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ABSTRACTS BOOK

LIVRE DES RÉSUMÉS

THE TERRY FOX RESEARCH INSTITUTE
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MARATHON OF HOPE CANCER CENTRES NETWORK
RÉSEAU DES CENTRES D'ONCOLOGIE DU MARATHON DE L'ESPOIR

Digital Health & Discovery Platform
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1. RESTORATION OF MASTICATION FUNCTIONALITY POST-MANDIBULAR RECONSTRUCTION: INSIGHTS FROM DYNAMIC COMPUTER MODELLING

Hamidreza Aftabi[1], *Benedikt Sagl*[2], *John E Lloyd*[1], *Eitan Prisman*[1], *Antony Hodgson*[1], *Sidney Fels*[1]

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Advanced head and neck cancers implicating the mandible necessitate surgical intervention, which significantly impacts the structural and functional integrity of the mandible. Restoring mastication functionality post-reconstruction is a complex endeavor, not fully addressed by current surgical planning methodologies. This study explores the efficacy of dynamic computer models in predicting the restoration of masticatory functionality post-mandibular reconstruction surgery.

Methods

This study developed dynamic biomechanical models of the reconstructed mandible, considering different defect types. The models were validated using literature data and aimed to estimate the degree of functionality achievable post-rehabilitation. The approach employed the Artisynth software platform (www.artisynth.org) for simulations, incorporating full skull CT and MRI scans. It modelled 24 muscles as Hill-type linear actuators and used a scarring model along with a validated temporomandibular joint (TMJ) model.

Results

The findings, validated against literature, reveal a promising capacity for the reconstructed mandible to regain effective masticatory function, with a substantial potential for the restoration of mastication across various defect types. The adapted functionality is comparable to that of a healthy individual. It shows a trajectory error below two millimetres and a bite force reduction ranging from 36 per cent to negligible. Additionally, there is improved condyle mobility and changes in muscle activation, capped at a maximum of 20 per cent. These results highlight the masticatory system's potential for remarkable adaptability to new conditions post-reconstruction.

Conclusions

The dynamic computer models provide a promising approach to predict mastication functionality restoration post-mandibular reconstruction. Additionally, the adaptability of the masticatory system plays a crucial role in recovering mastication functionality, underscoring the importance of personalized rehabilitation techniques.

Outcome/Impact

Computer simulations can enhance surgical planning and rehabilitation for mandibular reconstruction by predicting outcomes to prevent undesirable results in these costly procedures. It can assist surgeons in decision-making involving the muscles, bones and joints by offering insights into the masticatory system's adaptability and restoration potential. This capability has the potential to revolutionize care for patients with mandibular cancer.

KEYWORDS

mandibular reconstruction, computational models, surgical simulations

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2. EXAMINING THE THERAPEUTIC POTENTIAL OF PORPHYRIN-LIPID NANOPARTICLES FOR HEPATOCELLULAR CARCINOMA

Alyssa Apilan[1,2], Jeffrey To[1,2], Anita Bakrania[1], Yulin Mo[1], Anni Pan[1], James Bu[1], Juan Chen[1], Gang Zheng[1], Mamatha Bhat[1,2]

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Hepatocellular carcinoma (HCC) continues to rise in prevalence and is the most common form of liver cancer globally and in Canada. One important clinical consideration is that patients are often concurrently affected by cirrhosis, which greatly impairs liver function, making it difficult to receive systemic treatments. Of this population, 30 per cent have genetic mutations in the gene CTNNB1, which is considered undruggable. To target this, RNA nanomedicine, specifically through the use of Porphyrin-Lipid nanoparticles (Porphyrin-LNPs) loaded with anti-CTNNB1 siRNA presents itself as a potential approach.

Methods

Live fluorescence imaging was used to quantify Porphyrin-LNP biodistribution in both non-cirrhotic and cirrhotic mice. Then, the efficacy of Porphyrin-LNPs with CTNNB1 siRNA was evaluated ***in vitro*** using HCC cell lines. RT-qPCR and clonogenic assay studies were performed to assess the gene silencing efficacy and the survival and growth of cells post-treatment, respectively. Subsequently, non-cirrhotic environments were established in mice using the sleeping beauty transposon system combined with mutant oncogenic plasmids delivered via hydrodynamic injection.

Results

Biodistribution studies have demonstrated that the majority of these particles are uptake in both the non-cirrhotic and cirrhotic liver. From our results, we have showcased that Porphyrin-LNPs with CTNNB1 siRNA demonstrate significant gene silencing and that the targeted treatment significantly inhibits cellular proliferation. Finally, a non-cirrhotic mouse model was successfully developed with a tumour incidence rate of 100 per cent.

Conclusions

Overall, we have demonstrated the ***in vitro*** feasibility of utilizing Porphyrin-LNPs with CTNNB1 siRNA as a potential targeted treatment avenue for HCC, as well as an *in vivo* platform for examining therapeutic efficacy. Current work is being done to evaluate this treatment ***in vivo***.

Outcome/Impact

Through this therapeutic platform, HCC patients both in the presence and absence of cirrhosis, especially those restricted from current standards of care, may benefit from this targeted treatment. Current findings suggest potential applications ***in vivo***.

KEYWORDS

gene therapy, hepatocellular carcinoma, RNA nanomedicine, beta-catenin

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3. THE USE OF ARTIFICIAL INTELLIGENCE-BASED HISTOPATHOLOGY IMAGE ANALYSIS TO IDENTIFY A NOVEL SUBTYPE OF ENDOMETRIAL CANCER WITH UNFAVOURABLE OUTCOME

Maryam Asadi[1], Amirali Darbandsari[2*], Hossein Farahani[1], Samuel Leung[4], Stefan Kommoss[5], David Huntsman[3,4], Aline Talhouk[3], C. Blake Gilks[3,6], Jessica N. McAlpine[7], Ali Bashashati[1,3]* Equal contribution*

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The Proactive Molecular risk classifier for Endometrial cancer (ProMisE) was developed by our team as a pragmatic, cost-effective and clinically applicable molecular classifier for endometrial cancer (EC) patients. Despite ProMisE subtypes being associated with clinical outcomes, some subtypes have clinical/prognostic outliers, as is especially true in No Specific Molecular Profile (NSMP) (approx. 50 per cent of ECs), a subset of patients exhibits an aggressive disease course comparable to p53abn ECs.

Methods

We hypothesized that objective assessment of the digitized hematoxylin and eosin (H&E)-stained histopathology slides of NSMP could potentially identify clinical outcome outliers. Thus, we developed an artificial intelligence (AI)-based image analysis model for identifying NSMP cases with histopathological similarity to the p53abn subtype, as assessed by H&E stain.

Results

Our AI-based method identified 6.25 per cent out of the 416 NSMP cases with similar histopathological features as p53abn cases. We refer to these cases as 'p53abn-like' NSMPs. Compared to the rest of the NSMP cases, these cases had markedly inferior disease-specific survival (DSS) (five-year DSS 66.60 per cent vs. 99.4 per cent ($p<8.20e-13$)) and progression-free survival (PFS) (five-year PFS 62.55 per cent vs. 88.92 per cent ($p<5.41e-6$)).

Conclusions

Utilizing an AI-based approach for histopathology image analysis, we have discovered 'p53abn-like' NSMPs that resembles p53abn cases in morphology and have noticeably inferior outcome compared to the rest of the NSMP cases.

Outcome/Impact

By discovering a novel stratification of NSMP, the most clinically and prognostically diverse molecular subtype of EC, we provide opportunities for better contemporary and future therapeutic options.

KEYWORDS

endometrial cancer, ProMisE, NMSP, AI-based pathology

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4. ULTRA-DEEP SEQUENCING TO IDENTIFY DRIVERS OF GLIOBLASTOMA RECURRENCE AND EVOLUTION

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Glioblastoma (GBM) is the most prevalent and deadliest form of brain tumour with most patients surviving for 15 months after diagnosis. Single-nucleotide variants (SNVs), structural variants (SVs) and epigenetic alterations, such as aberrant genome methylation patterns, contribute to GBM development and recurrence. However, the mechanisms of how various molecular alterations contribute to therapy resistance and tumour recurrence remain poorly understood. To address this, we sequenced a novel cohort of matched primary and recurrent GBMs to understand how genetic and epigenetic alterations drive tumour evolution.

Methods

We performed ultra-deep whole transcriptome, whole genome and nanopore long-read sequencing of matched primary and recurrent GBMs of 12 adult patients with matching germline genomes from fresh-frozen blood. High-performance base-calling of nanopore sequencing data provided higher-accuracy base calls and single nucleotide resolution of methylated bases. We determined somatic SNVs, SVs and copy number alterations (CNAs), genome-wide DNA methylation patterns and transcriptomic profiles from our multi-omics datasets. We inferred GBM sub-clonal evolution of SNVs and CNAs. We integrated our novel dataset with publicly available datasets including Glioma Longitudinal Analysis (GLASS) and TCGA cohorts to extend and validate our results from our smaller cohort.

Results

We mapped a complex landscape of genetic and epigenetic alterations, potentially contributing to GBM recurrence and therapy resistance, in primary and recurrent GBMs. Major changes between primary and recurrent samples, such as SVs and sub-clonal methylation patterns, were only detectable using long-read sequencing strategies, emphasizing the value of long-read sequencing in sub-clonal evolutionary analyses. Extensive inter- and intra-tumour heterogeneity was detected from ultra-deep WGS sequencing as well as evidence of clonal selection in recurrent tumours. We found consistently differentially spliced genes between primary and recurrent GBMs, such as *EGFR* and hallmark genomic features of GBM, such as *PTEN* and *RB1* loss and chromosome seven gain / 10 loss. Our paired primary-recurrent GBM analysis reveals evidence of genetic, transcriptional and epigenetic drivers of recurrence.

Conclusions

Our multi-omic sequencing of a novel cohort of paired primary and recurrent tumours reveal mechanisms of tumour evolution across several levels of cellular regulation.

Outcome/Impact

This analysis will enhance our understanding of how genomic, epigenetic and transcriptomic alterations drive GBM evolution and recurrence.

KEYWORDS

glioblastoma, long-read sequencing, evolutionary analysis, sub-clonal analysis

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5. VIRTUAL INTERACTIVE PATIENT FOR OPTIMAL MANDIBULAR RECONSTRUCTION

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Mandibular resection and reconstruction surgery for the treatment of oral cancer is often performed using a free-hand surgical (FHS) approach, which can result in numerous complications regarding rehabilitation and flap health. To mitigate these risks, we have developed an in-house virtual surgical planning (VSP) platform which allows precise preoperative planning of the required cuts followed by the 3D printing of patient-specific cutting guides for both the resection and flap preparation.

Methods

The VSP platform is built using Artisynth, a biomechanical modelling toolkit (www.artisynth.org). Surface meshes of the mandible and donor bone are segmented from CT images and imported into the VSP. The resection cut plane locations are then identified and the outer contour of the resection is approximated by several line segments (typically one to three), which are used to automatically plan the shapes and locations of the flap segments within the donor. This plan can be manually adjusted to control for various metrics, such as volume overlap and bony contact between segments. STL files are then created for the 3D printing of the donor and resection cutting guides. Ongoing improvements to the VSP will include adding dental implants to the reconstruction, easing dental rehabilitation and removing the need for secondary implantation surgeries.

Results

The entire VSP process, from CT segmentation to generating the cutting guide STL files, typically requires 10 to 15 minutes. The guides improve surgical precision, resulting in better reconstruction fit and bone union; recent results have shown higher rates of union (75 per cent vs. 65 per cent) and lower rates of non-union (7.3 per cent vs 18.7 per cent) with VSP versus FHS [10.1002/hed.27759]. Our system is also currently being used by three other clinical sites in addition to VGH/UBC.

Conclusions

VSP involving the creation of patient-specific cutting guides has been shown to be a rapid process resulting in improved mandibular reconstructions and patient outcomes.

Outcome/Impact

The in-house VSP is a highly beneficial tool to optimize patient outcomes for head and neck cancers necessitating mandibular reconstructions.

KEYWORDS

mandibular reconstruction, virtual surgical planning, surgical cutting guides, oral cancer

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6. CHARACTERIZATION OF ESCAPE MECHANISMS FROM FOLFIRINOX-INDUCED SENESCENCE OF PANCREATIC TUMOUR CELLS

Bianca Bossé[1], Jordan Guillon[1], Gerardo Diez-Rodriguez[1] and Gerardo Ferbeyre

[1]CRCHUM

Pancreatic cancer is one of the deadliest cancers, with a five-year survival rate of 10 per cent in Canada. Few treatments are available for the advanced forms of the disease and FOLFIRINOX, the most widely used chemotherapy against this cancer, only confers a median overall survival of 11 months. The majority of patients that receive FOLFIRINOX respond to the treatment but develop chemoresistance. A better understanding of the mechanism behind this resistance can be useful to improve the treatment.

Methods

To investigate the mechanism responsible for the FOLFIRINOX resistance in pancreatic cancer cell (PDAC), we treated the PDAC cell lines KP4 and Panc-1 with FOLFIRINOX (5-fluoracil, oxaliplatin and irinotecan) and obtained FOLFIRINOX-resistant variants after repeated treatments. We performed transcriptomic and proteomic analysis on resistant and non-resistant cells to identified molecules and pathways implicated in chemoresistance.

Results

FOLFIRINOX treatment induces senescence in PDAC cells characterized by growth arrest, increase in the senescence associated ß-galactosidase, activation of the DNA damage response and secretion of inflammatory cytokines. Cells escape from this senescent arrest after around three weeks to generate escaper cell population. Killing senescent cells with senolytics prevented the escape, indicating that senescence is required to develop the chemoresistance stage. We compared the transcriptome of escapers with the parent sensitive cell line. GSEA analysis demonstrated a higher expression of molecules implicated in the PI3K-mTOR pathway in resistant PDAC cells. Interestingly, inhibition of mTOR increased the rate of senescence escape but the inhibition of S6K, a downstream effector of mTOR, significantly inhibited the escaping from senescence. Co-treatment with FOLFIRINOX and S6K inhibitor was necessary for delaying the escaping suggesting that the anti-senescence function of the S6 kinase occurs when cells are entering the senescence process.

Conclusions

Our results demonstrate that the S6K protein appears to be a key protein in the activation of mechanisms responsible for chemoresistance and its inhibition during treatment with FOLFIRINOX is sufficient to re-sensitize the cells to the chemotherapy.

Outcome/Impact

Our research opens doors for development of new therapy able to re-sensitize patients to FOLFIRINOX and to increase the effectiveness of already existing chemotherapy.

KEYWORDS

pancreatic cancer, FOLFIRINOX, senescence, escaping, drug resistance, S6K

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7. AN ANTIBODY-DRUG CONJUGATE TARGETING A TUMOUR-SPECIFIC GLYCOEPITOPE OF PODOCALYXIN FOR THE TREATMENT OF P53 ABNORMAL ENDOMETRIAL CANCER

Julyanne Brassard[1], Janet Wu[1], Diana Canals Hernaez[1], Michael R. Hugues[1], Pamela M. Dean[2], Dawn Cochrane[3], Spencer Martin[4], Farhia Kabeer[4], Jessica McAlpine[5], Calvin. D Roskelley[1,2], Kelly M. McNagny[2]

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Endometrial cancer (EC) can be segregated into four molecular subtypes using ProMisE (Proactive Molecular Risk Classifier for Endometrial Cancer) a classifier system developed by members of our research group. One molecular subtype regroups EC tumours with TP53 mutations or abnormal p53 protein expression (termed p53abn ECs). While p53abn EC tumours only represent 15 per cent of all EC cases, they account for 50 to 70 per cent of EC associated deaths indicating that there is an urgent need for new treatments.

Podocalyxin (Podxl) is a highly glycosylated sialomucin normally expressed on restricted cell types, such as endothelial cells and kidney podocytes. However, in cancer, Podxl expression is upregulated and this upregulation is associated with poor disease outcomes. We capitalized on the frequent dysregulation of glycosylation pathways in cancer to develop an antibody, PODO447, that targets a tumour-specific glycoepitope of Podxl. To assess its therapeutic potential, we coupled PODO447 to a chemotherapeutic agent and tested its efficacy as an antibody-drug conjugate (ADC). We previously demonstrated that the PODO447-ADC treatment can significantly reduce tumour growth and improve mice survival in patient-derived xenograft (PDX) tumour models of pancreatic and ovarian cancer. Interestingly, around 30 per cent of p53abn ECs expressed the PODO447 epitope. We, therefore, wanted to determine if the PODO447-ADC is an effective therapy for p53abn ECs.

Methods

We first needed to generate a PDX model of p53abn EC by transplanting fresh tumour tissue resected from a human p53abn EC tumour into NRG mice. To test the ADC efficacy, mice were segregated into control (Palivizumab-ADC) or treatment (PODO447-ADC) groups and treated twice a week by intravenous injection of four mg/Kg of the ADC for a total of five injections.

Results

We successfully generated a PDX model of p53abn carcinosarcoma expressing high levels of the PODO447 epitope. Using this PDX model, we observed a significant reduction in tumour growth and increased survival in PODO447-ADC treated mice compared to the control group.

Outcome/Impact

Our results indicate that the PODO447-ADC can effectively eliminate p53abn tumour cells in pre-clinical models and could eventually provide a new therapeutic option for p53abn EC patients.

KEYWORDS

podocalyxin, endometrial cancer, antibody-drug conjugate, patient-derived xenograft

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8. IMMUNE MODULATION IN HIPPO PATHWAY REGULATED BREAST TUMOUR MICROENVIRONMENTS

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Tumour microenvironment (TME) immune heterogeneity poses a challenge for immunotherapy success in breast cancer patients. Thus, understanding how breast cancers modulate their immune landscape remains an important task. The Hippo signalling pathway regulates tissue growth and is commonly deactivated in many cancers. However, whether Hippo deactivation influences breast cancer development and is responsible for modulating its immune landscape remains to be investigated.

Methods

To study this, I generated a mouse model of conditional Lats1/2 deletion and therefore Hippo deactivation. To induce mammary-specific Lats1/2 deletion, I performed mammary intraductal injections of Cre-expressing lentivirus into Lats1/2$^{floxed/floxed}$ mice, alongside LSL-Pik3ca^{H1047R} mice for Pik3ca activation as a known oncogenic driver and LSL-Pik3ca^{H1047R}-Lats1/2$^{floxed/floxed}$ mice. To study the cellular changes associated with Hippo-regulated mammary tumour initiation, the microenvironments of early lesions were analyzed using imaging mass cytometry of a 40-marker mouse epithelial and immune antibody panels and a custom, spatially-resolved, lesion-specific, single-cell analysis pipeline.

Results

I showed that while most Lats1/2-deleted lesions were resolved and those that did form tumours had longer latencies compared to Pik3ca-activated lesions (400 and 200 days, respectively), Pik3ca-activated; Lats1/2-deleted lesions showed extremely rapid tumour onset within 60 days. Lats1/2-deleted and Pik3ca-activated; Lats1/2-deleted lesions displayed loss of normal luminal-basal integrity and infiltration of diverse immune cell types. Next, using spatial transcriptomics and an ***in vivo*** CRISPR screening approach, we are currently investigating lesion-specific drivers involved in these epithelial changes and their associated recruitment of specific immune cell types.

Conclusions

Hippo pathway deactivation in the mammary gland induced epithelial changes that led to tumours with increased aggressiveness. This was coupled by the presence of an immune "hot" TME. However, what is still unclear and what we are currently investigating is how the tumours are able to circumvent the increased immune challenge.

Outcome/Impact

Altogether, this study will allow us to decipher Hippo-regulated factors involved in immune modulation that play a role in mitigating or accelerating mammary tumourigenesis. This will reveal Hippo pathway mediators that can be targeted to improve anti-tumour responses and immunotherapy success in breast cancer patients.

KEYWORDS

breast cancer, Hippo pathway, immune system, spatial biology

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9. INTERFERON-SIGNALLING NEIGHBOURHOODS WITHIN HUMAN CANCERS

Mathew Cormier[1], Diala Abd Rabbo[1], Wenxi Xu[1] , Giselle Boukhaled[2], David Brooks[1,2,3]

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Interferons (IFNs) are important regulators of cancer cells and anP-tumour immunity. Perplexingly, the restoration of IFN signalling is associated with the efficacy of immunotherapies as well as immune dysfunction, which leads to tumour progression. Identifying which cells respond to IFNs in the tumour and understanding how this signalling leads to divergent immune outcomes will shed light on how anti-tumour immune responses are regulated in the context of immune therapies.

Methods

To better understand the contrasting roles of IFN signalling in human cancer, we probed the enrichment of interferon-stimulated genes (ISGs) in publicly available single-cell RNA sequencing and spatial transcriptomic data. Sections of human tumours were analyzed by immunohistochemistry and immunofluorescence.

Results

There are high levels of IFN signalling present in select cells across multiple immune and non-immune cell subsets. This phenotype was also present in different cancer types, as demonstrated by the quantification of IFN-stimulated proteins (ISPs) by mass cytometry. Strikingly, the infiltrating immune cells of patients who had developed resistance to immune therapies showed higher levels of IFN signalling prior to therapy when compared to those of patients who responded to therapy. However, these findings were not specific to any one cell type, suggesting that cells exhibiting high levels of IFN signalling may be spatially localized within the tumour. In fact, the analysis of ISP expression by tumour imaging and ISG expression in spatial transcriptomic data revealed localized niches of high IFN signalling which dissipated to regions of minimal IFN signalling. Interestingly, these "IFN neighbourhoods" contained both immune and non-immune cells.

Conclusions

These data reveal that there are localized neighbourhoods of high IFN signalling within human tumours.

Outcome/Impact

A continuation of this work will provide an understanding of how the spatial localization of IFN neighbourhoods influences anti-tumour immune responses and how this biology may be targeted to enhance immunotherapy efficacy.

