell at a time, unique differences between single cells in a seemingly homogeneous population can be uncovered. Single-cell RNA sequencing has recently emerged as a promising tool to study cancer heterogeneity and to better understand the function of an individual cell in its microenvironment. This has thus far led to the discovery of intratumoral heterogeneity, elucidation of tumor evolutionary mechanisms, as well as identification of new driver mutations and novel biomarkers. In this study, we employed the microfluidics-based Fluidigm C1 and fluorescence activated cell sorting (FACS) workflows to sort and isolate single cells from primary gastric tumors and patient-derived xenografts. Through single-cell RNA sequencing, we identified different populations of cells in the tumor microenvironment, including tumor cells, stromal cells and immune cells. Computational analysis is in progress to delineate the mechanistic networks involved in the signaling crosstalk between different cell types. Understanding these information may provide insights to early cancer detection and monitoring of rare cancer cells, and help in the development of personalized and precise cancer therapeutic approaches. Collectively, our findings may uncover novel cell-specific mechanisms of gastric cancer at single-cell resolution and lead to the discovery of new biomarkers for therapeutic intervention.

BS-27

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The Theoretical Effects of Synergistic and Antagonistic Combination Therapies on the Evolution of Resistance in Cancer

Background

Synergistic combinations have been pursued and applied for cancer therapy due to their attractive property of eliciting high efficacy with low doses. However, the determinant of therapeutic success is often the evolution of drug resistance. How synergistic therapies impact the evolution of resistance, as opposed to additive or antagonistic therapies, is not yet understood.

Methods

We developed an abstract computational model simulating the evolution dynamics of resistance in a cancer cell population, treated with two-drug combinations. The model tracked cell numbers in 4 subpopulations: sensitive to drugs 1 and 2, resistant to drug 1, resistant to drug 2, and doubly-resistant.

Results

Simulations showed that when combination therapies were dosed at the same dose combination, synergistic combinations had a better initial efficacy and were more successful in delaying resistance evolution. However, when combination therapies with equal initial efficacy were compared (allowing dose combinations to differ), antagonistic combinations were better at suppressing the growth of resistant subpopulations. Our analysis revealed that the contrast between synergism and antagonism parallels the contrast between offensive and defensive treatment strategies. Synergism combats resistance through an offensive “pro-efficacy” approach; it can counter resistance evolution if it is efficacious enough to rapidly kill naïve cells before resistance arises. Meanwhile, antagonistic and redundant therapies combat resistance through a defensive “anti-resistance” approach of reducing the competitive evolutionary advantage of partially-resistant cells.

Conclusions

In this study, we demonstrated the intrinsic property of synergism as a pro-efficacy strategy and antagonism as an anti-resistance strategy for combating cancer drug resistance. The effect of synergistic and antagonistic combinations on the evolution of resistance and the long-term success of therapy was characterized.

BS-28

Madhura Kulkarni, Nurfarhanah Bte Syed Sulaiman, Xiaomeng Wang, Wanjin Hong ,Lee Soo Chin, Yoshiaki Ito

Cancer Science Institute of Singapore, NUS

Elevated YAP expression is required for EMT, stem-ness and angiogenic properties in triple negative breast cancer cell lines.

Background

Triple Negative Breast Cancer (TNBC) is considered one of the aggressive breast cancer subtypes with higher risk of recurrence and mortality compared to other breast cancer subtypes. Analysis of six TNBC cell lines has uncovered Yes-associated protein; YAP to be specifically associated with increased mesenchymal properties linked with recurrence.

Methods

Six TNBC cell lines with different degrees of YAP expression were selected for the study. After stable knockdown of YAP expression using retrovirus, six TNBC cell lines were assessed for trans-well migration, EMT marker expression, and mammosphere formation. Angiogenic potential was assessed by analysing media supernatants from vector and YAP knockdown cells. Further detailed characterisation of media supernatants was carried out to identify angiogenic markers altered after YAP knockdown.

Results

TNBC cell lines with high YAP expression (MDA-MB-231, MDA-MB-468) and medium YAP expression (MDA-MB-435, Hs578T) showed significantly reduced migration potential in trans-well assay, and reduced mammosphere formation after YAP knockdown. Similarly, media supernatants from high YAP and medium-YAP cell lines showed severely affected angiogenic potential towards endothelial cell migration and Choroid angiogenesis after YAP knockdown. Proteomic analysis of media supernatants revealed IGFBP-1 and ANG-1 expression to be down regulated after YAP knockdown in all 4 cell lines. TNBC cell lines with low YAP expression (MDA-MB-436, BT549) did not manifest significant reduction in EMT, mammosphere formation or angiogenic properties after YAP knockdown. We intend to validate these results in TNBC tumor samples by comparing EMT, stem-ness and angiogenic markers, stratified by YAP expression.

Conclusion

YAP indeed regulates recurrence-associated properties like EMT, stem-ness and angiogenic potential in TNBC cell lines. Further analysis will establish YAP as a potential marker to predict metastasis and recurrence in TNBC