KEYWORDS

spatial biology, melanoma, interferons, immunotherapy

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10. MOLECULAR TRIAGE OF SUSPECTED NEUROENDOCRINE LUNG CANCER LESIONS THROUGH MICRORNA-BASED LIQUID BIOPSY

Simona Damiani[1], Harrison Mayotte[1], Yousif Al-Aboosi[1], Alexis Fang[1], Dong Yao Wang[2], Xiaojing Yang[1], Kathrin Tyryshkin[1,3], Geneviève Digby[2], Neil Renwick[1]

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Lung neuroendocrine neoplasms (NENs) are a heterogenous group of increasingly common tumours and cancers that are difficult to diagnose. microRNAs (miRNAs, miRs) are small regulatory RNA molecules that are excellent biomarkers due to their abundance, disease-stage specificity and stability in tissues and biofluids. Based on our previous finding that miR-375 is higher in lung NEN compared to non-NEN tissues (Nanayakkara ***et al.***, ***NAR Cancer*** 2020), we hypothesized that plasma miR-375 levels are higher in individuals with lung NENs compared to non-NEN controls.

Methods

We generated platelet-depleted plasma from 84 patients [21 per diagnostic category: lung NEN; squamous cell carcinoma (SCC); lung adenocarcinoma (LAD); and non-neoplastic lung disease (NNLD)] who were recruited through the Lung Diagnostic Assessment Program, Kingston Health Sciences Centre. Following RNA isolation, we quantitated miR-375 and spike-in control ath-miR-159a abundance using miRNA real-time PCR and calculated their relative abundance. Differences in normalized miR-375 abundance between diagnostic categories were analyzed using the Kruskal-Wallis test and Dunn's multiple comparisons test.

Results

Plasma miR-375 levels are higher in lung NEN patients (-7.8±2.7) compared to patients with lung SCC (-11.9±1.4, $p=3.8 \times 10^{-6}$), LAD (-12.0±1.7, $p=3.6 \times 10^{-6}$) or NNLD (-12.0±1.5, $p=4.6 \times 10^{-6}$). Results are expressed as mean ± standard deviation; p-value <0.05 is considered significant.

Conclusions

Preliminary analyses indicate that plasma miR-375 is significantly more abundant in patients with lung NENs compared to controls. Next steps include determining inter-rater reliability within the test cohort described above and assessing generalizability in a separate validation cohort. As well, assessing other miRNA and non-miRNA targets to expand utility of liquid biopsies for lung NEN detection.

Outcome/Impact

This research will lay the foundation for a rapid and inexpensive diagnostic blood test that reduces barriers to diagnosis and accelerates time-to-treatment for patients with lung NENs.

KEYWORDS

lung cancer, neuroendocrine neoplasms, microRNA, liquid biopsy

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11. EXPLORING THE ROLE OF THE ACETYLTRANSFERASE TIP60 (KAT5) DURING DNA DAMAGE-INDUCED CELL FATE DECISIONS

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Senescent cells are characterized by multiple senescence-associated (SA) phenotypes including a pro-inflammatory secretory phenotype (SASP). Multiple signalling pathways contribute to the regulation of SASP factors including the DNA-damage response (DDR), which can modulate the activity of NF-kB, the master transcription factor controlling the SASP. TIP60 (KAT5) can acetylate multiple histone and non-histone proteins involved in the DDR, DNA repair, p53 signalling, NF-kB activity and general transcription.

Our objective is to test the idea that TIP60 modulate a dynamic cell fate decision program in response to DNA damage, which include a choice between senescence and apoptosis. We will clarify the regulation of senescence, particularly SASP and other senescence hallmarks by TIP60 in the context of DDR. To do so we will combine targeted and unbiased strategies in controlled contexts in response to DNA damage to allow the temporal separation of TIP60 regulation mechanism.

Methods

Using lentiviruses, we have overexpressed and genetically depleted (shRNA) TIP60. Senescence was induced using irradiation (DNA damage). We have characterized SA phenotypes including proliferation arrest using live-imaging, SA-beta-galactosidase activity, genomic instability and persistent DNA Damage Foci (DDF) using immunofluorescence of 53BP1/γ-H2AX, telomeric DDF (Telomeric FISH) and SASP (ELISA for Il-6/Il-8). We have also performed transcriptome and functional analysis to understand the mechanism involved in TIP60 regulation under the influence of senescence and DDR.

Results

We find that global histone acetylation decreases as cells enter senescence. We have observed that the genetic depletion of TIP60 has a negative impact on IL-6 secretion and others SASP factors (IL-8, MCP-1 and IL-1), genomic instability and SAPA but increased 53BP1/γ-H2AX accumulation induced by irradiation. We find opposite effects on SASP, SAPA and DDR when TIP60 is overexpressed.

Conclusions

Our results reveal the DDR regulator TIP60 as a novel modulator of cell senescence including downstream cellular functions like SASP. This connects genome stability to tissue-wide microenvironmental responses.

Outcome/Impact

Overall, our data refine the specific SASP regulatory network and elucidate new ways to manipulate senescence phenotypes, which can further be employed to generate therapeutics targets for cancer treatment response and aging-related pathologies.

KEYWORDS

TIP60, KAT5, apoptosis, secretome, senescence, acetyltransferases

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12. IRF5 DEFINES A NEW HIGH-RISK INFLAMMATORY T-LINEAGE ACUTE LYMPHOBLASTIC LEUKEMIA SUBTYPE

Gibran Edun, Mark Gower, Ximing Li, Alicia G. Aguilar-Navarro, Brian Lin, Minerva Fernandez, John E. Dick, Gregory Schwartz, Johann Hitzler, Tanya A. Gruber, Mark Minden, Courtney Jones, Igor Dolgalev, Soheil Jahangiri, Anastasia N. Tikhonova

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T-lineage acute lymphoblastic leukemia (ALL) is an aggressive cancer arising from distinct blocks in T cell development. T cell ALL (T-ALL), Early T cell precursor ALL (ETP-ALL) and T/Myeloid Mixed phenotype acute leukemia (T/My-MPAL) represent three distinct subtypes of T-lineage ALL, defined by their stages in developmental block. The clinical diagnosis of T-lineage ALL relies heavily on immunophenotyping techniques, which can face challenges due to phenotypic inconsistencies across subtypes. However, precise diagnosis is crucial because drug sensitivity in preclinical models of T-lineage ALL is closely tied to differentiation state, underscoring the critical necessity of identifying consistent phenotypic traits associated with unique therapeutic vulnerabilities.

Methods

To gain insights into subset-specific therapeutic vulnerabilities and potential therapeutic targets, we conducted an integrative multiomic analysis of bone marrow samples from 40 newly diagnosed T-lineage ALL patients, including T cell ALL, ETP-ALL, and T/My-MPAL. We investigated the heterogeneity of the transcriptomic landscape in our T-lineage ALL cohort and identified a distinct subset of patients characterized by activation of stem and inflammatory gene program. Next, we employed single-cell regulatory network inference and clustering (SCENIC), to identify regulatory networks differentially regulated between these subsets. Finally, to determine the therapeutic vulnerability of these subtypes, we performed ***in vitro*** and ***ex vivo*** drug sensitivity assays, and ***in vivo*** PDX treatments.

Results

We identified a subset of patients from all three subtypes transcriptionally resembling more stem-like states. Furthermore, this subset was distinguished by activation of inflammatory gene programs and exhibited distinct biological features, including the production of pro-inflammatory cytokines, prevalence of mutations affecting cytokine signalling and chromatin remodelling, poor treatment responses and expression of interferon regulatory factor 5 (IRF5). Strikingly, we found that while $IRF5^{HIGH}$ samples were significantly less sensitive to dexamethasone, they exhibited unique sensitivity to a BCL2 inhibitor, venetoclax. To facilitate T-lineage ALL patient classification, we developed a computational IRF5 scoring system, which effectively stratifies patients and predicts disease prognosis in three additional patient cohorts.

Outcome/Impact

By identifying high-risk T-lineage ALL patients based on IRF5 activity, our study provides a framework for targeted therapeutic approaches in these challenging-to-treat cancers.

KEYWORDS

interferon regulatory factor 5 (IRF5), t-lineage acute lymphoblastic leukemia, inflammatory program

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13. CHRONIC HYPOXIA DRIVES EPIGENETIC REPROGRAMMING AND ACQUISITION OF AGGRESSIVE BEHAVIOUR IN EWING SARCOMA

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Ewing Sarcoma (EwS) is an aggressive bone and soft tissue sarcoma primarily affecting children, adolescents and young adults. Despite intensive efforts, the survival rate for EwS patients with metastatic disease remains dismal at 15 to 20 per cent, which has not changed for decades. Current treatments for EwS rely on chemotherapy regimens and radiation that often cause developmental defects. We have previously shown that hypoxia in the tumour microenvironment may play a role in EwS metastasis, but the mechanism remains elusive.

Methods

In this study, we assessed effects of chronic hypoxia on epigenetic and transcriptomic profiles in EwS cells, which were exposed to chronic hypoxia for 30 days and then returned to either normoxia or continued hypoxic conditions for a further three days. Cells were then assessed by chromatin immunoprecipitation (ChIPseq) and RNAseq to determine the activating and repressive histone modifications and gene expression profiles, respectively. Results were then used to determine the molecular pathways that were persistently upregulated even after cells were returned to normoxic conditions.

Results

Our preliminary results identified several cellular pathways that are upregulated in EwS cells in response to extended periods of hypoxia *in vitro*. These expression changes were accompanied by elevated H3K4me3 and/or H3K27ac activating histone marks and loss of repressive H3K27me3 marks. Intriguingly, several of these alterations persisted 72h after removal of the hypoxic stress and a return to normoxia, including ribosomal biogenesis, oxidative phosphorylation, cell cycle control, DNA damage and vesicle organization pathways.

Conclusions

Based on our results, we hypothesize that extended exposure to hypoxia allows for selection of EwS cells with adaptive epigenetic and transcriptomic modifications that confer increased cellular fitness in the tumour microenvironment. These resistant cells have increased metabolic plasticity and proliferative capacity and are likely to give rise to treatment resistant cell clones.

Outcome/Impact

Our study indicates that low oxygen tension in the tumour microenvironment leads to persistent pathway alterations in EwS cells, even after removal of hypoxic stress, likely due to epigenetic reprogramming. This epigenetic memory is likely important in the metastatic phenotype of EwS and possibly other aggressive cancers.

KEYWORDS

Ewing sarcoma, metastasis, oxygen tension, tumour microenvironment

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14. EVALUATING INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN 3 (IGFBP3) AS A NOVEL DRUG TARGET IN HEAD AND NECK SQUAMOUS CELL CARCINOMA (HNSCC)

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Head and neck cancer is one of the deadliest and most prevalent cancers with rising incidence and limited treatment options. Given that the Notch tumour suppressive pathway is inactivated in approx. 61 per cent of patients, I performed an ***in vivo*** CRISPR-Cas9-based dropout screen in Notch-mutant autochthonous models of HNSCC to identify novel therapeutic gene targets.

Methods

Our group has developed HNSCC mouse models that integrate multiplexed *in vivo* CRISPR gene editing to test the transforming potential of hundreds of genes in a single mouse. Using ultrasound-guided microinjections, lentiviruses encoding the Cre recombinase and sgRNAs (LV-Cre-sgRNA) are delivered directly into the single-layered surface ectoderm of Lox-Stop-Lox-(LSL)-Pik3ca^{H1047R};Notch1$^{fl/fl}$;Cas9 mouse embryos. Activation of Pik3ca^{H1047R} and loss of *Notch1* results in HNSCCs. Using this model, I screened 600 Notch1 target genes that are de-repressed upon NOTCH signalling inactivation and identified Insulin-like growth factor binding protein 3 (IGFBP3) as the top hit essential for the selective proliferation of transformed cells. To further validate this finding, I performed IGFBP3-inhibition *in vitro* and *in vivo* assays.

Results

Overexpression of IGFBP3 triggered SCC development in Pi3Kca^{H1047R} Cas9, while vector-only control injected mice remained tumour-free, indicating that IGFBP3 both necessary and sufficient to drive SCC formation in ***Pik3ca***^{H1047R} mice.

Additionally, genetic ablation of IGFBP3 in NOTCH-mutant ***Pi3Kca***^{H1047R} Cas9 mice led to a significant extension in tumour-free survival. Furthermore, treatment with the IGFBP3 inhibitor NIB31772 resulted in wide-spread tumour necrosis and a dramatic extension of life in tumour-bearing NOTCH-mutant ***Pi3Kca***^{H1047R} mice. Inhibition of IGFBP3 also decreased human HNSCCs cell proliferation. Finally, NIB31772 administration in four Patient-Derived Xenograft (PDX) models effectively blocked tumour growth only in "predicted responders" Notch-mutant tumours and not in Notch-wild-type ones.

Conclusions

IGFBP3 is a key driver and promising therapeutic target in HNSCC.

Outcome/Impact

In vivo dropout screening can determine novel and biologically relevant therapeutic cancer targets and pave the way for drug discovery focusing on IGFBP3 in HNSCC.

KEYWORDS

head and neck squamous cell carcinoma, Notch pathway, ***in vivo*** SCRISPR dropout screening, IGFBP3

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15. INVESTIGATION OF *HLTF* LOSS IN PATHOGENESIS OF ACUTE MYELOID LEUKEMIA

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HLTF is a SWI/SNF gene involved in post-replication DNA repair. Our preliminary data analysis shows reduced *HLTF* expression in AML samples compared to healthy blood CD34+ cells. We investigate the role of *HLTF* as a tumour suppressor gene in Acute Myeloid Leukemia (AML) pathogenesis.

Methods

We use comet assays, gamma-H2AX detection assays and metaphase spreads to investigate genomic changes induced by *HLTF* loss. AML patient RNA-seq data across four cohorts (TCGA, PMP, Beat AML, Leucegene) was analyzed to investigate signalling and mutation signatures in low *HLTF* patients. Annexin-V and Ki67 expression was detected using flow cytometry to investigate cell death and changes in proliferation respectively.

Results

HLTF knockdown (KD) AML cell lines show increased double-strand breaks, chromosomal breakages and end-to-end fusions. Despite increased DNA breakage, *HLTF* KD cells do not show increased cell death or cell cycle arrest, compared to control (CTR) KD cells. AML patient samples with reduced *HLTF* expression had a significantly higher number of chromosomal fusions compared to patients with high *HLTF* expression. Patients bearing specific clinically relevant AML fusions also had a significantly lower *HLTF* expression compared to normal karyotype patients.

KEYWORDS

AML, ***HLTF***, DNA damage

Conclusions

HLTF loss induces significant genomic instability while mitigating the responses to genomic instability i.e., apoptosis and cell-cycle arrest. We suggest that *HLTF* acts as a tumour suppressor gene, loss of which potentially induces leukemogenic chromosomal translocations and promotes survival and expansion of these mutated cell clones.

Outcome/Impact

Chromosomal translocations drive oncogenesis in hematopoietic cancers via formation of chimeric proteins or dysregulating expression of oncogenes or tumour suppressor genes. However, the molecular conditions preceding the formation of these chromosomal aberrations are not well understood. Here we aim to elucidate the involvement of *HLTF* loss as a preliminary step that facilitates the formation of these chromosomal aberrations.

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16. INVESTIGATING THE ROLE OF CHD4 IN THE MTOR/AR TRANSCRIPTIONAL CROSSTALK IN PROSTATE CANCER

Lingwei (Stephanie) Han[1,2], Catherine R. Dufour[1], Anthony Alfonso[1,2], Megan Katz[1,2], William Pastor[2], Vincent Giguère[1,2]

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Prostate cancer (PCa) is the third leading cause of cancer deaths in Canadian men. Its progression is largely regulated through androgen acting on the androgen receptor (AR), leading to a global change in gene transcription. Mammalian target of rapamycin (mTOR) is another key factor in PCa progression and emerging studies revealed that mTOR directly regulates gene expression by associating with chromatin in the nucleus (nmTOR).

However, the detailed mechanism on how nmTOR regulates gene expression with AR in PCa remains unclear. Recently our group uncovered a nmTOR-AR functional crosstalk in PCa cells where upon androgen stimulation, nmTOR associates with chromatin and activates an oncogenic metabolic gene program in an AR-dependent manner. Using a proteomic approach referred to as rapid immunoprecipitation mass spectrometry of endogenous protein (RIME), we previously identified the nucleosome remodelling and deacetylase (NuRD) complex as a partner of nmTOR-AR bound on chromatin in PCa cells. The NuRD complex is known to promote or suppress cancer progression through recruitment to tumour suppressors and oncogenes, however, its potential roles in PCa and involvement in the mTOR/AR crosstalk remains to be investigated.

Methods

Our study widely utilizes ChIP-seq, RNA-seq and ATAC-seq approaches to investigate the dynamic transcriptional crosstalk of mTOR, AR and CHD4 in prostate cancer cells.

Results

Our investigation reveals a dynamic transcriptional complex formed by CHD4, the catalytic subunit of NuRD, nmTOR and AR at pre-accessible regions in a time-dependent manner upon androgen stimulation. The depletion of CHD4 triggers a widespread transcriptional rewiring in PCa cells, impacting a significant portion of androgen-response genes.

Outcome/Impact

This underscores the novel role of CHD4 in orchestrating gene expression in PCa and its interplay with mTOR and AR, leading to a deeper understanding of prostate cancer development.

KEYWORDS

mTOR, AR, CHD4, PCa

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17. INCREASED SENSITIVITY TO FERROPTOSIS AND EMT IN BREAST CANCER CELLS SURVIVING APOPTOSIS

Rachel Hausman[1,2], Wells Brown[2], Paul McDonald[2], Shannon Awrey[2], Shoukat Dedhar[1,2]

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Survival of cancer cells post-chemotherapy is a barrier to effective treatment. Most chemotherapeutics kill cells by apoptosis, and apoptotic resistance can cause recurrent tumours. Executioner caspase release was previously thought to be the point of no return from apoptotic cell death. However, it has been shown that removal of the reagent causing caspase release can lead to recovery of cells from caspase activation, a phenomenon which has been termed "Anastasis". Further investigation is needed to probe surviving anastatic cells and identify vulnerabilities to be targeted by potential novel therapeutics.

Methods

To identify and enrich for anastatic breast cancer cells, we used the CasExpress biosensor to permanently label cells surviving executioner caspase activation with GFP. This system was used to identify and isolate a population of anastatic cells surviving caspase-3 activation resulting from chemotherapeutic stress.

Results

Downstream analysis demonstrated that anastatic cells have increased resistance to chemotherapy, decreased active caspase-3, a mesenchymal phenotype with up-regulation of transcriptional regulators ZEB-1 and Snail, decreased GPX4 expression and increased sensitivity to ferroptosis. Inhibition of epithelial to mesenchymal transition (EMT) by targeting ZEB-1 rescued GPX4 expression and reduced sensitivity to ferroptosis. To determine if EMT plays a mechanistic role in ferroptosis sensitivity, we induced EMT in NMe mouse mammary epithelial cells using TGF-ß. This EMT induction lead to a synergistic decrease in GPX4 expression and an increase in ferroptosis sensitivity.

Conclusions

Together, these data suggest a link between apoptotic survival of chemotherapy treated cells, ferroptosis sensitivity and EMT. Current investigations are focused on elucidating the mechanisms behind this vulnerability to ferroptosis, acquisition of a more mesenchymal phenotype and apoptotic survival.

Outcome/Impact

Recurrent tumours are more aggressive and difficult to treat. By identifying surviving anastatic cells and pathways involved in their evasion of cell death, we hope to identify novel therapeutic strategies to prevent their survival and overcome resistance.

KEYWORDS

drug resistance, anastasis, EMT, ferroptosis

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18. CELL-OF-ORIGIN EPIGENOME UNDERLIES SS18-SSX-MEDIATED TRANSFORMATION

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Synovial sarcoma is an aggressive soft-tissue malignancy that is characterized by a pathognomonic t(X;18)(p11.2;q11.2) translocation, which produces a fusion ***SS18-SSX*** oncogene. Despite recent advancements in our understanding of synovial sarcoma biology, the cell-of-origin remains undefined.

Methods

A mesenchymal stromal cell (MSC) specific, *Hic1*^{CreERT2}, line was used to drive expression of SS18-SSX in fibroblasts and related cell types. Multi-omics analyses, including scRNA-, scATAC- and ChIP-seq, were utilized to study the trajectory of the cells through the transformation process. In addition, we performed single cell spatial transcriptomics (Xenium, 10X Genomics) on normal and tumour-laden tissue, using a probe set that consisted of the pre-designed mouse brain panel and a custom add-on of 100 genes, selected based on scRNA-seq data.

Results

The novel mouse model exhibited 100 per cent penetrance in synovial sarcoma formation, with a median latency period of 16.2 ± 2.5 weeks. Murine tumours exhibited high concordance with human synovial sarcoma sub-types at the histological and omics levels. Genetic refinement of the cell-of-origin revealed that synovial sarcomas derive from a rare Hic1^{+} Pdgfra^{+} Lgr5^{+} fibroblastic population. Multi-omic profiling along the transformation continuum elucidated the step-wise acquisition of a transformed phenotype, initiated by the loss of a mature fibroblastic profile and subsequently, the gradual unmasking of an epigenetically embedded embryonic MSC program, including a neurodevelopmental transcriptional signature. SS18-SSX recruitment to developmental H2AK119ub marked loci culminated in the widespread loss of H3K27me3 at bivalently marked genes, and their transcription. Spatial transcriptomic analyses revealed the presence of clusters representing normal epithelial, stromal and neural populations, along with multiple clusters with tumour specific signatures. Notably, several tumour related subpopulations emerged that were mapped to specific histological features; monophasic cells were defined by *Nkd2* expression, *Epcam*+ *Sall3*+ cells organized into glandular structures and poorly differentiated cells were enriched for *Sall3* and *Pax2*.

Conclusions

Collectively, these studies define a rare MSC context, conducive for SS18-SSX-mediated transformation, and demonstrate that synovial sarcoma tumourigenesis involves induction and maintenance of an embryonic-like MSC phenotype.

Outcome/Impact

By better understanding the mechanisms of action of synovial sarcoma, the ultimate goal is to develop novel therapeutic strategies.

KEYWORDS

synovial sarcoma, mouse model, multi-omics, spatial transcriptomics

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19. SEX DIFFERENCES PRIMARILY ALTER PRIMITIVE HAEMATOPOIETIC PROGENITORS IN WILDTYPE AND GENETICALLY MODIFIED MICE

Samantha Holmes, Amy McNaughton, Christopher Wells, Michael Rauh, Sheela Abraham

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Understanding the regulation of haematopoietic stem and progenitor cells (HSPCs) is vital for deciphering haematopoiesis and its dysregulation in hematological diseases. DNMT3A and TET2 serve as pivotal epigenetic modulators identified in clonal haematopoiesis and other diseases like acute myeloid leukemia (AML), myelodysplastic syndromes (MDS) and lymphomas, highlighting their significant role in HSPC regulation. We are interested in deciphering how mutations in DNMT3A and TET2 alter HSPC behaviour.

Methods

In this study, we used DNMT3A and TET2 knockout (KO) mice to characterize the impact of these mutations on HSPC populations using flow cytometry. Bone marrow cells were harvested from femurs, tibias and iliac crests of DNMT3A and TET2 KO mice, age-matched wildtype and floxed controls. HSPC populations were identified based on the expression of Sca-1, c-Kit (CD117), CD150, CD48, CD16/32 and CD34.

Results

Flow cytometric analysis uncovered alterations in HSPC populations, suggesting a connection between sex and genetic modifications. Our findings revealed sex differences in Lin− Sca1+ cKit+ (LSK) cells among wildtype C57BL/6 mice, along with a notable increase in long-term HSCs (LT-HSCs) in DNMT3A KO mice, consistent with literature. We also confirmed previous reports of increased multipotent progenitors (MPPs) in TET2 KO mice, and a decrease in DNMT3A KO MPPs, highlighting divergent regulatory mechanisms in these transgenic models. Interestingly, our results did not reveal an increase in LSK cells in the KO models as previously published, however male mice exhibited drastically higher LSK percentages across both TET2 and DNMT3A genotypes. Finally, when analyzing differentiated progenitor populations, both DNMT3A and TET2 KO mice displayed increased megakaryocyte-erythroid progenitors (MEPs) with a decrease in common myeloid progenitors (CMPs) compared to their wildtype counterparts.

Conclusions

Our findings underscore the complex interplay between sex and genetic modifications in shaping HSPC populations, revealing sex biases, ultimately leading to distinct alterations in HSPC populations.

Outcome/Impact

These results serve as a valuable guideline for designing experiments involving genetically modified mice by highlighting the significant impact of genetic modifications on HSPCs, while providing insights into the pathogenesis of hematological malignancies associated with DNMT3A and TET2 mutations.

KEYWORDS

haematopoietic stem cells, epigenetic modifiers, DNMT3A, TET2

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20. DIFFERENCE IN PULMONARY MICROBIOME BETWEEN NON-SMALL CELL LUNG CANCER PATIENTS AND CANCER-FREE PATIENTS

Yongjia Hu[1], Babacar Mbaye[1], Meriem Messaoudene[1], Myriam Benlaifaoui[1], Diogjena Katerina Prifti[1], Jade Maillou[1], Sreya Duttagupta[1], Arielle Elkrief[1,2], Julie Malo[1], Mayra Ponce[1], Pedro Guimaraes Rocha Lima[2], Thomas Vandemoortele[2], Moishe Liberman[2], Bertrand Routy[1,2]

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The gut microbiome has emerged as a key determinant in the development of various cancers. However, the role of the lung microbiome in non-small cell lung cancer (NSCLC) development remains unclear. In this study, we investigate the role of the lung microbiome in NSCLC oncogenesis by coupling lung microbiome profiling techniques and culturomics in both NSCLC and cancer-free patients.

Methods

We collected 48 bronchoalveolar lavage (BAL) samples at the CHUM endoscopy suit and performed 16S rRNA sequencing on 45 samples. In addition, we conducted extensive culturomics using Columbia agar with five per cent sheep blood plate at 37 °C under both aerobic and anaerobic conditions. Bacteria were isolated and identified using MALDI-TOF. Diversity and LEfSe bacterial difference between NSCLC and cancer-free patients were analyzed. Next, intravenous injection of B16F10 cell line was performed with treatment of dual immune checkpoint blockade (ICB) as well as anti-LAG3. Antibiotic (ATB) solution containing ampicillin, streptomycin and colistin was administered to mitigate the impact of microbiome.

Results

16s rRNA results revealed no difference in lung microbiome diversity indexes between NSCLC and cancer-free patients. At the genus level, Staphylococcus and Veillonella were numerically increased in NSCLC patients. Next, we isolated an average of 26 bacteria using culturomics, but we did not observe any difference in the number of bacteria under either aerobic or anaerobic conditions. Moreover, there was a numerical increase in Staphylococcus as well as Prevotella melaninogenica in NSCLC patients compared to cancer-free patients. To elucidate the role of the lung microbiome in murine cancer model, we performed preliminary experiments and demonstrated that combination of anti-CTLA-4 and anti-PD-1 as well as anti-LAG3 significantly inhibited B16F10 lung metastases. Notably, this anti-tumour efficacy was abrogated by oral intake of ATB.

Conclusions

Our preliminary results showed that patients with NSCLC exhibit an enrichment of distinct bacteria compared to cancer-free patients. Using our murine tumour models, we plan to further demonstrate the critical role of the lung microbiome in ICB response.

Outcome/Impact

Unravelling the key role of the lung microbiome in NSCLC development and response to ICB.

KEYWORDS

lung microbiome, non-small cell lung cancer, immune checkpoint blockade, culturomics

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21. COMPARING *IN VIVO* PORPHYSOME QUANTIFICATION USING DIFFUSE OPTICAL SPECTROSCOPY AND T1 MAPPING

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Porphysomes are liposome-like nanoparticles that hold promise as theranostic agents for cancer imaging and light-based cancer therapy. They exhibit structure-dependent optical properties, wherein they are either intact and inert, or disaggregated and activated.

In intact form, the porphyrin-lipid subunits are densely packed, quenching their porphyrin fluorescence and passively accumulating in tumours through the enhanced permeability and retention effect. As the porphysomes are processed within the tumour they enter their dissociated form, unquenching fluorescence and photoactivity, enabling porphysome mediated photodynamic therapy (PDT). However, a major obstacle in delivering porphysome PDT is that no means exists of measuring porphysome concentration ***in vivo*** that distinguishes between intact and dissociated states, which is a key parameter for PDT dosimetry.

Methods

A handheld fiber optic probe has been developed that uses diffuse reflectance spectroscopy (DRS) and quantitative fluorescence spectroscopy (QFS) to measure ***in vivo*** concentrations of intact and dissociated porphysomes. DRS exploits the structure-dependent absorption spectra of porphysomes to distinguish and quantify each state, while QFS is employed as a more sensitive measure of dissociated porphysome concentration. The spectroscopic system was evaluated in a pilot study through comparison against T1 mapping of gadolinium-labelled porphysomes administered to a set of 5 mice with MOC2 xenograft tumours in their tongues. T1 maps of each mouse tongue were captured with a 7T MRI and with the DRS/QFS system at time points immediately before, and 5, 25, 50 and 72 hours post injection.

Results

Strong contrast of 24 ± 4.2 per cent was observed between the tumour pre injection and 24 hours post injection in T1 map. Agreement between total porphysome concentration measured with DRS/QFS and MRI showed marginal agreement, with the probe overestimating porphysome concentration by a factor of 2.2 on average. However, pharmacokinetics measurements were closer in agreement: the rate of tumour PS clearance after peak concentration was 0.1 ± 0.01 μM/hr measured by T1 map, 0.16 ± 0.19 μM/hr measured by DRS.

Conclusions

Further refinements are necessary to improve accuracy, but DRS/QFS offers distinct benefits over other quantitative modalities like PET and T1 mapping, including the ability to distinguish between intact and dissociated porphysomes, it's compact portable form and measurement speed, taking only several seconds.

Outcome/Impact

The DRS/QFS system is an enabling technology for porphysome PDT. It also offers utility as an aid during porphysome fluorescence-guided resection of tumours.

KEYWORDS

porphysomes, quantitation, diffuse reflectance spectroscopy, quantitative fluorescence spectroscopy, instrumentation

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22. RISK PREDICTION MODELS FOR LUNG CANCER IN PEOPLE WHO HAVE NEVER SMOKED: A SYSTEMATIC REVIEW

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With the increasing proportion of people who have never smoked among lung cancer cases, there is a pressing need to develop prediction models to identify high-risk people who have never smoked and include them in lung cancer screening programs. The present systematic review is intended to provide a comprehensive summary of the evidence on existing risk prediction models for lung cancer in people who have never smoked.

Methods

Electronic searches, unrestricted by language, were conducted in MEDLINE (Ovid), Embase (Ovid), Web of Science Core Collection (Clarivate Analytics), Scopus and Europe PMC and Open Access Theses and Dissertations databases to retrieve relevant results from their inception to 14 November 2023. The review is ongoing: two reviewers will independently perform title and abstract screening, full-text review and data extraction using the Covidence review platform. Data extraction will be performed based on the Checklist for Critical Appraisal and Data Extraction for Systematic Reviews of Prediction Modelling Studies (CHARMS). The risk of bias will be evaluated independently by two reviewers using the Prediction model Risk of Bias Assessment Tool (PROBAST) tool. If a sufficient number of studies are identified to have externally validated the same prediction model, we will combine model performance measures, using a random-effects approach, to evaluate the model's average predictive accuracy (e.g., calibration, discrimination) across diverse settings and populations and explore sources of heterogeneity.

Results

The review protocol has been registered in PROSPERO under the registration number CRD42023483824. From 25,302 identified reports, 6,199 duplicates were removed.

Conclusions

The results of the review will identify risk prediction models and will contribute to a better understanding of risk factors for lung cancer in people who have never smoked.

Outcome/Impact

These will be useful for researchers planning to develop novel prediction models, and for clinical practitioners and policy makers seeking guidance for clinical decision-making and the formulation of future lung cancer screening strategies for people who have never smoked.

KEYWORDS

lung cancer, prediction model, never smokers, systematic review

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23. PRE-TREATMENT PREDICTION OF BREAST CANCER RESPONSE TO NEOADJUVANT CHEMOTHERAPY USING MR AND CT RADIOMICS

Deok Hyun Jang[1,2], Christopher Kolios[1], Lakshmanan Sannachi[1,3], Laurentiusoscar Osapoetra[1,3], Gregory Czarnota[1,3]

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Early prediction of breast cancer response to neoadjuvant chemotherapy has the potential to enable adaptive therapy and ultimately improve the survival outcome of the patients. The aim of this study is to predict the response of breast tumours to NAC based on patients' clinical characteristics and radiomic analysis of pre-treatment magnetic resonance imaging (MRI) and computed tomography (CT) data.

Methods

Pre-contrast T2-weighted and post-contrast T1-weighted MR images were collected from 256 patients with breast cancer. In addition, CT images of 176 patients from the same cohort were collected. Intratumoural and peritumoural regions were segmented from the three image sets. Shape-based features, first-order features and second-order (texture) features were extracted for each segmentation. The response classification model was trained with a clinical feature set, a radiomic feature set and a combined feature set using XGBoost classifier and 10 iterations of nested cross-validation were employed for model evaluation. In this study, two response criteria were assessed: pathologic complete response (pCR) vs. non-pCR and response vs. non-response.

Results

The best classification performance was observed when clinical features and radiomic features were combined for both modalities and for both response criteria. With MR radiomic and clinical features from 256 patients, AUC of 0.86 and 0.76 were achieved for pCR vs. non-pCR criterion and response vs. non-response criterion, respectively. With CT radiomic and clinical features from 176 patients, AUC of 0.83 and 0.71 were achieved for pCR vs. non-pCR criterion and response vs. non-response criterion, respectively.

Conclusions

Machine learning analysis of the clinical information and radiomic features obtained from pre-treatment CT and MR images, may be a useful clinical tool for breast cancer response prediction.

Outcome/Impact

Radiomic data acquired from pre-treatment MRI and CT, which are readily acquired during the standard treatment process, complement the clinical information in prediction of breast cancer response to NAC.

KEYWORDS

breast cancer, neoadjuvant chemotherapy, MRI, CT, radiomics

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24. UNCOVERING THE ROLE OF LONG NON-CODING RNA *LNC-35682/PAN3-AS1* IN ACUTE MYELOID LEUKEMIA

Zhen Jin[1,2], Zongmin Liu[1,2], Yilin Liu[2], Jennifer Grants[3], Leo Escano[2], Maryam Ghashghaei[1,2], Kyle McPherson[2], Sandra Spencer Miko[3], Glenn Edin[2], Florian Kuchenbauer[2], Gregg Morin[3], Aly Karsan[3], Ly P Vu[1,2]

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Acute myeloid leukemia (AML) is an aggressive blood cancer with a five-year survival rate under 30 per cent. Long non-coding RNAs (lncRNAs), defined as transcripts > 200nt without protein coding potential, has recently emerged as an important class of regulators of tumourigenesis. While there are more than 100,000 lncRNAs reported in human, most of them have not been functionally characterized. Insights into identities and mechanisms of action for lncRNAs can uncover novel pathways and targets for treatment of cancers.

Methods

We identified a lncRNA, *Lnc-35682*, which is expressed in mouse hematopoietic stem/progenitor cells and highly upregulated in leukemia stem cells (LSCs)-enriched population via transcriptome profiling of murine normal and leukemia cells. We generated a genetic mouse model of *Lnc-35682* germline knockout (KO) and evaluated requirement of *Lnc-35682* during normal and malignant hematopoiesis. Syntenic analysis revealed *PAN3-AS1* as the putative human homolog of *Lnc-35682*. Functional analyses were performed using lentivirus carrying shRNA for knockdown (KD) and gene sequence for overexpression (OV) in human cell lines and primary cells and in transplantation/xenograft mouse models. To explore the molecular mechanisms of *PAN3-AS1* in AML, we performed transcriptome profiling, ATAC-seq, and iDRiP (identification of direct RNA interacting proteins). siRNAs encapsulate in lipid nanoparticles are currently tested to target *PAN3-AS1* in AML cells *in vitro* and *in vivo*.

Results

We found that depletion of *Lnc-35682/PAN3-AS1* significantly inhibited survival of both mouse and human leukemia cells ***in vitro*** and delayed leukemogenesis *in vivo*, while sparing normal hematopoiesis. Conversely, overexpression of *Lnc-35682/PAN3-AS1*-OV promoted cell growth. High expression of *PAN3-AS1* correlates with unfavourable prognosis in AML patients. Forced expression of *Lnc-35682* rescued *PAN3-AS1* ablation in human leukemia cells, indicating conserved function in mouse and human. Mechanistically, transcriptome profiling demonstrated that *PAN3-AS1* promotes expression of its neighboring genes, *FLT3, PAN3* and *FLT1* while suppressing expression of genes involved in mediating immune responses.

Conclusions

We identified a functionally conserved oncogenic lncRNA, *Lnc-35682/PAN3-AS1* and nominated *PAN3-AS1* as vulnerability to be exploited for AML therapy.

Outcome/Impact

This study will provide insights into mechanisms of lncRNAs in pathogenesis of AML and identify novel targets for treatment of AML.

KEYWORDS

lncRNA, leukemogenesis, acute myeloid leukemia, leukemia stem cell

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25. BREATH BIOMARKER DISCOVERY IN LUNG CANCER

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Lung cancer remains the number one cancer killer globally. Breath testing has potential as a non-invasive, universal and simple lung cancer screening test. Volatile organic compounds (VOCs) in exhaled breath provide a rich matrix for biomarker discovery. One of the current limitations in breath analysis is VOC 'contamination' from the gastrointestinal tract and ambient environment. To mitigate contamination, we designed a novel bronchial brushing method for collecting VOCs directly from the tumour environment.

Methods

38 patients were recruited for the study: 10 with early-stage lung cancer (stage I & II, age 67.91 ± 7.29) and 28 healthy controls (age 67.2 ± 5.26). To collect VOCs from the tumour, a cytology brush was directed bronchoscopically via an ultrasound sheath proximal to the tumour. The same protocol was performed for the contralateral lung and control participants. Extracted VOCs were analyzed using thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS). TD-GC-MS data was processed using VOCcluster, an unsupervised algorithmic method used to cluster features with similar mass spectra and retention indices (RI) from deconvolved GC-MS data.

Results

A total of 263 samples were analyzed, including 60 bronchial brushes (10 cancer/10 contralateral/40 control), blanks, RI standards and environmental samples. Clustering resulted in 3,804 features from all samples, and 358 were selected for statistical analysis following environmental assessment and data preprocessing. 22 molecular features were found to be significantly different (Dunn's test, $p < 0.05$) between cancerous lung, contralateral lung, or control lung brushings. Validation of the clustering process using 35 target features yielded a clustering accuracy of 98 per cent.

Conclusions

In exhaled breath, VOCs specific to the lung can be differentiated from a larger number of VOCs derived from non-lung sources, using a novel bronchial brushing method. Importantly, this also allowed for differentiation between cancer and non-cancer participants. Our discovery strategy has important implications for breath biomarker discovery for the early detection of lung cancer.

Outcome/Impact

The identified target VOCs from this study will be validated on a larger population of early lung cancer patients as part of our TFRI funding opportunity. Specifically, we will be analyzing never-light smokers who do not currently qualify for lung cancer screening.

KEYWORDS

breathomics, lung cancer, mass spectrometry, volatile organic compounds

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26. *TP53* R217H AND R242H MUTANT ZEBRAFISH DISPLAY DYSFUNCTIONAL P53 HALLMARKS AND RECAPITULATE LFS PHENOTYPES

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Li-Fraumeni syndrome (LFS) is a hereditary disorder associated with nearly a 100 per cent lifetime cancer risk. LFS is characterized by a diverse, early onset tumour spectrum and germline mutations in the *TP53* tumour suppressor gene.

Methods

We generated two ***tp53*** zebrafish point mutants, R217H and R242H, (representing commonly mutated human LFS and sporadic residues, R248 and R273H) and obtained ***tp53*** null mutants (Langenau lab). Native p53 tumour suppressive functions (induction of p53 target genes, apoptosis and cell cycle arrest) were evaluated in ***tp53*** mutants to determine the functional consequences of the mutations. RNA sequencing and whole genome bisulfite sequencing (WGBS) on mutant larvae were performed to define tumour-driving mechanisms.

Results

R217H and R242H mutants recapitulate LFS phenotypes, including partial-to-no induction of p53 target genes; resistance to p53-mediated apoptosis; and G_1 checkpoint dysfunction. The loss of these functions resulted in spontaneous tumour development beginning at six to nine months post-fertilization that histologically resemble human sarcomas. Tumour kinetics differ with R242H mutants exhibiting earlier tumour onset, higher life-time incidence and tumour fish sex ratios skewed towards females compared to R217H and null mutants. Additionally, R217H and R242H mutants each present with a distinctive anatomic tumour distribution compared to nulls, altogether indicating unique tumour-driving mechanisms. Bulk larvae RNA sequencing and WGBS together implicated metabolic reprogramming and deregulated biological macromolecule synthesis and degradation in ***tp53*** mutants.

Conclusions

tp53 R217H and R242H zebrafish recapitulate human LFS phenotypes and hold tremendous potential to define underlying tumour-driving mechanisms and identify novel molecular targets for therapeutic intervention to prevent tumour evolution.

Outcome/Impact

As individuals with LFS face nearly a 100 per cent risk of developing cancer, a better understanding of key mechanisms that predispose to cancer development in LFS will identify novel prospective therapies that can be used to treat, and ultimately prevent cancer for this high-risk population.

KEYWORDS

zebrafish, p53, cancer, cancer prevention

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27. MULTI-OMIC ANALYSIS IDENTIFIES DEVELOPMENTAL HIERARCHIES OF RENAL SARCOMAGENESIS IN A TRANSGENIC MOUSE MODEL OF *DICER1* SYNDROME

Felix KF Kommoss[1,2], Joyce Zhang[1,2], Shary Chen[1,2], Branden Lynch[1], Lesley Ann Hill[3], Yana Moscovitz[1], T Michael Underhill[3], Yemin Wang[1,2], David G Huntsman[1,2]

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DICER1 syndrome is a tumour predisposition syndrome linked to various cancers, mostly mesenchymal tumours, arising in different anatomical sites of children and young adults. These cancers can progress to sarcomas, for which effective treatments are lacking due to poorly understood disease biology.

Methods

We generated a genetically engineered mouse model that can express a hemizygous RNase IIIb missense mutation of *DICER1* (p.D1709N), fully recapitulating the genetic state of *DICER1* gene in *DICER1* syndrome-associated neoplasms. We crossed these mice with Hic1^{CreERT2} mice, which activates *DICER1*^{D1709N} in Hic1+ mesenchymal progenitors upon tamoxifen treatment, to trigger tumour development. We performed targeted single cell spatial analysis (Xenium, 10x Genomics) on non-neoplastic tissues, and tumours of different stages and histotypes and whole transcriptomic single cell analyses on fresh tumours. We also performed targeted shallow whole genome and cancer gene mutational analysis. These data are integrated to understand sarcomagenesis in *DICER1* syndrome.

Results

Induction of *DICER1*^{D1709N} mutation in neonatal and juvenile mice, but not adult mice, leads to the development of renal tumours with a latency of eight to 15 months. Histologically, these tumours closely resemble renal tumours in *DICER1* syndrome, including low-grade mesenchymal tumours (cystic nephroma) and sarcomas with heterologous differentiation (rhabdomyosarcoma and anaplastic sarcoma). Unsupervised clustering of spatial analysis identified distinct histotypes of neoplastic cell populations: primitive low-grade mesenchymal cells (primarily in early-stage lesions), sarcoma, heterologous sarcoma with rhabdomyoblastic differentiation and high-grade sarcoma with blast-like appearance, exclusive to high-stage tumours. Single cell whole transcriptomic analysis identified differences between tumour cells and Hic1+ cells in normal kidneys, pseudo-differentiation trajectory of spatially-annotated tumour cell populations and differentially expressed genes alongside enriched biological functions in each population.

Conclusions

Our transcriptomic analyses reveal that *DICER1* mutation alters the fate of renal mesenchymal progenitors towards rhabdomyoblastic differentiation, which can be escaped to develop high-grade sarcoma. Ongoing genomic analysis will inform on the acquisition of concurrent genomic events and subsequent oncogenic pathway activation associated with sarcomagenesis.

Outcome/Impact

By investigating events driving biological programs in different developmental hierarchies of *DICER1* renal sarcoma, we will understand the mechanisms involved in sarcomagenesis and tumour progression to inform disease management.

KEYWORDS

DICER1, GEMM, sarcoma, spatial, transcriptomics

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28. GENERATION OF CHIMERIC MVA VIRUS FOR GLIOBLASTOMA TREATMENT

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The Vaccinia virus is a widely used oncolytic platform, with different strains having varied safety and effectiveness. Tian Tan (TT) is potent against cancer but can also grow in normal tissues. Modified Vaccinia virus Ankara (MVA) is safe for normal tissues and boosts immune responses against tumours but lacks TT's cancer oncolytic activity. Our study aims to create a chimeric virus merging the strengths of both strains to improve cancer virotherapy safety and effectiveness, especially for glioblastoma.

Methods

We use a CRISPR-Cas9 library to target different regions of the TT and MVA genomes, aiming to boost their ability for homologous recombination. This approach aims to generate new chimeric viruses combining features from both MVA and TT. We then identify viruses that address the limitations of each original virus and study their interactions with normal and glioblastoma cells, both *in vitro* and *in vivo*.

Results

We successfully developed and validated a comprehensive CRISPR-Cas9 library targeting every 100 bp of the TT genome. Co-infection of MVA and TT with the library in permissive cell lines produced various chimeric MVA viruses with enhanced oncolytic activity against cancer cells. Using Nanopore sequencing, we confirmed the top 10 chimeric viruses. Our next steps involve optimizing these chimeric viruses in glioblastoma cell models to isolate strains with targeted oncolytic activity.

KEYWORDS

glioblastoma, oncolytic virotherapy, TT, MVA

Conclusions

Our research pioneers the development of chimeric MVA viruses with enhanced oncolytic properties and safety through precise genetic engineering with CRISPR-Cas9. These viruses hold potential for targeting glioblastoma cells while sparing normal tissue. The next step involves refining these chimeric strains in glioblastoma models to select the optimal oncolytic virus (OV) for effective treatment. This work lays the groundwork for advanced humanized mouse studies and the translation of findings into clinical practice, offering new hope for cancer patients with safer and more effective oncolytic virotherapy strategies.

Outcome/Impact

We aim to identify the optimal OV for glioblastoma treatment with reduced non-specific growth in normal cells. Our findings will support humanized mouse studies targeting glioblastoma and eventual clinical translation for cancer patients.

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29. ELUCIDATING THE ROLE OF HIGH MOBILITY GROUP BOX 3 (HMGB3) IN TUMOUR PROGRESSION AND RESPONSE TO RADIATION THERAPY IN PANCREATIC NEUROENDOCRINE TUMOURS

Nilakshi Kulathunga[1], Zoey wang[1,2], Areeba Qureshi[1], Benjamin Lok[3], Kathrin Tyryshkin[4], Anne-Claude Gingras[5], Iacovos Michael[1,2]

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HMGB3, a high mobility group superfamily protein, shows increased expression in high-grade metastatic cancers, including in Pancreatic Neuroendocrine Tumours (PanNETs). Our previous transcriptomic analysis on mouse and human models identified HMGB3 upregulation during PanNET dedifferentiation, highlighting its potential contribution to the transition from benign to metastatic tumours, yet its intrinsic role and mechanisms remain elusive.

Methods

We have used gain-of-function and loss-of-function approaches to assess the functional role of HMGB3. Here, we utilized PanNET cell lines for ***in vitro*** assays and immunodeficient mice for ***in vivo*** orthotopic tumour assays. In collaboration with Dr. Anne-Claude Gingras, we established a proximity-dependent biotin identification (BioID) assay to analyze the HMGB3 protein interactome.

Results

We found that HMGB3 knockdown reduces the colony formation ability of high-grade PanNET cell lines and hinders their ability to form tumours in mice. Moreover, the analysis of the HMGB3 protein interactome unveiled its interaction with chromatin modifiers, thus supporting its involvement in PanNET dedifferentiation. Surprisingly, we also discovered interactions between HMGB3 and proteins in the DNA repair pathway, suggesting its contribution to radiotherapy response in PanNETs. Consistently, Hmgb3 knock-down in PanNET cells sensitizes them to radiation, as assessed by colony formation assays.

Conclusions

Overall, this study provides mechanistic insights into HMGB3 role during tumour progression and radiation therapy response, potentially by controlling chromatin modification and DNA damage response. We are currently performing further analysis to understand the role of Hmgb3 and characterize its protein interactome.

Outcome/Impact

Our studies will validate the potential therapeutic role of HMGB3 and identify interacting molecules as potential therapeutic targets.

KEYWORDS

pancreatic neuroendocrine tumours, HMGB3, tumour progression, DNA damage

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30. PROFILING LINEAGE-DEFINED CELL CYCLE DYNAMICS AND DNA DAMAGE RESPONSE IN PRIMARY BREAST EPITHELIAL CULTURES

Abhijith Kuttanamkuzhi[1], Bowen Zhang[1,2], Shane M. Harding[1,2,3], Rama Khokha[1,2*]*

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Lineage specification causes the development of multiple epithelial cell types that comprise the normal breast and underlie tumour subtypes in breast cancer. Studies from our labs and others have identified lineage-based differences in DNA damage responses, stress response and drug resistance in breast epithelial cells *in vivo*.

Methods

A current limitation that hinders our understanding of lineage-based differences and their downstream signalling is the difficulty of maintaining lineage fidelity of primary epithelial cells *ex vivo*. We have developed a monolayer culture system that can expand lineage-sorted primary breast epithelial cells and maintain their lineage identity over multiple passages. We have also established organoid cultures derived from primary tissues that recapitulate the bi-layered epithelium and can be passaged for multiple generations. Utilizing these cultures, we analyzed the cell cycle dynamics and DNA damage response signalling in primary human breast epithelial cells, measured by flow cytometry, transcriptomics and biochemical analyses.

Results

The organoid and monolayer culture systems were validated for the presence of both lineages and 3D organization. Upon gamma radiation, basal cells showed an accumulation of cells in the G2/M phase of the cell cycle. Moreover, ionizing radiation-induced differential cell cycle checkpoint signalling in the basal versus the luminal lineage. Interestingly, these differences are rewired in BRCA1 mutation carrier-derived primary cells, where the basal cells did not accumulate in the G2/M phase upon irradiation.

Conclusions

We found that breast epithelial cells exhibit differences in cell cycle dynamics. These differences could be associated with differential checkpoint signalling in these cells. Results from BRCA1 mutation carrier samples indicate that molecular pathways can be affected before loss of heterozygosity. We predict such differential signalling can be a key contributing factor to DDR, stress response signalling and drug response in the mammary lineages.

Outcome/Impact

Understanding baseline distinctions in mammary lineage-specific cell cycle dynamics and the DDR pathways will provide new insights into how genomic instability during carcinogenesis may offer a targetable strategy for early intervention and prevention of cancer development.

KEYWORDS

breast cancer, DNA damage, cell cycle, breast progenitor cells

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31. EDITING NK CELLS TO OVERCOME THE ADENOSINE PATHWAY DEPLOYED BY SENESCENT CELLS

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Cells get damaged by different factors often resulting in permanent cell cycle arrest also known as cellular senescence and as we age, these damaged cells tend to accumulate and weaken our immune system. Notably, in the tumour microenvironment (TME), these cells can aid in tumourigenesis and contribute to immune evasion by potentially increasing extracellular levels of adenosine, an immunosuppressive metabolite. We hypothesize that senescence interferes with immune cell functions by increasing the production of adenosine.

Methods

We conduct our experiments using human dermal fibroblasts. To induce senescence, we used three validated Methods: exposure to ionizing radiation and doxorubicin and the expression of the H-RAS^{v12} oncogene. NK cells were collected from various human healthy donors. Experiments mainly include cytotoxicity assays by co-culture, flow-cytometry and CRISPR editing.

Results

In support of our hypothesis, our initial findings show that NK cell killing is reduced when cells are put in contact with senescent human fibroblasts. We also observed that senescent fibroblasts expressed high levels of membrane enzymes CD39 and CD73, which convert Adenosine Triphosphate (ATP) to adenosine. Consist with these findings, we found that these senescent cells produced higher levels of extracellular adenosine compared to their non-senescent counterparts.

KEYWORDS

natural killer cell, adenosine, senescence, CRISPR editing

To overcome this resistance mechanism, using CRISPR, we generated NK cells deficient for the expression Adenosine A2A receptor (A2AR), the main receptor that recognizes adenosine on these cells. These engineered NK cells were resistant to adenosine-induced immune inhibition.

Outcome/Impact

Our goal will be to evaluate if engineered NK cells resist to the suppression caused by senescent cells and adenosine in the context of therapy-induced senescence. We foresee that A2AR knockout NK cells may be more potent at killing cancer and senescent cells in high adenosine environments.

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32. IMMUNE INFILTRATION IS ASSOCIATED WITH IMPROVED SURVIVAL IN P53 ABNORMAL ENDOMETRIAL CARCINOMA

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Our group and others previously demonstrated that endometrial carcinoma (EC) is comprised of four subtypes that are biologically distinct, diagnostically reproducible and highly significantly prognostic. Among these subtypes, p53 abnormal (p53abn) EC is by far the deadliest, with over 50 per cent of patients succumbing to disease within five years. Recent clinical trial results suggest that a subset of patients with p53abn EC respond to immune checkpoint and angiogenic inhibitors. Furthermore, trials of PARP inhibitors and HER2 targeted therapies show benefit in EC. However, the immune cell response to p53abn EC and associations to these targetable pathways have yet to be thoroughly investigated.

Methods

We built tissue microarrays from 256 treatment-naïve p53abn EC samples gathered from across Canada. Guided artificial intelligence analysis of three multiplex immunofluorescent panels identified the cell types, functional status and tissue localization of the immune infiltrate and angiogenic markers. Results were compared with shallow whole genome sequencing data of the same patient samples.

Results

Mixture modelling divided p53abn EC into lymphocyte-high and lymphocyte-low subsets, and increased intraepithelial T lymphocytes (TIL) correlated with increased stromal lymphocytes. The immune cell high subset associated with longer overall and disease-specific survival in multivariate analysis, which accounted for age, stage and treatment, and was particularly pronounced in advanced stage disease. Furthermore, high TIL associated with increased frequencies of PDL1+ macrophages, potentially providing mechanistic rationale for the effectiveness of PD-1/PDL1 checkpoint inhibitors. In contrast to high grade serous carcinoma of the ovary, TIL were not associated with homologous recombination deficient mutational signatures or HER2 amplification in p53abn EC.

Conclusions

p53abn EC can be subdivided based on an immune signature that correlates with improved survival and does not correlate with homologous recombination signatures or HER2 amplification.

Outcome/Impact

These findings may lead to improved selection of p53abn EC patients for immune checkpoint inhibition, PARP inhibitors and HER2 targeted therapies, leading to improved outcomes and reduced adverse effects.

KEYWORDS

endometrial carcinoma, p53-abnormal subtype, anti-tumour immunity, shallow whole-genome sequencing

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33. HEAD AND NECK CANCER OUTCOME TREATMENT PREDICTION USING FEATURE LEVEL FUSION AND MACHINE LEARNING: MRI, CT AND QUANTITATIVE ULTRASOUND IMAGING MODALITIES FUSION

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Radiation therapy is a common treatment for head and neck cancer, but outcomes vary among patients. Predicting outcomes before treatment can help reduce undesired results. Radiomic features extracted from biomedical images can be used to train machine learning systems for outcome prediction. This study aims to predict treatment outcomes for head and neck cancer cases using a fusion of radiomics features from MRI, CT and quantitative ultrasound imaging.

Methods

Radiomic features were extracted from MRI, CT and quantitative ultrasound images using open-source python package Pyradiomics. High-dimensional data were generated and then radiomic features were extracted from three different imaging modalities. In order to tackle this challenge, we applied an autoencoder with a two-layer encoder and two-layer decoder to fuse these features. The bottleneck of autoencoder was considered as the fused features. A support vector machine (SVM) classifier was employed to train a model to differentiate complete responders (CR) from partial responders (PR) amongst head and neck cancer patients.

Results

This study involved 63 head and neck cancer patients, with a total of 496 radiomic features determined (356 from quantitative ultrasound, 70 from MRI and 70 from CT). The SVM model using fused features achieved an accuracy of 85 per cent, with sensitivity at 81 per cent, specificity at 90 per cent, F1-score at 87 per cent and balanced accuracy at 85 per cent.

Conclusions

The study here demonstrated that fusing information at the feature level with an SVM classifier is an effective method for predicting treatment outcomes in head and neck cancer patients. This fusion technique generated new features that encapsulate information from all imaging modalities. By applying information fusion, the fused features contained information about both primary tumours and lymph nodes, enhancing the predictive capability beyond what is available in individual imaging modalities alone.

KEYWORDS

head and neck cancer, radiation therapy, machine learning, radiomics, information fusion

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34. ASCL1-DRIVEN EPIGENETIC AND METABOLOMICS PROGRAMS IN NEUROENDOCRINE PROSTATE CANCER

Shaghayegh Nouruzi[1,2], Dwaipayan Ganguli[2], Olena Sivak[2], Alastair Davies[1], Seth Parker[3,4], Amina Zoubeidi[1,2]

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Androgen receptor (AR) pathway inhibitors (ARPIs), such as enzalutamide (ENZ), have significantly improved survival for castration-resistant prostate cancer (CRPC) patients. However, approximately 20 per cent relapse with AR-independent tumours, exhibit activation of alternative lineage programs and progress to aggressive neuroendocrine prostate cancer (NEPC) without targeted therapies.

This progression, characterized by the limited genomic aberrations, distinct chromatin state and DNA methylation patterns unique to NEPC, strongly suggests that NEPC is driven by lineage plasticity and epi-transcriptomic reprogramming. Our previous work identified the transcription factor (TF) ASCL1's essential role in lineage plasticity, influencing the chromatin landscape in favour of NEPC. Exploring the dichotomy between NEPC ASCL1-low and ASCL1-high patients, we found ASCL1-high to be associated with metabolic processes supporting the biosynthesis of S-adenosylmethionine (SAM), the universal methyl donor. However, the mechanism by which ASCL1 dictates cell fate through epigenome and metabolome is unknown.

Methods

We investigated ASCL1's impact on the metabolome and epigenome by measuring changes in transcriptomic, chromatin accessibility, metabolism and ASCL1 binding in cell lines and patient-derived xenografts.

Results

We observed that silencing ASCL1 in NEPC cells altered metabolomics pathways linked to the methionine cycle. In support of ASCL1 regulating SAM bioavailability, silencing ASCL1 in NEPC led to a decrease of global histone methylations, along with repression of pathways involved in histone methylation. Metabolomic profiling of a small group of patient-derived NEPC xenografts showed a unique metabolic profile in ASCL1-high NEPC, distinct from ASCL1-low.

Conclusions

While much focus has been placed on the impact of epigenetic "writers" and "erasers", less is known about the regulation of metabolic pathways that impact epigenetics and provide the "fuel" for this machinery. Our preliminary data suggests that ASCL1 may orchestrate a symphony of metabolic and epigenetic regulation that facilitates the emergence of NEPC.

Outcome/Impact

We will elucidate the mechanism by which ASCL1 facilitates a metabolic switch that alters epigenetic programming to support NE differentiation in human clinical samples confer ENZ resistance, and provide a mechanistic proof-of-principle for targeting the resultant metabolic-epigenetic vulnerabilities in ASCL1hi patients.

KEYWORDS

castration-resistant prostate cancer, ASCL1, cancer plasticity, treatment-resistance

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35. UNVEILING METABOLIC REWIRING ASSOCIATED WITH RECURRENCE IN DUCTAL CARCINOMA *IN SITU*

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Following diagnosis of ductal carcinoma ***in situ*** (DCIS), prophylactic breast surgery is typically performed to decrease the risk of invasive tumours—albeit with significant recurrence rates of 13 to 25 per cent 10 years following breast-conserving lumpectomies. In addition to oft-studied genetic and epigenetic markers in cancer progression, the identification of metabolic vulnerability in recurrent subtypes might provide a complementary approach to improve patient outcome.

Methods

Leveraging a publicly available dataset from the Translational Breast Cancer Research Consortium (TBCRC), we performed differential gene expression analysis on bulk RNA to characterize common expression patterns amongst those patients with recurrence within five years versus those without recurrence. Next, we employed constraint-based metabolic modelling to characterize how differences in gene expression may result in differences in cellular growth. Specifically, we started by applying this mathematical simulation framework to a genome-scale human metabolic reconstruction to identify metabolic reactions that fulfil key cellular tasks. Then, by mapping the log fold change associated with differentially expressed genes onto these reactions, we identified metabolic tasks undertaken either more or less within cells from patients with recurrence. Last, we deconvolute the bulk RNA sequencing data to identify the cell specificity of these differentially regulated tasks.

Results

529 genes are differentially expressed between individuals with recurrent and non-recurrent DCIS, with an overlapping set of 149 and 128 genes differentially expressed in recurrence amongst ER-positive and PGR-positive individuals respectively ($p<0.05$). We further identified metabolic tasks in fatty acid and phospholipid metabolism to be upregulated and downregulated (respectively) more than randomly expected amongst patients with recurrence. Importantly, this metabolic rewiring in high-risk individuals can be stratified by cell type specificity.

Conclusions

Cells implicated in recurrent subtypes of DCIS are associated with significant differences in gene expression and lipid metabolism.

Outcome/Impact

Our simulation framework highlights metabolic vulnerabilities associated with DCIS recurrence, with potential application to improving patient outcome.

KEYWORDS

ductal carcinoma *in situ* recurrence, metabolism, differential gene expression, constraint-based metabolic modelling

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36. UNCOVERING MOLECULAR FEATURES OF HIGH-RISK BREAST TISSUE TO PREVENT BREAST CANCER

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Breast cancer (BC) continues to pose a substantial global health challenge as the most common malignancy in women and second-leading cause of cancer-related deaths in women worldwide, thus emphasizing the need for innovative strategies that facilitate early detection and intervention.

Recent single-cell RNA sequencing (scRNAseq) studies have provided insights regarding the cellular heterogeneity present within the mammary gland, highlighting the intrinsic differences between the two main mammary epithelial lineages – basal and luminal. Luminal progenitor expansion has been widely reported in *BRCA1*^{mut} carriers and are linked to aggressive basal-like BC; however, the exact molecular alterations that contribute to a malignant luminal progenitor state is not yet clear. We propose that molecular profiling of high-risk tissues will allow us to identify unique molecular features within the mammary epithelium which can be targeted for clinical benefit in women at high-risk of BC.

Methods

We will use the 10X Chromium single-cell multiome sequencing platform to obtain both gene expression (scRNAseq) and chromatin accessibility (scATACseq) from viably cryopreserved *BRCA1*^{mut}, *BRCA2*^{mut} and normal/non-carrier samples.

Results

Currently, we have generated pilot data using cryopreserved breast specimens from *BRCA1*^{mut} (n=3) and non-carrier (n=3) patient cohorts. After quality control and filtering of the 10X multiome data, a total of 49,372 cells were detected, and unsupervised clustering of the integrated dataset revealed 19 cell clusters. We identified eleven mammary cell types through canonical marker gene expression. Also, using differential expression and differential accessibility analysis, we found that classical mammary stem-like expression markers were more accessible in luminal progenitors.

Conclusions

Our high-risk breast samples contain the expected mammary cell lineages and classical mammary stem-like markers were more accessible in luminal progenitors.

Outcome/Impact

Lineage-specific vulnerabilities within mammary stem and progenitor compartments can potentially be harnessed for clinical benefit in women at high-risk of BC.

KEYWORDS

breast cancer, precision medicine, single-cell multiome, epigenetic regulation

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37. VIRTUAL PREPLANNING OF MANDIBULAR RECONSTRUCTION: UPDATE ON A PHASE II/III RANDOMIZED CONTROL TRIAL

Diane Pan[1], *Antony Hodgson*[1], *Sidney Fels*[1], *Eitan Prisman*[1]

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Patients diagnosed with oral cavity cancer are often required to undergo mandibular reconstruction involving transplantation of the fibula or scapula, where surgeons have conventionally shaped the donor bone to recreate the native mandible's curvature in a free hand fashion (FHS). Virtual surgical planning (VSP) has emerged as a new method to improve surgical outcomes by planning the reconstruction in a virtual controlled setting and 3D printed surgical cutting guides to facilitate execution of the virtual plan.

Methods

Patients undergoing mandibular reconstruction with a fibula or scapula free flap are randomized between the current standard of care using FHS and VSP in a 1:1 ratio. Phase II of this trial involves an internal pilot conducted at Vancouver General Hospital to assess feasibility of patient recruitment and retention, and fidelity of intervention. Phase III will compare union rate of reconstruction using VSP vs FHS, patient-reported quality of life (QOL), complication rates, reconstruction accuracy and functional outcomes.

Results

Forty patients have been recruited, with 21 VSP patients and 19 FHS patients at an average recruitment rate of three patients per month. Fifteen patients have been followed to one year with 87 per cent retention due to mortality, and crossover rate from VSP to FHS at 5 per cent. The average operative and ischemic times for VSP vs. FHS are 230.5 vs. 231.2 minutes and 55.7 vs. 60.8 minutes, respectively. Patient-reported outcomes at one month assessed by the University of Washington QOL questionnaire show higher scores for VSP vs FHS in the activity and recreation domains with average scores of 63 per cent vs. 33 per cent and 52 per cent vs. 31 per cent ($p < 0.05$), respectively.

Conclusions

An interim review of the Phase II trial shows that the recruitment, retention and intervention fidelity is within the progression criteria and that VSP does not significantly differ from the standard of care using FHS in terms of surgical outcomes.

Outcome/Impact

VSP is a feasible novel method to be implemented in a Phase III multi-institutional trial and has potential to demonstrate improved clinical outcomes and quality of life for patients undergoing mandibular reconstruction.

KEYWORDS

virtual surgical planning, mandibular reconstruction, oral cavity cancer, osseous free flap

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38. LEVERAGING LIQUID BIOPSY CIRCULATING TUMOUR DNA (CTDNA) ANALYSIS TO PREDICT TREATMENT RESPONSE IN POOR-PROGNOSIS METASTATIC PROSTATE CANCER PATIENTS

Karan Parekh[1], Kim van der Zande[2], Sarah W.S. Ng[1], Vincent van der Noort[2], Gillian Vandekerkhove[1], Wilbert Zwart[3], Andre M. Bergman[3], Alexander W. Wyatt[1,2]

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Despite significant therapeutic advancements in metastatic castration-resistant prostate cancer (mCRPC), there remains a lack of biologically-informed treatment strategies. Circulating tumour DNA (ctDNA) profiling may enable the development of minimally-invasive prognostic and predictive biomarkers, potentially aiding in treatment planning for mCRPC patients who are candidates for standard-of-care cabazitaxel (CBZ) or androgen receptor targeted agents (ARTAs) following progression on docetaxel.

Methods

The OSTRICh trial, a prospective, phase IIb study, randomized 106 poor-prognosis mCRPC patients between second-line CBZ and ARTA. The primary endpoint was Clinical Benefit Rate (CBR) at 12 weeks, and secondary endpoints included progression-free (PFS) and overall survival (OS) using the Kaplan-Meier method. We conducted deep targeted next-generation sequencing on 100 baseline plasma ctDNA samples alongside patient-matched germline DNA using a custom panel spanning 76 mCRPC genes, including tumour suppressors (***TP53, PTEN, RB1***), DNA repair genes (***BRCA2, CDK12, ATM, MSH2***) and oncogenes (***SPOP, FOXA1, AR***). ctDNA fraction (ctDNA per cent) was calculated using somatic mutations and copy-number alterations.

Results

No differences in CBR between CBZ and ARTA at 12 weeks were observed (***P*** = 0.54). Median PFS was 2.53 months with CBZ and 2.99 months with ARTA (***P*** = 0.30), with comparable median OS (14.9 months ***vs*** 13.9 months; ***P*** = 0.53). Decrease in serum PSA ⇢50 per cent (i.e. PSA50) was noted in 26.9 per cent of patients in the CBZ arm and 45.3 per cent in the ARTA arm (***P*** = 0.04). Deep targeted sequencing revealed a median ctDNA per cent of 21 per cent, consistent with aggressive disease. Commonly mutated genes included ***TP53*** (50.6 per cent), ***ATM*** (13.8 per cent), ***AR*** (12.6 per cent) and ***BRCA2, RB1*** and ***PTEN*** (each observed in 6.9 per cent of patients), with whole genome doubling detected in 44 per cent of assessable cases. CtDNA per cent ⇢30 per cent was strongly correlated with unfavourable OS and PFS, independent of clinical prognostic factors. Deep PSA50 responses were more frequent in ARTA-naïve patients, regardless of ***AR*** genotype or ctDNA per cent.

Conclusions

ctDNA per cent remains a well-established genomic prognostic tool in mCRPC, while prior exposure to ARTAs is a key predictor of subsequent therapy benefit.

Outcome/Impact

ctDNA profiling is a robust genomic prognostic tool, capturing tumour biology and disease burden beyond clinical parameters.

KEYWORDS

biomarker profiling , circulating tumour DNA, metastatic castration resistant prostate cancer, liquid biopsy

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39. DECIPHERING THE ROLE OF DRUG TOLERANT PERSISTERS IN CANCER RELAPSE

Sumaiyah Rehman[1], Jennifer Haynes[1], Evelyne Collignon[2], Kevin Brown[3], Yadong Wang[1], Miguel Ramalho-Santos[2], Sidhartha Goyal[4], Jason Moffat[3], Catherine O'Brien[1]

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Colorectal Cancer (CRC) is the third highest cause of cancer-related deaths in North America, with the greatest challenge being non-genetic processes that drive resistance to chemotherapy. Drug Tolerant Persister (DTP) cells are recognized as key players of non-genetic heterogeneity across many tumour types and represent a reversible cell state, where they exhibit an impressive initial response to standard-of-care chemotherapies but resume tumour growth upon treatment cessation; however, the underlying mechanisms by which DTPs confer drug tolerance and tumour recurrence remains unknown.

Methods

We labelled patient-derived tumour CRC lines with a DNA barcode library to track cell subclones within a tumour, established xenografts and treated them with standard-of-care chemotherapy. Resulting tumours were subject to next-generation sequencing to assess barcode composition, and gene expression analysis to investigate the biology of DTPs.

Results

Barcode composition analysis showed no difference in barcode complexity in chemotherapy-treated tumours (versus controls), indicating that all cells in a tumour possess an equipotent capacity to enter the DTP-state, survive chemotherapy and drive tumour recurrence. Gene expression (RNAseq) analysis revealed that DTP tumours exhibited significant downregulation of mTOR and MYC signalling, with functional dependency on the autophagy program for survival. Single cell RNAseq analysis revealed a transcriptionally distinct sub-population of cells in DTP state tumours (versus controls) with increased invasive and migratory profiles, indicating metastatic potential. We developed a DTP-signature that stratified TCGA CRC patients with poor prognosis and identified patients with minimal residual disease. The signature is now being used to identify relapse-prone patients, and guide therapy in a Phase III trial for metastatic colon cancer patients (OICR CATA grant) at UHN.

Conclusions

Tumour cells enter the reversible DTP state to survive chemotherapy and drive tumour recurrence. Targeting DTPs represents a therapeutic opportunity prior to the development of classical irreversible mutation-driven chemo-resistance.

Outcome/Impact

We identified DTPs as being responsible for CRC tumour relapse and established a DTP-signature which is currently guiding therapy for relapse-prone colon cancer patients in a Phase III clinical trial at UHN.

KEYWORDS

drug tolerant persisters, colorectal cancer, tumour relapse, DTP signature

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40. TARGETING THE YAP-DEPENDENT REVIVAL STEM CELL TO IMPROVE COLORECTAL CANCER THERAPIES

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This study aims to further understand the role of the hippo pathway, the revival stem cell (revSC) and the efficacy of targeting this population during colorectal cancer therapy to mitigate recurrence.

Methods

Investigations utilized the Nuak1/2 inhibitor HTH-02-006 and the commonly used chemotherapeutic agent, 5-Fluorouracil (5-FU), on APC mutant mouse intestinal organoids which have constitutive WNT activity resembling early colorectal adenomas. Preliminary work utilized both fluorescence microscopy to monitor revSC dynamics as well as brightfield imaging to assess organoid viability.

Results

RevSCs possess the ability to reconstitute damaged LGR5+ cells after damage and are quiescent and transiently induced by Yap and TGF-ß signalling. Preliminary research has shown that the treatment of 5-FU is effective in killing the LGR5+ stem cells in colorectal adenomas however this drug alone is insufficient in targeting the revSC population. These 5-FU treated organoids become dormant but recover approximately 10 days after treatment causing budding in regions of the organoid. The Yap-dependent revSC was targeted using the Nuak inhibitor HTH-02-006 in conjunction with 5-FU. The results from this experiment showed that individual 5-FU and HTH-02-006 treatments were insufficient in killing the organoids. Dual-treatment of HTH-02-006 and 5-FU were able to cause a loss of both the LGR5+ and revSC population leading to organoid death.

Conclusions

This data indicates that 5-FU is insufficient in causing death to adenoma organoids due to the remaining revSC population that persists after LGR5+ stem cell loss. This remaining revSC population may act as the main driver of recurrence after chemotherapy treatments. The inhibition of Nuak utilizing HTH-02-006 allows for sensitization of adenomas to 5-fluorouracil, specifically in the revSC population.

Outcome/Impact

Targeting of the Hippo pathway and the revSC in the context of colorectal cancer will allow for a deeper understanding of the mechanisms and cell type driving recurrence, while also providing a novel pathway for colorectal cancer therapies.

KEYWORDS

colorectal cancer, Hippo pathway, revival stem cell, mouse intestinal organoid

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41. GLOBAL PROTEOMICS REVEALS BIDIRECTIONAL INTEGRIN SIGNALLING AS A DRIVER OF GLIOBLASTOMA INVASION

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Glioblastoma (GBM) is the most aggressive form of adult brain cancer and is particularly difficult to treat owing to the diffuse infiltration of therapeutically resistant tumour cells into healthy brain tissue. Recent single-cell RNA sequencing and spatial transcriptomics studies have revealed that tumour cells actively migrate away from hypoxic areas, suggesting that hypoxia may drive GBM invasion. While these studies have advanced our understanding of GBM invasion, the significant heterogeneity of clinical tissue samples makes it difficult to prioritize migratory signalling pathways for therapeutic targeting.

Methods

We utilized a diverse cohort of 17 patient derived GBM stem cell (GSC) lines and advanced experimental systems to stimulate more controlled models of GBM invasion to identify actionable targets that impede tumour infiltration. To investigate invasive signalling pathways promoted by hypoxia, we leveraged mass spectrometry-based proteomics to profile GSCs cultured under 20 per cent, 1 per cent and 0.2 per cent oxygen concentrations. We then utilized the GSC and cerebral organoid (GLICO) co-culture model of GBM brain infiltration to investigate hypoxia independent GBM invasion through global proteomic profiling and spatial transcriptomic analysis.

Results

After confirming that our GSC cohort models hypoxia-induced invasion, we performed differential expression analysis between oxygen conditions, which revealed the enrichment of integrin-related signalling pathways in hypoxia. We then investigated the importance of integrin signalling for hypoxia independent GBM invasion by profiling the proteomes of GLICOs, which revealed the upregulation of proteins involved in both outside-in and inside-out integrin signalling pathways. In addition, we spatially correlated integrin signalling to the GSC compartment of GLICOs using spatial transcriptomics. Finally, we functionally evaluated the role of integrin signalling in GBM invasion by treating GLICOs with a chemical inhibitor against inside-out integrin signalling, which resulted in significantly reduced GSC infiltration.

Conclusions

This study highlights the pivotal role of bidirectional integrin signalling as a driver of GBM invasion. Further exploration of key regulators presents a promising therapeutic avenue.

Outcome/Impact

As GBM treatments have remained largely unchanged in the past few decades, the actionable target critical for GSC invasion identified in this study could therefore represent a much-needed new therapy for GBM.

KEYWORDS

glioblastoma, glioma stem cells, mass spectrometry, spatial transcriptomics

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42. INVESTIGATING CAR T CELL EFFICACY AND PHENOTYPE ACROSS DISTINCT EXTRANODAL SITES OF DIFFUSE LARGE B CELL LYMPHOMAS

Gillian Savage[1,2], Pauline Loos[1], Nicole Wretham[3], Lauralie Short[1,2], Grace Bernard[1,2], Laura Evgin[1,4]

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Recent studies exploring predictive factors for relapse or resistance to Chimeric Antigen Receptor (CAR) T cell therapy have shown that diffuse large B cell lymphoma (DLBCL) patients who present with two or more sites of extranodal (EN) disease experience inferior outcomes to CD19 CAR T cell therapy. Therefore, we intend to determine the differences in efficacy and phenotype of CD19 CAR T cells across distinct anatomical sites of disease in multiple B cell lymphoma mouse models to elucidate possible reasons for CAR T cell relapse at EN sites.

Methods

With the syngeneic A20 lymphoma mouse model we looked at CD19 CAR T cell engraftment and phenotype over time within a wide range of lymphoid and nonlymphoid tissues. We performed spectral flow cytometry and evaluated marker expression on CAR T cells associated with memory, fate specification, activation, exhaustion and function, allowing us to evaluate phenotypic differences that may be contributing to different CAR T responses across distinct tissues.

Results

We observed differential clearance and relapse of lymphoma tumours depending on the anatomical site and the CAR construct. This was not linked to CAR T cell engraftment but instead could be described by phenotypic differences of the CAR T cells within each tumour site and construct. Specifically, CAR T cells within the lymph nodes took on a central memory phenotype, which correlated with complete clearance of lymphoma cells. Meanwhile, the liver showed significant disease burden at relapse and consisted of CAR T cells that lacked memory markers, and instead displayed higher levels of certain exhaustion and activation markers, such as CD39 and CD69.

Conclusions

We observed that anatomical site is a major driver of CAR T cell phenotype which contributes to the differential efficacy of CAR T cells at distinct sites.

Outcome/Impact

These results provide insight to better understand CAR T cell function within EN sites to hopefully provide better CAR T treatment outcomes for DLBCL patients presenting with EN disease.

KEYWORDS

immunotherapy, CAR T cell therapy, diffuse large B cell lymphoma, extranodal

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43. MYCN IS AN ESSENTIAL IMMATURE T-ALL ONCOGENIC DRIVER MARKED BY BROAD H3K4ME3

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T cell acute lymphoblastic leukemia (T-ALL) is an aggressive leukemia of immature T cells. The outcome for patients with intrinsic therapy resistance or disease relapse remains poor, and the biological underpinnings for aggressive disease remain ill-defined.

Methods

Using our recently developed synthetic leukemia model from human CD34+ cord blood (CB) cells (Nat Comms 2019), we performed whole transcriptomic (RNA-Seq) analysis of 32 synthetic leukemias (SynLs) generated with various combinations of Notch1 (N), LMO2 (L), TAL1 (T) and BMI1 (B) oncogenes. Genetic loss-of-function approaches, including shRNA-mediated knock-down (KD) or CRISPR/Cas9-mediated knock-out (KO), were used to assess potential dependencies of oncogene-transduced CB cells and SynLs.

Results

Transcriptomic analysis of SynLs revealed two major types of disease corresponding roughly to early (ETP-like) and later (post beta-selection) T cell development stages. Interestingly, early vs. late SynLs could be distinguished by MYCN expression level, with higher expression in early SynLs. This observation was also notable in that T-ALL patients with high MYCN expression presented shorter survival (hazard ratio=5.2, n=264; TARGET cohort). To assess MYCN's contribution to T-ALL biology, we performed MYCN KD/KO in early SynLs and observed significantly reduced bulk cell growth and clonogenic activity.

KEYWORDS

acute leukemia, T-ALL, MYCN, H3K4me3

Conversely, overexpression of MYCN in combination with Notch1 in CB cells increased clonogenic activity *in vitro* but was insufficient to generate leukemia *in vivo*. To explore mechanisms of MYCN gene regulation in this setting, we performed histone ChIP-Seq on NLTB-transduced CB cells and early SynLs. NLTB transduction resulted in a global gain of H3K4me3 marks, often distributed over gene bodies including MYCN. KD of the H3K4 methyltransferase complex components WDR5 and KMT2D led to reduced growth/survival of NLTB-transduced CB cells and early SynLs, while normal T-progenitors appeared unaffected by KMT2D KD.

Conclusions

Genetic modelling of human T-ALL revealed MYCN as a critical oncogene associated with less mature tumours. Epigenetic regulation may be key to facilitate consistent oncogene expression and define a malignant identity.

Outcomes/Impact

These results emphasize developmental stage-specific oncogene dependencies and identify the epigenome as a therapeutic target in T-ALLs with high MYCN expression.

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44. DLG5 AS A NUAK2 INTERACTOR AND REGULATOR OF THE HIPPO SIGNALLING PATHWAY

Catherine Song[1,2], Mandeep Gill[2], Siyuan Song[2], Mohammad Inanlou[2], Amber Couzens[3], Anne-Claude Gingras[3], Liliana Attisano[1,2]

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The Hippo pathway plays a critical role in organ growth and tissue size control, with dysregulation implicated in tumour development. Previous research from our lab identified NUAK2, an AMP kinase family member, as a negative regulator of the Hippo pathway. However, the regulatory mechanism of NUAK2 activity remains to be understood.

Methods

By employing a proximity-dependent biotinylation (BioID)-mass spectrometry screen using NUAK2 as the bait protein, we identified Discs large homolog 5 (DLG5), a member of the membrane-associated guanylate kinase (MAGUK) family, as a potential interactor of NUAK2. Validation of this interaction was performed through co-immunoprecipitation and GST pull-down assays. Functional characterization of DLG5 in the Hippo pathway involved immunofluorescence microscopy, real-time quantitative PCR, cell proliferation and migration assays.

Results

We confirmed the interaction of full length DLG5 with NUAK2 by immunoprecipitation and immunoblotting lysates from transiently transfected HEK293T cells. Mapping of interacting domains using DLG5 deletion constructs revealed strong interactions with two PDZ domains and the SH3-GUK domain. Additionally, GST pull-down assays indicated a potential direct interaction between the PDZ domains of DLG5 and purified GST-NUAK2. To explore the functional role of DLG5 in the Hippo pathway, we screened a panel of cell lines from various cancer types (breast, bladder, head and neck and colorectal cancers) for the effect of DLG5 knockdown using siRNA. In all the cell lines, siDLG5 suppressed the expression of Hippo target genes such as ANKRD1 and CTGF. Importantly, siDLG5 also induced cytoplasmic localization of YAP/TAZ, two key transcriptional regulators in the Hippo pathway, in MDA-MB-231 and T24 cells. Moreover, siDLG5 and siYAP/TAZ inhibited cell proliferation and reduced cell migration in head and neck cancer cells (HSC3, SCC25 and SCC9).

Conclusions

DLG5 interacts with NUAK2, and the C-terminal region of DLG5 displaying stronger interaction. Loss of DLG5 restores Hippo activity and attenuates cell proliferation and migration in diverse cancer cell lines.

Outcome/Impact

The interaction between DLG5 and NUAK2 opens up a possibility for inhibiting the tumour-promoting kinase and restoring Hippo pathway function, thereby offering novel strategies for suppressing tumour initiation and progression.

KEYWORDS

Hippo pathway, BioID, DLG5, NUAK2

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45. CELL-FREE DNA FRAGMENTOMICS FOR CANCER DETECTION IN LI-FRAUMENI SYNDROME

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Li-Fraumeni syndrome (LFS) is a cancer predisposition syndrome associated with germline mutations in TP53. LFS patients are a high-cancer-risk population that contends with weak genotype-phenotype correlations (i.e. different tumour types, variable ages of onset) and can benefit from novel screening avenues. Previous work in the lab leveraging an integrated cell-free DNA (cfDNA) approach shows that plasma sWGS analysis at a depth of 1X can help to detect cancer in patients with LFS in advance of current clinical surveillance Methods (positive predictive value = 67.6 per cent; Wong et al., 2024). To increase the sensitivity and specificity of blood-based cancer detection, we hypothesize that the sensitivity of cfDNA fragmentation analysis can be improved and act as a functional genomics predictor of tumour occurrence and risk as the depth of plasma WGS increases.

Methods

cfDNA extracted from the blood plasma of cancer-negative (n = 26) and cancer-positive (n = 14) LFS patients was sequenced along with plasma from healthy controls (n = 18) using two whole-genome sequencing (WGS) technologies: Illumina and Ultima Genomics. DNA fragmentation was used as a marker to elucidate whether increased sequencing depth allows for extracting additional information from a plasma sample, as well as validate that observed differences are indeed biological differences between cancer-positive and cancer-negative mutation carriers.

Results

Plasma samples from cancer-positive LFS patients (n = 14) had an increased proportion of short 10–150 bp fragments compared to healthy controls (n = 18), a pattern that suggests increased fragmentation within nucleosomes (in review).

Conclusions

Two different sequencing technologies that use competing chemical reactions were used to validate the differential fragmentation in cfDNA from LFS patients with an active cancer diagnosis, suggesting the fragment length distribution shifts towards shorter fragments in sporadic cancers occurring in individuals with LFS.

Outcome/Impact

Our pilot work successfully demonstrated the equivalency of more cost-effective Ultima WGS technology to the current gold-standard, Illumina. Employing this new sequencing technology in upcoming projects with significantly greater depth of coverage for cfDNA fragmentomics analysis will enable improved early cancer detection and *de novo* mutation calling for patients where a biopsy is not available.

KEYWORDS

Li-Fraumeni syndrome, TP53, cell-free DNA, fragmentomics

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46. CIRCULAR RMST COOPERATES WITH LINEAGE-DRIVING TRANSCRIPTION FACTORS TO GOVERN NEUROENDOCRINE TRANSDIFFERENTIATION

Mona Teng[1,2], Jiacheng Guo[3], Xin Xu[2], Xinpei Ci[2], Yulin Mo[2,4], Sujun Chen[5], Wang Yuan Guo[2], Martin Bakht[6], Shengyu Ku[6], Michael Sigouros[7], Ziting Xia[8], Moliang Chen[2], Sami Ul Haq[2], Wen Yang[9], Alejandro Berlin[2], Theo van der Kwast[2], Leigh Ellis[10], Amina Zoubeidi[11,12], Gang Zheng[2,4], Jie Ming[9], Yuzhuo Wang[11,12], Benjamin H. Lok[1,4,13], Brian Raught[1,2], Himisha Beltran[6], Jun Qin[3,], Housheng Hansen He[1,2]

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Circular RNA (circRNA) is a class of noncoding RNA with regulatory functions. However, it is unknown if they play a functional role in promoting the neuroendocrine transdifferentiation of prostate and lung cancer.

Methods

Total RNA-sequencing of neuroendocrine prostate cancer (NEPC) and small cell lung cancer (SCLC) was performed to profile the circRNA landscape, which led to the identification of circular RMST (circRMST) as one of the most abundant circRNAs with neuroendocrine-specific expression. The function of circRMST was then characterized in NEPC and SCLC cell line models using RNA-interference and CRISPR-based knockdown approaches. The effect of circRMST on cancer cell growth and gene expression was examined using ***in vitro*** growth assays and RNA-sequencing. The functional role was further examined in genetically engineered mouse models for NEPC. To understand its mechanism, the protein interactors of circRMST was identified with RNA pulldown followed by mass spectrometry.

Results

We showed that circRMST is essential for neuroendocrine tumour growth and maintaining the neuroendocrine cell identity. The knockdown of circRMST leads to downregulation of major neuroendocrine markers including ASCL1. In mouse, circRMST is conserved and its genetic knockout in NEPC mouse model leads to complete inhibition of neuroendocrine transdifferentiation. Through RNA pulldown assays, we identified that circRMST physically interacts with transcription factors SOX2 and NKX2-1 which are implicated in neuroendocrine biology. In the absence of circRMST, NKX2-1 presents decreased expression while SOX2 level is unchanged. However, the genomic binding of SOX2 and NKX2-1 at ASCL1's promoter is reduced, leading to the downregulation of ASCL1.

Conclusions

We identified circRMST as an essential component of a regulatory network that governs neuroendocrine tumour development.

Outcome/Impact

We present one of the first functional studies of circRNA in neuroendocrine tumours of the prostate and lung. We propose circRMST as a potential therapeutic target given its essential role in driving neuroendocrine transdifferentiation.

KEYWORDS

neuroendocrine tumours, noncoding RNA, circular RNA, transcription factors

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47. COMBINING ONCOLYTIC VIRUS AND TUMOUR-INFILTRATING LYMPHOCYTE (TIL) THERAPIES FOR THE TREATMENT OF COLORECTAL CANCER

Vallati, Sydney[1,2], Crupi, M.J.F.[2], Taha, Z.[1,2], Petryk, J.[2], Austin, B.[2], Gilchrist, V.[1,3], Alluqmani, N.[1,2], Boulton, S.[1,2], & Bell, J.C.[1,2]

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While showing promise in select solid cancers, tumour-infiltrating lymphocyte (TIL) therapy has been limited to a subset of patients due to the scarcity of tumour specific TILs in the tumour microenvironment. The combination of TILs and oncolytic viruses (OVs) shows promise in overcoming these challenges and improving patient outcomes.

Methods

We have established a pipeline for the isolation, expansion, characterization and adoptive cell transfer of TILs. Using an MC38 colorectal model in C57BL/6 mice, we determined the impact of parental OV vs. OV encoding an immunostimulatory cytokine pre-treatment on TILs. The number of lymphocytes isolated per gram of tumours in combination with immunohistochemistry was used to assess the impact of OVs on TIL recruitment. The relationship between OV pre-administration and TIL composition was characterized using flow cytometry and immune assays. We evaluated the efficacy of OV-induced TILs compared to conventional TILs when adoptively transferred in an ***in vivo*** disseminated model of disease.

Results

Pre-administration of an OV encoding a cytokine results in TILs that show increase anti-tumour reactivity, shown through an IFN-γ ELISpot. In the metastatic model of disease, adoptively transferred TILs induced by the cytokine encoding OV show increased tumour control compared to the parental OV induced TILs.

Conclusions

Our results show that pre-administration of an OV encoding an immune stimulatory cytokine induces changes in the TIL population that are beneficial for use in adoptive cell therapy.

Outcome/Impact

Pre-treatment with an OV encoding immune stimulatory transgenes can be used to overcome some of the challenges limiting the use of TIL therapy as a viable treatment option, making it more accessible to more patients.

KEYWORDS

tumour-infiltrating lymphocytes (TILs), oncolytic virus (OV), adoptive cell therapy

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48. DROPLET-BASED PROTEOMICS REVEALS CD36 AS A MARKER FOR PROGENITORS IN MAMMARY BASAL EPITHELIUM

Matthew Waas[1]; Amanda Khoo[1, 2]; Pirashaanthy Tharmapalan[1]; Curtis W McCloskey[1]; Meinusha Govindarajan[1, 2]; Bowen Zhang[1, 2]; Shahbaz Khan[1]; Paul Waterhouse[1]; Rama Khokha[1, 2]; Thomas Kislinger[1, 2]

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Breast cancer, a heterogeneous disease of multiple origins, is the most common cancer in women. Stem and progenitor cells essential for mammary ductal expansion constitute < 5 per cent of the epithelium. These rare cells are the cells-of-origin for the most aggressive, deadly subtypes of breast cancers. An attractive prevention strategy for breast cancer is to develop therapeutic modalities to monitor and target the oncogenic precursors as they arise. Though various markers have been reported to enrich stem/progenitor capacity, there remains no consensus as to how the cells enriched by distinct markers relate to each other. Improving our knowledge of the features and regulation of stem/progenitor cells has the potential to improve clinical outcomes for breast cancer patients.

Methods

We have developed an accessible low-input proteomic method for studying rare populations of cells, Droplet-based one-pot preparation for proteomic samples (DROPPS). Mass spectrometry-compatible detergents and reagents are used to digest samples in a single-pot preparation. Mammary glands are dissected and dissociated using standard protocols. Lineage-positive cells are excluded and epithelial compartments are distinguished using established markers.

Results

DROPPS performance is robust across operators and sample processing batches, and it is easy to implement,relying exclusively on common or affordable laboratory equipment. Notably, DROPPS outperforms other recently described accessible low-input proteomic Methods. We uncovered CD36 as a candidate marker for basal cells with elevated mitochondrial membrane potential and functionally validated enhanced clonogenicity.

Conclusions

DROPPS has utility to uncover biological insights of rare epithelial cell populations. DROPPS affords the capacity to assess mouse-to-mouse variability, differences between subsets of cells within each mouse and rare populations of cells thus enabling proteomic insights into the functional heterogeneity that were precluded by previous sample requirements. DROPPS has widespread applicability to various sample-limited systems and is well positioned to transform our ability to map the proteomic landscapes of rare cell populations.

Outcome/Impact

We anticipate the results of this research will harmonize our understanding of mammary stem cell biology and support breast cancer prevention, prognosis and treatment efforts.

KEYWORDS

breast cancer, proteomics, stem cells, progenitors

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49. UNDERSTANDING THE IMPACT OF AIR POLLUTION ON THE GENOMIC LANDSCAPE OF LUNG CANCER IN NEVER SMOKERS

Peiyao Wang, Stephen Lam, Wan Lam, Callum MacAulay, Anna McGuire, Julia Naso, William Lockwood

University of British Columbia

Up to 25 per cent of lung cancer patients have never smoked, yet effective screening Methods for this subpopulation are lacking and the etiology of this disease is largely undefined. Outdoor air pollution has been correlated with higher lung cancer incidence and this study aims to investigate its impact on the genome of lung cancer.

Methods

Up to 200 lung tumour and adjacent normal tissue samples from patients who have never smoked in British Columbia will be collected for whole genome sequencing (WGS) and bulk RNA sequencing. Detailed questionnaire data will be collected from these patients regarding their residential history to classify them according to their levels of cumulative $PM_{2.5}$ exposure. Lung epithelial cells will undergo acute and chronic $PM_{2.5}$ exposure to investigate malignant transformation and genomic and transcriptomic alterations in parallel with the clinical samples.

Results

Acute $PM_{2.5}$ exposure causes significant DNA damage ***in vitro***, suggesting that exposure may lead to mutations that drive lung cancer initiation. Analysis of future WGS data will potentially yield mutational signatures associated with high $PM_{2.5}$ exposure. Correlation of somatic variants with differentially expressed genes in clinical samples paired with ***in vitro*** findings will provide insight into the influence of outdoor air pollution on lung cancer in patients who have never smoked.

Conclusions

There may be significantly different mutational and transcriptional landscapes within lung cancer patients who have been exposed to high levels of $PM_{2.5}$. This will elucidate how outdoor air pollution influences the initiation and development of lung cancer.

Outcome/Impact

Genomic and transcriptomic differences between lung tumours with high and low levels of $PM_{2.5}$ exposure have the potential to inform screening strategies and novel therapeutic targets in patients who have never smoked.

KEYWORDS

lung cancer, never smoked, outdoor air pollution, genomics

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50. PLASMABLASTIC LYMPHOMA (PBL) DOES NOT RELY ON B-CELL RECEPTOR SIGNALLING FOR SURVIVAL

Jasper Wong, F Frontzek, BJ Collinge, LK Hilton, S Ben-Neriah, GW Slack, P Farinha, Y Li, JR Cook, I Salaverria, E Campo, G Ott, A Rosenwald, C Amador, ES Jaffe, TC Greiner, PW Raess, JY Song, G Inghirami, DD Weisenburger, WC Chan, H Holte, K Beiske, K Fu, J Delabie, S Pittaluga, AL Feldman, KJ Savage, AJ Mungall, C Steidl, G Lenz, LM Rimsza, RD Morin, DW Scott

Lymphoma and Leukemia Molecular Profiling Project consortium

Plasmablastic lymphoma (PBL) is an aggressive type of non-Hodgkin lymphoma. It is more common in HIV+ patients, and is often associated with Epstein-Barr virus (EBV). The standard of care for PBL is immunochemotherapy but this is rarely curative. Other than ***MYC*** translocations, the molecular drivers of PBL are poorly understood, partly owing to the small cohort sizes available for genomic analyses. Here, we provide a comprehensive analysis of the genomic and transcriptomic landscape of PBL using a collection of 178 PBL exomes and genomes and 64 transcriptomes from archival diagnostic tissue biopsies.

Methods

Somatic mutations (SNVs/Indels) were identified using an ensemble of four variant callers (Strelka2, Lofreq, Mutect2, SAGE). MiXCR and igBlast were used to infer the immunoglobulin (Ig) constant gene rearrangement sequences. Salmon and DESeq2 were used to identify differentially expressed genes. We compared PBL to genomes and transcriptomes of other known B-cell malignancies including 92 multiple myelomas (MM), 238 Burkitt lymphomas (BL) and 208 diffuse large B-cell lymphomas (DLBCL). ***In vitro*** experiments were conducted on PBL-1, the only known PBL cell line.

Results

In 64 per cent of the PBL transcriptomes, we were unable to reconstruct a dominant clonotype expressing both Ig heavy and light chain constant genes. This result suggests these tumours lack a B-cell receptor (BCR). Consistent with this, flow cytometry analysis of the Ig light chain showed that 32 per cent of PBL do not express either a lambda or kappa light chain. Analysis of exome sequencing data revealed mutations affecting the JAK/STAT pathway (***STAT3***, ***SOCS1***) and MAPK pathway (***NRAS***), as opposed to mutations affecting the NF-kB signalling pathway commonly observed in activated B-cell-like (ABC) subtype of DLBCL, the entity thought to be the most closely related lymphoma to PBL. Through comparisons with transcriptomic data of other aggressive B-cell neoplasms (DLBCL, BL, MM), we identified a PBL signature associated with reduced expression of genes in both the canonical and non-canonical NF-kB signalling pathway. ***In vitro*** data showed lack of NF-kB signalling and resistance to therapeutics that inhibit B-cell receptor signalling pathways.

Conclusions

Taken together, PBL is a distinct B-cell malignancy that, unlike ABC-DLBCL, does not rely on constitutive activation of the BCR-dependent NF-kB pathway.

Outcome/Impact

These findings may explain why current chemotherapeutic drugs targeting the NF-kB pathway will have limited efficacy in PBL.

KEYWORDS

plasmablastic lymphoma, genomics, B-cell receptor signalling, bioinformatics

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51. INTEGRATED SINGLE-CELL ANALYSIS REVEALS CO-EVOLUTION OF MALIGNANT B CELLS AND THE TUMOUR MICROENVIRONMENT IN TRANSFORMED FOLLICULAR LYMPHOMA

Shaocheng Wu, Clémentine Sarkozy, Katsuyoshi Takata, Tomohiro Aoki, Susana B Neriah, Katy Milne, Talia Goodyear, Celia Strong, Tashi Rastogi, Laura K Hilton, Daniel Lai, Laurie H Sehn, Pedro Farinha, Brad H Nelson, Andrew Weng, Marco Marra, David W Scott, Jeffrey W Craig, Christian Steidl, Andrew Roth

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Histological transformation of follicular lymphoma (FL) to aggressive form occurs two to three per cent per year with poor outcome. Divergent evolution and an altered tumour-microenvironment (TME) have been implicated during transformation. However, phenotypic consequences of this evolution and its impact on the TME remain unknown.

Methods

We performed single-cell whole genome sequencing (scWGS) and transcriptome sequencing (scWTS) of 11 paired pre/post-transformation patient samples and scWTS of additional samples from patients without transformation. The malignant B cell phylogenetic trees were constructed based on the copy number profiles extracted from scWGS data. Unbiased clustering of the scWTS data was performed to segregate non-malignant B cells, malignant B cells and the TME cells. The association of scWGS and scWTS in malignant B cells was inferred using the clonealign statistical modelling. The cell-cell interactions among malignant B and TME cells were inferred by timepoints using CellChat. Then we used multi-colour immunofluorescence to find a transformation-related biomarker. The findings were validated using multi-colour immunofluorescence data from two independent pretreatment cohorts.

Results

We reveal evolutionary dynamics of FL transformation at single-cell resolution, highlighting a shifting TME landscape, with an emerging immune-cell exhaustion signature, co-evolving with the shifting malignant B phenotype in a regulatory ecosystem. Integration of the scWGS and scWTS data identifies malignant B cell pathways upregulated during clonal tumour evolution. With multi-colour immunofluorescence, we transfer these findings to a TME-based transformation biomarker, subsequently validated in two independent pretreatment cohorts.

Conclusions

Taken together, our results provide a comprehensive view of the combined genomic and phenotypic evolution of malignant cells during transformation, and shifting cross-talk between malignant cells and the TME.

Outcome/Impact

Our study provides a comprehensive view of the combined genomic and phenotypic evolution of malignant cells during follicular lymphoma transformation, and the shifting cross-talk between malignant cells and the tumour-microenvironment. Findings are translated to a TME-based transformation biomarker, independent of treatment type.

KEYWORDS

follicular lymphoma, lymphoma transformation, single cell

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52. THE TRANSCRIPTIONAL AND FUNCTIONAL LANDSCAPE OF N6-METHYLADENOSINE IN LOCALIZED PRIMARY PROSTATE CANCER

Xin Xu[1], Helen Zhu[1,2], Rupert Hugh-White[3], Julie Livingstone[3], Stefan Eng[3], Paul C. Boutros[2,3], Hansen He[1,2]

[1]Princess Margaret Cancer Centre; [2]University of Toronto; [3]University of California

Cancer progression involves intricate genomic dysregulation, with emerging evidence pointing towards the role of epitranscriptomic modifications such as N^6-methyladenosine (m^6A) in driving tumour initiation and progression. Understanding the landscape of m6A modifications in specific cancer types, such as prostate adenocarcinoma, offers insights into underlying molecular mechanisms and potential therapeutic targets.

Methods

We profiled 162 localized prostate tumours by using m^6A antibody based methylated RNA Immunoprecipitation Sequencing (MeRIP-Seq). Comprehensive analysis was done to characterize the m^6A landscape of prostate cancer with matched multi-modal DNA, RNA and protein profiling.

Results

Our study reveals diverse m^6A abundances across tumours and intricate germline-somatic interactions regulating m^6A patterns. These patterns were closely associated with prognostic clinical features, establishing the biomarker potential of m^6A modifications. Furthermore, we observed widespread dysregulation of m^6A profiles under tumour hypoxia conditions, bridging genomic and proteomic observations. Importantly, specific m^6A sites within key genes like *VCAN* were linked to disease aggressiveness, highlighting their functional significance in prostate cancer progression.

Conclusions

Our findings underscore the multifaceted role of m^6A dysregulation in prostate cancer, implicating germline risk, microenvironmental factors, somatic mutations and metastasis. The identification of specific m^6A modifications driving disease aggression suggests potential targets for therapeutic intervention. Overall, elucidating the role of m^6A modifications enhances our understanding of prostate cancer biology and may pave the way for personalized treatment strategies.

Outcome/Impact

This study links m^6A modifications to prostate cancer, informing potential therapies. Identified m^6A patterns may refine risk assessment and treatments. Targeting specific m^6A sites could enhance prostate cancer management.

KEYWORDS

prostate cancer, N^6-methyladenosine (m^6A), m^6A landscape, meRIP-seq, RNA modification, VCAN, transcriptomic dysregulation

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53. ULTRA MINIMALLY INVASIVE TRANSBRONCHIAL NANOPARTICLE ENABLED THERANOSTICS AND IMAGE GUIDANCE FOR PERIPHERAL LUNG CANCER

Fumi Yokote[1], Nicholas Bernards[1], Andrew Effat[1], Theodore Husby[2], Yoshihisa Hiraishi[1], Takamasa Koga[1], Kate Kazlovich[1], Takahiro Yanagihara[1], Kenta Nakahashi[1], Yusuke Fujibayashi[1], Hironori Hinokuma[1], Robert Weersink[2,3], Gang Zheng[2,3], Brian Wilson[2,3], Kazuhiro Yasufuku[1]

[1] Division of Thoracic Surgery, Toronto General Hospital, UHN; [2] Department of Medical Biophysics, University of Toronto; [3] Princess Margaret Cancer Center, UHN

Lung cancer accounts for 25 per cent of all cancer deaths in Canada. While lobectomy is standard of care for early-stage non-small cell lung carcinoma (NSCLC), many patients are ineligible due to comorbidities. Our innovative solution utilizes Porphysome (PS)-enabled transbronchial fluorescence image-guided photodynamic therapy (PDT), offering an ultra minimally invasive (UMI) approach. To address the challenge of targeting peripheral lung tumours precisely, we've developed a parallel-type ultrathin composite optical fiberscope (COF), allowing accurate delivery of therapeutic light via the bronchoscope working channel.

Methods

In our investigation of utilizing the COF for peripheral lung cancer, we evaluated its efficacy in visualizing tumours and its capability to reach peripheral lung regions across three preclinical models. In an ***in vivo*** New Zealand White rabbit lung tumour model, VX2 cells induced tumours via transbronchial inoculation, followed by an intravenous injection of PS once tumours reached 2 cm in diameter. We captured simultaneous white light and fluorescent images using the COF 24 hours post-injection. In another large animal model, using Yorkshire pigs, we assessed image quality and reach, verified by fluoroscopy. Additionally, we leveraged ***ex vivo*** human lungs, unsuitable for transplant, to evaluate visualization, accessibility and technical feasibility under surgical conditions with fluoroscopy.

Results

In the rabbit lung tumour model, the tumour area could be observed simultaneously in white light and fluorescence images, and the spectral data was analyzed to verify PS emission light. The pig model demonstrated that this new COF system can access the 6th to the 9th generations of the bronchial. In the ***ex vivo*** human lung model, the COF reached the periphery just beneath the pleura and the alveolar level, observable in white light images.

Conclusions

Here we demonstrated the technical feasibility of UMI transbronchial nanoparticle-enabled theranostics and image guidance for peripheral lung cancer. This device may enable UMI transbronchial treatment of peripheral lung cancer.

Outcome/Impact

Our research aims to develop highly precise and UMI treatments for peripheral lung cancer.

KEYWORDS

lung cancer, photodynamic therapy, transbronchial

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54. AMINO ACID METABOLISM DISTINCTIONS IN MAMMARY EPITHELIAL CELL TYPES

Bowen Zhang[1,2], Curtis W. McCloskey[1], Abhijith Kuttanamkuzhi[1], Pirashaanthy Tharmapalan[1], Hal K. Berman[1], Courtney L. Jones[3], Rama Khokha[1,2*] *Co-senior authors*

[1]Princess Margaret Cancer Centre, UHN; [2]Department of Medical Biophysics, University of Toronto; [3]Cincinnati Children's Hospital Medical Centre

The mammary epithelium is a functionally diverse bilayer consisting of basal cells and luminal cells, the latter subdivided into secretory or hormone sensing populations. While the importance of metabolic reprogramming in breast cancer progression is well established, the metabolic programs of normal mammary epithelial cell types from which breast cancers arise remain understudied.

Methods

We have generated metabolic profiles of mouse and human mammary epithelial populations using untargeted metabolomics. Mammary epithelial cells from virgin hormone cycle-staged mice (n = 4 for each stage) were FACS purified into basal and luminal populations. Similarly, epithelial cells from follicular stage human breast tissue specimens (n = 6 non-carrier, n = 5 *BRCA1* mutation carrier) were FACS purified into basal, luminal secretory and luminal hormone sensing populations. Additionally, we leveraged our human breast single cell RNA-seq dataset to infer metabolic pathway utilization. The functional relevance of metabolites of interest was assessed using primary ***in vitro*** model systems.

Results

Regardless of hormone stage, amino acids and their derivatives are more abundant in mouse luminal cells compared to basal cells. Hormone sensing luminal cells of human non-carriers are also enriched in amino acids, a trend that is lost in *BRCA1* mutation carriers. Among the luminal-enriched amino acids, branched chain amino acids and tryptophan are imported by SLC7A5. These amino acids play an important role in promoting cell proliferation and survival. Interestingly, metabolic flux prediction using single cell RNA-seq suggest that active catabolism of these amino acids occur in luminal secretory cells. We found that limiting import of these amino acids by SLC7A5 using JPH203 differentially decreased clonogenicity of mouse and human mammary epithelial cell types.

Conclusions

There may be differences in the import and utilization of amino acids between mammary epithelial lineages. Utilization of individual amino acids transported by SLC7A5 is being investigated in primary human monolayer and organoid models through isotopic labelling.

Outcome/Impact

Elucidating lineage-specific metabolic preferences will lay the foundation to target metabolic alterations of cancer-prone epithelial populations in the *BRCA1* breast.

KEYWORDS

mammary stem cells, metabolomics, amino acid transport, breast cancer prevention

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55. DIGITAL HEALTH AND DISCOVERY PLATFORM (DHDP)

No abstract. See poster and visit dhdp.ca to learn more.

**This poster will not be judged.*

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56. INTRAOPERATIVE REAL-TIME IMAGE-GUIDED FIBULAR HARVEST AND MANDIBULAR RECONSTRUCTION: A FEASIBILITY STUDY ON CADAVERIC SPECIMENS

Khanh Linh Tran[1], Georgia Grzybowski[2], Molly Stewart[2], Thomas D. Milner[1], Anat Bahat Dinur[1], Orla M. McGee[2], Amir Pakdel[1], Sidney S. Fels[3], Antony J. Hodgson[2], Eitan Prisman[1]

[1]Division of Otolaryngology, Department of Surgery, Faculty of Medicine, University of British Columbia (UBC); [2]Department of Mechanical Engineering, Faculty of Applied Science, UBC; [3]Department of Electrical and Computer Engineering, Faculty of Applied Science, UBC, Vancouver, BC, Canada

While virtual surgical planning (VSP) using 3D-printed guides has improved the accuracy of mandible reconstruction using the fibula free flap (FFF) technique, this method has disadvantages, including the lead time between planning and surgery. This study assesses the feasibility of using a customized surgical navigation system (including both hardware and software components) to plan and guide sequential steps during mandible reconstruction.

Methods

We designed an image-guided surgical system (IGS) that included customized mandible and fibula fixation devices with navigation reference frames as well as an accompanying image-guided software system. At the core of the IGS is an automated algorithm to generate a geometrically optimized reconstruction plan using FFF for any given mandibular defect. The mandibular and fibular segmental osteotomies and the positioning of the fibular segments at the donor site were performed using the IGS in five cadaveric specimens. In three cadavers, the surgeon used the IGS to guide the final positioning of the reconstructed fibula at the mandible prior to plating. In the remaining two cadavers, the surgeon used a "freehand" approach to position and plate the reconstructed fibula within the mandible defect. The time to task completion and cephalometric measurements of the reconstructions were recorded and the performance of the segment positioning approaches were compared to one another.

Results

Five real-time IGS mandibulectomy and fibular reconstructions were successfully performed. The mean Dice-score and Hausdorff-95 distance between the planned and actual mandible reconstructions was 0.8±0.08 and 7.29±4.81mm, respectively. Intercoronoid width, interangle width and mandible projection differences were 1.15±1.17mm, 0.90±0.56mm and 1.47±1.62mm, respectively. The segment positioning differences were comparable between the "guided" and "freehand" positioning Methods, suggesting that the accuracy in performing the osteotomies is currently the limiting step in this procedure.

Conclusions

This study presents the first demonstration of a comprehensive image-guided workflow for mandibulectomy and FFF reconstruction using cadaveric specimens and resulted in comparable cephalometric accuracy compared to VSP outcomes using 3D-printed guides that have been presented in the literature.

Outcome/Impact

The IGS system has the potential to be implemented clinically to improve reconstructive accuracy and clinical outcomes for patients with head and neck cancer.

KEYWORDS

mandible reconstruction, image guided surgery, fibula free flap, surgical technology

**This poster will not be judged.*

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57. CD83 MARKS ACUTE INFLAMMATORY STRESS ACTIVATION IN HUMAN HEMATOPOIETIC STEM CELLS AND ITS DEPLETION DAMPENS INFLAMMATION RESPONSE

Murtaza S. Nagree[1], Andy G.X. Zeng[1,2]*, Angelica Varesi[1,2]*, Sayyam Shah[1], Michael W.T. Zhang[1], Isabel N.X. Lim[1], Hyerin Kim[1], Liqing Jin[1], Mason Boulanger[1], John E. Dick[1,2], Stephanie Z. Xie[1] *Equal contribution*

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Inflammation activates many blood cell types, driving aging and malignancy. Surface markers that are responsive to inflammatory stress that we can target on human hematopoietic stem cells (HSC) and sustain lifelong blood production through a lifetime of infections are lacking. Here, we have identified CD83 as transcriptionally enriched in human HSC upon aging and in a subset of human HSC that respond to acute inflammation with heritable molecular changes in a xenograft model of inflammation-recovery ((Zeng ***et al***, ***in revision***). CD83 is linked to inflammation response and resolution on immune cells, but has not studied in human HSC.

Methods

Flow cytometry analysis of cord blood (CB) hematopoietic stem and progenitor cells (HSPC) coupled to gene expression studies and functional assays were performed.

Results

CD83 is low or absent in HSC and their downstream progeny at steady state. However, robust CD83 surface expression was observed in HSC and other HSPC subpopulations within 16 hours of ***ex vivo*** culture, with highest expression in long-term (LT)-HSC; further enhanced following culture with TNFα. Moreover, acute ***in vivo*** LPS treatment of mice xenografted with CB-derived HSC was sufficient to upregulate CD83 expression after 16 hours on LT-HSC/HSPC. To elucidate the potential function of CD83-expressing HSC, CD19-CD34+CD38-CD45RA- HSC-enriched cells were sorted from xenografts following acute inflammatory challenge for low and high CD83 expression and subjected to bulk RNA sequencing. We found enrichment of a signature specific to activated human HSC within CD83-positive cells. Strikingly, signatures of stimulated vs resting T cells and effector T cells signatures were enriched in CD83-positive compared to CD83-negative cells. TNFα stimulation enhanced both CD83 and MHC class II surface expression on human LT-HSC ***ex vivo***. Importantly, Cas9-sgRNA-mediated knockout studies shows CD83 deficiency restrains TNFα-induced HSPC functional defects in colony assays and lipopolysaccharide challenge within xenografts.

Conclusions

CD83 may mark an 'effector' state of acute inflammatory stress response in human HSC that could be a target to preserve HSC fitness.

Outcome/Impact

CD83 represents a long-sought functional biomarker of inflammatory stress activation in human HSC that may have significant potential for eradication of inflammation-responsive HSC in normal and malignant hematopoiesis.

**This poster will not be judged.*

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58. ESTABLISHING A COMPREHENSIVE LOGISTICS AND SEQUENCING FRAMEWORK FOR MICROBIOME ANALYSIS IN COLORECTAL CANCER PATIENTS IN NEWFOUNDLAND

Akinola A. Alafiatayo[1], Matthew Dyer[1], Azra Hasan[2], Anthony Reiman[3,4,5], Sidney E. Croul[6], Daniel Gaston[67], Stephen M. Lewis[8,9], Robin Urquhart[10], Thomas J. Belbin[1,11], Kara Laing[11], Sheila Drover[1], Sherri L. Christian[12], Atlantic Cancer Consortium, John Thoms[11], Sevtap Savas[1,11], Touati Benoukraf[1]

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Gut microorganisms can have significant roles in biological processes related to human health and diseases. Colorectal cancer is the fourth most common cancer worldwide. Within Canadian provinces, Newfoundland and Labrador (NL) has the highest incidence rate of colorectal cancer. Novel approaches to understanding the pathobiology of this disease are therefore important for better disease control and prognosis. This study leverages advanced long-read sequencing technologies to comprehensively characterize the microbiome landscape at the strain level by establishing robust protocols and logistics that span the entire process, from clinical sample collection to sequencing and data analytics.

Methods

Ten patients from NL recruited to the TFRI's Atlantic Cancer Consortium (ACC) were included. We established a logistics system for collecting stool specimens from patients' homes, which are then mailed to Biobank NL. This enables convenient and non-invasive sample acquisition. DNA from the patient's stool was extracted, and libraries were prepared and sequenced using Oxford Nanopore Minion MK1C. With an in-house bioinformatics tool, abundance of bacteria taxa was determined.

Results

Our preliminary findings observed considerable variability in the sequencing reads generated from stool specimens, ranging from one to five million reads. The initial analysis of the microbiome landscape in our cohort revealed the presence of several common species, such as Phocaeicola vulgatus, Bacteroides stercoris, Odoribacter splanchnicus, Oscillospiraceae bacterium and Bacteroides fragilis.

Conclusions

These early results provide an initial map of the microbiome in our cohort. In conclusion, we established a feasible system for collecting patient stool specimens and mapping their microbiome profiles.

KEYWORDS

gut microbiome, stool, colorectal cancer, long-read sequencing, bacteria

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59. COMPREHENSIVE WHOLE GENOME AND TRANSCRIPTOME ANALYSIS OF ADVANCED SOLID TUMOUR PATIENTS TREATED WITH IMMUNE CHECKPOINT INHIBITOR THERAPY IN THE PAN-CANCER COHORTS FROM THE MARATHON OF HOPE CANCER CENTRES NETWORK STUDY (MOHCCN)

Khadjah AL Shankati[1], Jeffrey P Bruce[1], Pamela Ohashi[1], Marcus O. Butler[1], David Cescon[1], Aaron Hansen[1], Linh Nguyen[1], Ming Tsao[1], Benjamin Haibe-Kains[1], Celeste Yu[1], Peter Sabbatini[1], Albiruni Razak[1], Anna Spreafico[1], Ben Wang[1], Trevor Pugh[1], Lillian Siu[1], Philippe L. Bedard[1]

[1]Princess Margaret Cancer Consortium (PM2C)

Immune checkpoint inhibitors (ICI) improve survival in multiple advanced solid tumours but many patients do not benefit. We conducted a whole genome transcriptome sequencing (WGTS) analysis to identify predictors of immune sensitivity.

Methods

Clinical and molecular data from archival or pre-treatment FFPE tumour tissue were available for analysis MOHCCN. It includes patients (Pts) with advanced solid tumours and ECOG PS 0 or 1 treated with ICIs targeting PD-1, PD-L1, or CTLA-4 in the INSPIRE and OCTANE cohorts. Response (R) to ICI was defined as radiological and clinical response without PFS event at six months; versus non-response (NR) as radiological or clinical progression within six months. Responders without evidence of progression for >12 months were categorized as durable responders (DR). Nucleic acids were sequenced using Illumina NovaSeq 6000 system. WGTS data were integrated with clinical data to identify associations with response to ICIs.

Results

59 Pts were included in this analysis: 28 (R) and 31 (NR). The most common tumour types were head and neck (n = 19) and melanoma (n = 7). The most frequent ICI was pembrolizumab 76 per cent, with 90 per cent of all pts receiving ICI monotherapy and 10 per cent combination. Higher tumour mutation burden (TMB) was observed in R vs NR (median 13 vs 5 coding mut/Mb, p=0.001), with the highest TMB in patients with DR (median 14 mut/M). Differential RNA gene expression analysis showed NR had increased expression of several oncogenic pathway signatures, including MYC targets, G2M checkpoint, and E2F targets. Responders had significant enrichment in mutations in WNT/ß-catenin pathway genes in both coding (APC, AMER1, LZTR1, TCF7L2) and promoter regions (CTNNB1). Notably, the 6 Pts with CTNNB1 promoter mutations had significantly increased CTNNB1 gene expression (p = 0.005).

Conclusions

ICI responders showed enrichment in coding mutations of several negative regulators of the Wnt/ß-catenin pathway and non-coding promoter mutations in CTNNB1 compared with non-responders. Further investigation is ongoing to biologically validate how these mutations in the Wnt/ß-catenin pathway may lead to improved response to ICI.

KEYWORDS

immune checkpoint inhibitors (ICI), WNT/ß-catenin pathway, WGTS, pan-cancer

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60. OUTLINING THE ROLE OF HYPOXIA IN BLADDER CANCER DEDIFFERENTIATION AND PROGRESSION

Andrew Garven[1], Hamid Ghaedi[1], Julia Vassalakis[2], Amber Simpson[2,3], Lynne Marie Postovit[2], David Berman[1]

[1]Department of Pathology and Molecular Medicine, Division of Cancer Biology and Genetics, Queen's University Cancer Research Institute; [2]Department of Biomedical and Molecular Sciences, Queen's University; [3]School of Computing, Queen's University, Kingston, ON, Canada

Arising from the urothelium, consisting of basal stem cells and their differentiated luminal progeny, bladder cancer (BC) predominantly manifests as a luminal urothelial phenotype in early-stage non-muscle invasive bladder cancer (NMIBC). Conversely, the aggressive basal subtype emerges almost exclusively in late-stage muscle-invasive bladder cancer (MIBC), incurring a heightened metastatic potential and mortality. Therefore, the objective of this study is to uncover the mechanisms underlying the transition from luminal NMIBC to lethal basal MIBC.

Methods

Utilizing the transcriptional expression profiles of GATA3 (luminal) and KRT5 (basal) for molecular subtype classification, we conducted differential expression and pathway enrichment analyses comparing luminal and basal MIBC tumours (n=419). Additionally, we analyzed flow-sorted luminal and basal cells from the luminal cell line SW780. Our investigation unveiled a notable upregulation of oxidative stress signalling in basal BCs, a finding currently undergoing validation on MOHCCN BC genomic data. Further, trajectory interference analysis of tumour epithelial cells from a single-cell dataset (SRA: PJRNA662018; 4 NMIBC, 4 MIBC) delineated the emergence of the basal BC lineage from a luminal precursor lesion. This dedifferentiation was again correlated with heightened oxidative stress signalling. To assess causality, SW780 cells were subjected to hypoxic culture conditions (five per cent, two per cent, per cent, 0.5 per cent) for 24 hours, and the expression of differentiation markers (GATA3, KRT5), hypoxia markers (HIF1α, LOX), and cellular stress markers (eIF2α) were evaluated using qPCR and western blots.

Results

Lineage plasticity, wherein luminal cells adopt basal-like phenotypes, delineates the progression of certain luminal NMIBCs to basal MIBC. This transition towards a more primitive cancer stem cell-like phenotype aligns with the expression of oxidative stress signatures, including response to hypoxia, cellular response to oxidative stress, and CAIX expression. Moreover, exposure of the luminal BC cell line SW780 to hypoxic conditions led to dedifferentiation and the emergence of the basal phenotype.

Conclusions

Basal MIBC originates from a better-differentiated luminal-like precursor lesion, driven by hypoxia and associated oxidative stress signalling.

Outcome/Impact

Outlining the mechanisms underlying the luminal to basal transition in BC offers valuable insights into developing lethal BCs, enabling earlier diagnosis of high-risk cancers, and identifying novel therapeutic targets to impede disease progression.

KEYWORDS

bladder cancer, epithelial plasticity, hypoxia, integrated stress response

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61. IMMUNE PROFILING BY BULK RNA-SEQ AND MULTIPLEXED IMMUNOHISTOCHEMISTRY IMAGING ACROSS MULTIPLE CANCER TYPES

Catia Gaspar, Simone Stone, Ben Wang, Aileigh Kay, Prabhjit Basra, Emily Van de Laar, Trevor J. Pugh, Pamela Ohashi, Lillian L. Siu, Jeffrey P. Bruce

Princess Margaret Cancer Consortium (PM2C)

There is emerging interest to characterize immune cell subtypes in the tumour microenvironment (TME) to identify potential prognostic and predictive biomarkers of anticancer therapy, especially immunotherapy. Traditionally, immune cell subtype estimation relied on quantitative immunohistochemistry (IHC), using single antibody markers for immune cell populations.

Increasingly, multiplexed IHC (mIHC) allowing for detection of multiple markers on a single tissue section, enables the evaluation of TME composition, immune contexture and cell-cell interactions. Recently, in silico tools developed to analyze bulk RNA-Seq data are also being used to characterize the immune compartment in the TME. Concordance between these Methods are not yet established and investigations have been limited to specific tumour types, with concordance dependent upon histology type and with overall better correlation for T/B cells rather than for macrophages.

Methods

This study will assess concordance between immune cell subtype frequencies estimated by i) a 6-marker mIHC panel (CD3, CD8, FOXP3, CD20, CD68 and PanCK/or melanoma cocktail) by multispectral image analysis and ii) Tumour whole transcriptome ('bulk RNA-Seq') data; analyzed using multiple state-of-the-art immune cell type deconvolution tools. The concordance of five major immune cell subtypes (CD4/CD8 T cells, Tregs, B cells and macrophages) will be assessed. Clinicopathological data regarding tumour type, histology, anatomical site of biopsy and treatment history/exposure will be collected for correlation.

Results

We have gathered a total of 363 patient samples, representing 20 different cancer types, from 13 unique studies/cohorts included in the Marathon of Hope Cancer Centres Network (MOHCCN) initiative at PM2C. All sample slides have been clinically annotated, stained for mIHC markers and are undergoing multispectral image analysis. RNA-Seq analysis is completed and bioinformatics analysis is ongoing.

Conclusions

Data generation is in progress and preliminary results will be presented.

Outcome/Impact

The immune compartment of the TME can influence tumour cell survival and interfere with cancer treatments. Current standard of care pathology reports does not offer information on immune infiltrates. Benchmarking studies such as this will be crucial to guide the selection of appropriate technologies and algorithms for specific use cases as clinical TME assays are developed.

KEYWORDS

TME, mIHC, RNAseq, pan cancer

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62. A HIGH-RISK SUBGROUP MULTIPLE MYELOMA CLASSIFICATION BASED ON THE DETECTION OF PR MINOR SUBCLONES

Noémie Leblay[1], Sheri Skerget[2], Sungwoo Ahn[1], Rémi Tilmont[1], Holly Lee[1], Mansour Poorebrahim[1], Daniel Penaherrera[2], Elie Barakat[1], David Jung[1], Nizar Bahlis[1], Jonathan J. Keats[2], and Paola Neri[1]

[1]Arnie Charbonneau Cancer Institute, University of Calgary, AB, Canada; [2]Translational Genomics Research Institute, Phoenix, Arizona, US

The development of new treatments has dramatically improved the overall survival (OS) of Multiple Myeloma (MM) patients. However, it remains an incurable disease and it is currently difficult to identify patients with high-risk (HR) features associated to poor survival. In this study we have evaluated a new method to identify HR disease based on the presence of proliferative (PR) subclones characterized by the presence of a highly proliferative gene signature.

Methods

Bulk RNA analysis was performed on primary MM cells obtained from 718 newly diagnosed patients (CoMMpass study); while single cell RNA (scRNAseq) was done in 32 relapsed/refractory MM (RRMM) patients with available serial samples. For bulk RNAseq, we used the mRNA HyperPrep kit. For scRNAseq studies, unbiased mRNA profiling was performed using the GemCode system.

Results

In the bulk RNA dataset, the PR subtype comprised 7.1 per cent of patients with a mix of classic genetic subtypes and a very poor clinical outcome (OS of 21.3 months). Of note, 25.5 per cent of patients belonging to a non-PR subtype at diagnosis transitioned to the PR subtype. Regardless of initial subtype, patients who progressed to the PR subtype succumbed to their disease rapidly (88 days after detection of progression). In the scRNAseq dataset, the PR signature, defined as present in at least five per cent of cells, was identified in 38 per cent of RRMM cases, including some with favourable prognostic groups. Importantly, poor OS was observed in patients with the PR subtype (six months) compared to patients without the PR subtype (36 months). Furthermore, all PR patients retained their signature over time, and in 44 per cent of patients an enrichment of PR cells was observed at subsequent relapse, consistent with Darwinian clonal evolution due to therapeutic pressure.

Conclusions

PR subclones, defined by bulk or scRNA transcriptional signatures, can be detected in patients with different molecular subgroups of MM, including those classified as standard risk by the currently used classifications. The presence of these minor PR subclones is associated with HR features and very poor patients' survival.

Outcome/Impact

Future classifications should take into account the presence of these PR subclones for better disease prognosis and development of novel therapeutics.

KEYWORDS

multiple myeloma, high-risk disease, single-cell RNAseq, new classification

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63. MECHANISMS OF RESISTANCE TO T CELL IMMUNOTHERAPIES IN MULTIPLE MYELOMA

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Targeted T cell immunotherapies exhibit promising efficacy in inducing deep remissions in relapsed refractory multiple myeloma (MM); however, clinical responses are not universal and most patients relapse. Elucidating the biological mechanisms underpinning MM resistance to T cell therapies is critical for optimizing clinical outcomes.

Methods

In order to investigate the tumour-intrinsic and -extrinsic factors contributing to MM resistance to T cell therapies, we collected serial bone marrow (BM) aspirates and peripheral blood (PB) from patients treated with anti-BCMA/GPRC5D CAR T or TCE. MM cells were profiled using bulk whole genome sequencing (WGS), scRNA and scCNVseq. BM or PB T cells were subjected to scTCRseq and tested in ***in vitro*** functional assays.

Results

Our analysis focused on 40 relapsed refractory MM patients treated with anti-BCMA or GPRC5D TCE/CAR T. Biallelic ***TNFRSF17*** loss was noted in 20 per cent of cases post anti-BCMA CAR T. Among progressors post-anti-BCMA TCE, 42.8 per cent had biallelic or monoallelic deletion of ***TNFRSF17*** coupled with mutations in extracellular domain of BCMA. Recurrent BCMA mutations included p.Arg27Pro, p.Ser30del, p.Pro34del. At relapse post anti-GPRC5D TCE, convergent evolution of antigen escape clones was noted in four out of five cases. ***In vitro*** assays demonstrated that BCMA extracellular domain mutations prevented the binding and cytolytic activity of anti-BCMA TCEs. GPRC5D nonsense/missense mutations led to dysregulated ER trafficking, preventing their expression on cell surface. Transcriptomic analysis of patient T cells at relapse post TCE revealed phenotypic markers of activation and exhaustion. Despite this, functional assays conducted ***in vitro*** with TCEs showed that the T cells effectively eliminated MM cells, indicating that their cytolytic function was maintained at clinical relapse.

Conclusions

Convergent evolution of antigen escape clones is a predominant tumour-intrinsic mechanism of MM relapse post anti-BCMA or -GPRC5D TCE/CAR T. T cells from patients at relapse maintain their effector function, suggesting that T cell exhaustion is not a critical limitation in TCE therapy.

Outcome/Impact

Serial monitoring for antigenic escape and evaluation of T cell fitness are essential in optimizing the sequencing of T cell immunotherapeutic agents in MM.

KEYWORDS

cellular and t cell engager immunotherapy, multiple myeloma

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64. HETEROGENEITY IN CHROMATIN STATES DEFINE A DISEASE SPECTRUM IN SYNOVIAL SARCOMA

Lee, Kiera[1,2,3], Hofvander J[1,2]*, Qiu A[1,2,3]*, Carles A[1], Cao Q[1], Moksa M[1], Steif J[1], Su E[1], Goytain A[3], Singer S[4], Andrulis IL[5], Wunder JS[6], Mertens F[7], Nielsen TO[3], Hirst M[1,2] *Equal contribution*

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Synovial sarcoma (SyS) is a soft-tissue malignancy characterized by a pathognomonic chromosomal translocation and the SS18-SSX fusion oncoprotein. Research shows that SS18-SSX associates with BAF, a chromatin remodelling complex, deregulating of the chromatin architecture. SyS show few recurrent mutations beyond SS18-SSX but have varying clinical and histological presentation. We hypothesized that, given the lack of driving secondary alterations, the clinical heterogeneity may be explained by epigenetic heterogeneity.

Methods

We performed multi-omics analysis on 52 primary human SyS tumours using RNA-seq, WGS, WGBS and ChIP-seq (up to eight histone marks). Survival data was acquired. NanoString and immunohistochemistry was done on pathology samples. Machine learning was leveraged to develop a signature for prognostication. Cell viability assays provided drug vulnerability data.

Results

Our analysis revealed a continuum of epigenomic states across the cohort at fusion target genes independent of recurrent genetic changes. We identify subtypes of SyS defined by enhancer states and reveal unexpected relationships between H2AK119Ub and active marks. The number of bivalent promoters, dually marked by the repressive H3K27me3 and activating H3K4me3 marks, has strong prognostic value and outperforms tumour grade in predicting outcome. Finally, we identify SyS epigenomic features including H3K4me3 expansion associated with DNA hypomethylation of promoters in which SyS display the lowest mean methylation level of any sarcoma subtype. We explore this feature as a potential vulnerability in SyS cell lines and identify WRD5 inhibition through OICR-9427 treatment as a promising therapeutic strategy.

Conclusions

Primary pretreatment SyS epigenomes are highly heterogenous harbouring a continuum of bivalent promoter number at SS18-SSX target genes. Distinctive, aspects of the SyS epigenome include aberrant repressive mark relationships, and DNA hypomethylation. These novel insights have uncovered potential prognostic and therapeutic opportunities for SyS.

Outcome/Impact

SyS tumours have distinct and heterogenous epigenomic landscapes that can inform prognosis and have revealed WDR5 inhibition as a potential therapeutic vulnerability.

KEYWORDS

synovial sarcoma, epigenomics, genomics, precision oncology

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65. MULTI-OMIC ANALYSIS OF LONGITUDINAL LIQUID BIOPSIES FROM MELANOMA PATIENTS UNDERGOING IMMUNE OR TARGETED THERAPY

Rong Ma[1,2], Rached Alkallas[1,2], Jamie Magrill[1,2], Mathieu Lajoie[1,3], Vanessa Gaspar[1,3], Paméla Thébault[4,5], Nicolas Montpas[6], Ann Brasey[6], Pauline Cassart[6], Vincent Richard[7,8], Alexandra Lissouba[1], Nebras Koudieh[9], Nicholas Rozza[9], Eric Xu[9], Nicholas R. Bertos[9], Philippe Lefrançois[8,10], Wilson H. Miller Jr[8], Sonia Del Rincon[8], Margaret Redpath[11], Alan Spatz[8], Rahima Jamal[4,12], Anne-Marie Mes-Masson[4,13], Simon Turcotte[4,13], Marie-Christine Guiot[1,9,14], Kevin Petrecca[14], May Chergui[9], Ari-Nareg Meguerditchian[9], Sarkis Meterissian[9], Keith Richardson[9], Beatrice Wang[9], Kevin Watters[9], Marathon of Hope Cancer Centres Network – Quebec Node (MOH-Q), Guillaume Bourque[2], Lambert Busque[6,13,15], Christoph Borchers[7,8], Réjean Lapointe[4,5,13], Ioannis Ragoussis[2], Hamed Najafabadi[2], John Stagg[4], Catalin Mihalcioiu[9], Ian R. Watson[1,3,9]*

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Immune checkpoint inhibitors (ICIs) and BRAF/MEK inhibitors targeting the BRAF p.V600E mutation are standard-of-care (SOC) therapies for melanoma patients; however, both forms of therapies are limited by inconsistent response rates and the development of resistance. We hypothesized that by analyzing dynamic blood changes in patients receiving these treatments we could identify novel biomarkers predictive of therapy response, further improving personalized patient care.

Methods

In our study, we analyzed longitudinal blood samples from 22 melanoma patients treated with either anti-PD1 (n = 12), anti-PD1 + anti-CTLA4 (n = 10), or BRAFi + MEKi (n = 4), part of the MOH-Q cohort 6. We performed multiple assays, including flow cytometry, TCR sequencing, and the single-cell 10x multiome assay on peripheral blood mononuclear cells (PBMCs), as well as cytokine profiling and mass-spec proteomics on plasma. Additionally, whole genome and transcriptome sequencing (WGTS) were performed on surgical resections for a subset of these patients (n = 13).

Results

Firstly, when comparing TCR sequencing data from Responders (R, n=13) and Non-Responders (NR, n=9) to ICI therapy, we observed the greatest difference in the early phase of ICI treatment (***i.e.,*** one to four months on-treatment), showing a significant reduction in T cell clonotype diversity exclusively among responders. Secondly, upon examining plasma biomarkers, we found that IL17A, IL12/IL23p40, and IP10, along with a cluster of proteins involved in the 'Complement Cascade' pathway, exhibited higher concentrations in NR than in R. Thirdly, in comparing complete response patients receiving ICIs or targeted therapy, we detected differences in specific cytokines (***e.g.,*** IL7, VEGF) and proteins (***e.g.,*** ICAM1, IGHG1) from samples taken three to four months on treatment.

Conclusions

Our preliminary survey of the various profiling assays on liquid biopsies revealed that ICI treatment had a weaker effect in promoting T cell clonal expansion in NR compared to R patients. Furthermore, NR patients tended to exhibit higher levels of specific cytokines and proteins associated with the innate immune system.

Outcome/Impact

We anticipate that a more comprehensive understanding of ICI and BRAF/MEK inhibitor longitudinal effects via liquid biopsies could significantly refine treatment strategies, enhancing the effectiveness of melanoma treatment by optimizing therapy selection and timing for evaluation.

KEYWORDS

melanoma, immunotherapy, liquid biopsy, multi-omic analysis

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66. UTILIZING AN IPSC-DERIVED BONE MARROW-LIKE ORGANOID MODEL TO INVESTIGATE DRUG RESISTANCE MECHANISMS IN ACUTE MYELOID LEUKEMIA

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Acute myeloid leukemia (AML) is a severe blood cancer with poor outcomes and five-year survival rates under 35 per cent overall and approximately 10 per cent in patients above 60 years old[1]. Although classification and patient risk stratification have improved due to increased genomic characterization, progress in treatment has lagged, largely due to the heterogeneity of disease-initiating leukemic stem cells (LSCs) in an individual patient[2]. LSCs escape chemotherapies in the hematopoietic niche[3], while comprehensive modelling of this mechanism remains challenging due to a lack of physiological human models.

Methods

Human cell-derived organoids represent a potential bridge between 2D ***in vitro*** studies and animal models. Recent breakthroughs in scaffold technologies, hydrogel engineering and improvements in induced pluripotent stem cells (iPSCs) culture formed the basis for the development bone-marrow like organoid models, that this study is based on[4]. Immunofluorescence imaging on laser scanning confocal and 3D Thunder microscopes as well as fluorescently activated cell sorting (FACS) are utilized to validate cellular composition and evaluate treatment response[4].

Results

Existing bone marrow-like differentiation protocols[4,5] were used to establish a reliable, high-throughput screening platform with uniform organoid formation. Reproducible cellular composition across different iPSC, human embryonic stem cell and patient-derived iPSC cell lines was shown. Vascular sprouting, which is traditionally difficult to achieve ***in vitro***, was consistently generated across cell lines, with primary AML samples showing preferential homing close to vascular sprouts. Generated organoids were resistant to 5-Azacytidine and maintained three-dimensional cellular structure, which emphasizes suitability for drug screening studies. Patient samples seeded and treated in organoids replicated clinical response state towards 5-Azacytidine.

Conclusions

We were able to establish a reliable, organoid differentiation protocol from three different human stem cell sources. Drug treatment of patient samples within the organoids reliably replicated clinical response, while showing superiority over a simultaneous ***in vivo*** xenograft model.

Outcome/Impact

Utilizing a novel differentiation protocol, we simulate the bone marrow niche to investigate drug resistance mechanisms in AML. The established, high-throughput platform can be used for drug screening and ***in vitro*** treatment of primary AML samples with advanced translational capacity compared to existing preclinical models and may also reduce the necessity of using animal models.

KEYWORDS

acute myeloid leukemia, leukemic stem cells, organoids, 5-azacytidine

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67. UNDERSTANDING THE MOLECULAR BASIS OF IMMUNE DYSREGULATION IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Chronic lymphocytic leukemia (CLL) affects 2,500 older adults in Canada annually, and many more live with this condition, altering quality of life and simultaneously exerting pressure on strained healthcare systems. Patients with this condition are at risk of progressive disease or symptoms that require treatment. Many who don't require treatment still suffer from the immune dysfunction which leads to second malignancies or infections. There are key known molecular poor prognostic markers in CLL; none that predict immune dysfunction. The omic natural history of the immune environment nor the impact of various therapies on its' cellular composition is known.

Methods

Multi-omics data integration with CPTAC-recommended analytical tools as well as other programs from bioconductor packages like sparse PCA, sparse PLS and spars PLS-DA and weighted gene co-expression network analysis (WGCNA) will be employed to identify biomarkers for better predictability of treatment outcomes. Significant features will be correlated to potential CLL specific available clinical molecular marker data like IGHV mutational status, ZAP70 expression level and genomic instability like del11q, del17p, del13q, trisomy 12 will be accounted for by determining their respective roles in immunological interferences along with clinical outcomes.

Results

Analysis includes two cohorts, one never treated (at time of selection) with clinical outcomes of interest (death, treatment or second cancers) and a second where patients in remission were assessed post various treatments. Available data from whole genome sequencing (WGS) and bulk RNAseq will help shed light on association between mutational status of CLL driver genes like ATM, TP53, NOTCH1, BRAF and BIRCA, and predicted biomarkers involved in microenvironment-CLL cross-talks. Transcriptomics of the non CLL immune cells of the patients in remission will be evaluated.

Conclusions

Mutational status of driver genes and presence of molecular features enhances interaction of CLL cells with microenvironment leading to CLL proliferation with poor prognosis. Future work will evaluate the expression pattern of immune cells in both cohorts.

Outcome/Impact

Predicting treatment options based on the expression of discovered molecular features will improve duration of response in time limited therapies and predict resistance mechanisms in continuous therapies.

KEYWORDS

chronic lymphocytic leukemia, immune dysregulation

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68. DEFINING THE SPATIAL ORGANIZATION OF IMMUNE INFILTRATES IN COLORECTAL CANCER AND ITS RELATIONSHIP WITH TUMOUR SUBTYPES

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Colorectal cancer (CRC) is the fourth most common cancer among Canadians. In CRC, accumulating genomic mutations – often a consequence of microsatellite instability – can enhance the immunogenicity of tumours, making them potential targets for the immune system. T cells are typically front-of-mind as leading anti-cancer immunity; however, we and others have demonstrated that natural killer (NK) cells, B-cells, and macrophages in the tumour microenvironment (TME) are also associated with improved patient outcomes. The collaboration and organization of these immune cell subsets is poorly understood but may be key to anti-CRC immunity.

Methods

CRC Tissue microarrays (TMAs) were created from a retrospective cohort of surgically resected stage III node positive tumours (1997-2009, n=117). TMAs were assessed using Opal multiplex immunohistochemistry (IHC) to identify immune cell populations, including T cells, NK cells, macrophages, and B cell subsets. Tumours were processed with digital pathology and bioinformatics to identify individual cells and their phenotypes. Immune populations and potential interactions within the tumour were assessed using neighbourhood analysis via unsupervised clustering algorithms.

Results

Initial assessment of CRC tumours (n=26) shows distinct and non-random cellular coinfiltration immune cell types. To assess co-localization patterns, we performed neighbourhood analysis and identified six unique neighbourhoods based on the co-localization of specific immune cell subtypes. N0: high infiltration of all identified immune phenotypes; N1: epithelium infiltrating CD68+CD16+ macrophages and CD94+CD16+ NK cells; N2: stroma with only CD68+ cells; N3: tumour epithelium devoid of immune cells; N4: budding CRC cells (panCK+) surrounded by stromal immune cells; and N5: epithelium infiltrating CD20+ B cells and CD94+CD16+ NK cells. Microsatellite unstable CRC displays a significantly increased frequency of N0 (immune infiltrated epithelium) compared to microsatellite stable CRC (p=0.0175).

Conclusions

CRC tumours display distinct patterns of the types, arrangement and localization of immune cells beyond the known patterns of macrophage and T cell infiltration. Genomic tumour features may predict arrangement of immune cells in the tumour epithelium and stroma.

Outcome/Impact

Linking the immune landscape in the TME with clinical characteristics may inform disease progression and illuminate opportunities for precision medicine.

KEYWORDS

colorectal cancer, spatial biology, microsatellite instability, immune infiltration

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69. SYMPTOM BURDEN IN 784 PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS: CORRELATION WITH INFLAMMATORY/GENETIC BIOMARKERS AND REDUCED SURVIVAL

Alisa Poullet[1,2], Lambert Busque[1,2,3], Michaël Harnois[3], Natasha Szuber[1,2,3]

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Myeloproliferative neoplasms (MPN) are rare blood cancers that significantly impact patients' quality of life. Data from large registries, however, are limited and associations with genomics, unclear. We aimed to analyze symptom burden and its correlation with age, disease biology/genetics, treatment and overall survival (OS) in a large, real-world population.

Methods

Multicentre prospective study analyzing MPN-Symptom Assessment Form Total Symptom Scores (MPN-SAF TSS; validated questionnaire, ten symptoms graded 0-10) from patients diagnosed with polycythemia vera (PV), essential thrombocytosis (ET), or myelofibrosis (MF); enrolled in the Quebec CML-MPN Research Group registry, with 1+ questionnaire completed between 2013-2022 (conventional statistics; JMP® Pro 14.1.0, US).

Results

4105 MPN-SAF were completed by 784 patients (n=285 PV, n=422 ET, n=77 MF). Clinically significant total scores (>20), were associated with females ($p<0.0001$) and MF cases ($p=0.03$). Those <40 years vs older patients had significantly higher fatigue scores ($p=0.03$) and abdominal pain ($p=0.007$). Higher symptom scores associated significantly with increased C-reactive protein (CRP) ($p=0.03$), anemia ($p<0.05$), delayed treatment initiation ($p=0.0009$) and absence of antiplatelet agents ($p=0.0006$), as well as – importantly - *JAK2V617F* variant allele frequency >50 per cent (VAF) ($p=0.03$). Of those with high scores, a considerable proportion remained untreated (39 per cent). Over time, 43 per cent experienced worsening scores despite interventions. Higher mean MPN-SAF score was associated with significantly worse OS ($p=0.05$). Multivariate analysis revealed absence of antiplatelet therapy, age at diagnosis greater than 65 years, male sex and high-level inactivity as independent predictors of inferior OS (HR 29, 6, 3 and 3 respectively).

Conclusions

This large-scale study discloses novel findings such as severe symptoms in younger patients as well as potential symptom burden biomarkers: CRP, anemia, and for the first time, genetic marker – *JAK2* VAF. Moreover, it confirms the negative survival impact of high symptom burden in a real-world setting.

Outcome/Impact

Deterioration of symptoms despite therapy highlights the need for new therapies to alleviate MPN symptom burden. Furthermore, deep genomic profiling (TFRI supported) is currently underway with the goal of linking symptom scores and specific genetic markers, enabling state-of-the-art, personalized patient assessment and management.

KEYWORDS

myeloproliferative neoplasms, symptom burden, genomics, real-world evidence

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70. SEQUENCING OF PRIMARY TUMOURS AND CELL-FREE TUMOUR DNAS REVEALS GENOMIC ALTERATIONS RELATED TO AGGRESSIVE PROSTATE CANCER

Samira Rahimirad[1], Eleonora Scarlata[1], Lucie Hamel[1], Seta Derderian[1], Ginette McKercher[1], Fadi Brimo[2], Fred Saad[3], Armen Aprikian[1,4], Simone Chevalier[1,4,5]

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Prostate cancer (PCa) is a cause of death for patients failing current therapies and reaching the metastatic stage. Detecting genomic modifications over the disease course may help control progression. This study identifies alterations in primary tumours of patients with lethal PCa and shared and/or exclusive ones in serial cell-free tumour (cft)DNAs of one case.

Methods

Tumours of high cellularity were identified in prostate tissues from radical prostatectomy (RP) cases (n=25) and processed for whole-genome sequencing along with matched germline (blood) DNA. Cell-free (cf)DNA was isolated from serial plasma of a lethal case and sequenced. Mutations and copy number variations (CNVs) were identified through bioinformatics.

Results

Already established PCa-related CNVs were found in our cases: losses in PTEN(72 per cent), NKX3-1(68 per cent), TP53(48 per cent), RB1(32 per cent) and gains in MYC(40 per cent) and NCOA2(32 per cent). Deletions at 21q22.3(80 per cent) harboring SIK1 and at 11p15.5(84 per cent) containing MRPL23 and H19 were discovered. A new genomic loss at 22q11.21(44 per cent) with PRODH promoting PCa cell survival was detected. In the selected patient for cfDNAs sequencing, 52 somatic mutations were identified in the tumour, of which 17 were found in cftDNAs. The cftDNA fraction increased over his trajectory. Somatic mutations in PCa-related genes (PRSS3, FOLH1, KMT2C) were differentially detected in tumour versus cftDNAs. No gain in MYC was found in either. Seven mutations and CNVs promoting neuroendocrine and stem-like cell phenotypes were detected solely in cftDNAs prior to death, supporting a metastatic origin. His germline DNA contained mutations in DNA damage response genes (not in BRCA1/2) and PDGFRB. Overall, there was an over-representation of cell cycle, DNA repair and senescence genes in this patient.

Conclusions

The sequencing of primary prostate tumours pinpoints recurrent and new genomic alterations in patients with lethal PCa, without addressing the issue of residual disease. This was overcome by sequencing cftDNAs in serial liquid biopsies of a patient and led to the discovery of novel modifications at late stages of progression.

Outcome/Impact

The identification of genomic changes by sequencing cftDNAs will improve our understanding of lethal PCa. Ongoing studies on more patients will lead to genomic signatures to test during progression, and thereby pave the way to precision oncology.

KEYWORDS

prostate cancer progression, liquid biopsy, cell-free tumour DNA, genomic alterations

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71. ARTIFICIAL INTELLIGENCE FOR EFFICIENT, HIGH FIDELITY VARIANT INTERPRETATION IN PRECISION ONCOLOGY

Reisle, Caralyn[1,2], Grisdale, Cameron J.[1], Krysiak, K.[3], Danos, A.M.[3], Khanfar, M.[3], Pleasance, E.[1], Saliba, J.[3], Bonakdar, M.[1], Griffith, M.[3], Griffith, O.L.[3], Jones, Steven J.M.[1,2]

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Interpretation of genomic findings remains one of the largest barriers to automation in processing precision oncology patient data due to the high level of expertise in cancer biology, genomics, and bioinformatics required. Accurate cancer knowledge bases (KBs) are essential to interpreting genomic findings by facilitating matching patient variants to their known therapeutic, prognostic, diagnostic, and biological relevance in literature; but reliable KBs still require expert review, leading to a bottleneck in expanding content.

Methods

In an international collaboration with the team responsible for the cancer KB CIViC (https://civicdb.org), we have curated a companion dataset of cancer facts and their corresponding evidence in the literature using the web-based tool hypothesis (https://web.hypothes.is). Cancer facts represent individual entries in CIViC and are composed of therapeutic, prognostic, diagnostic, and biological associations of variants. Therapeutic associations are used to describe the response or resistance of tumours harbouring a particular variant treated with a particular drug. Using this bespoke dataset, we have fine-tuned large language models (LLMs) (ex. LLama2) to act as an expert KB reviewer.

Results

The cancer fact dataset contains annotated data curated by 11 experts from more than 500 publications. Our fine-tuned LLM KB reviewer model has achieved promising results in verifying cancer facts and continues to improve as further data is collected. The reviewer model can be used in tandem with human experts to streamline the review process and prioritize content for review.

Conclusions

Artificial intelligence can be used to facilitate matching patient variants to therapies described in the literature by ensuring accurate data collection into cancer KBs.

Outcome/Impact

Artificial intelligence and natural language processing will play a key role in the future of evidence-based medicine enabling precision oncology programs to efficiently and accurately interpret genomic data. Increased capacity and reduced turn-around-time are critical in preparing precision oncology programs to transition from clinical trials to the standard of care.

KEYWORDS

artificial intelligence, large language models, precision oncology, variant interpretation

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72. DEFINING DIFFERENCES IN KYNURENINE METABOLISM ACROSS BREAST CANCER TUMOURS

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Emerging evidence shows that metabolites have functional, or bioactive roles that may be harnessed therapeutically. Here, I will focus on how bioactive metabolites of the kynurenine pathway (KP) of tryptophan metabolism differs across breast cancer tumours and define their phenotypic effects ***in-vitro***.

Methods

LC-MS/MS-based metabolomics was performed across a cohort of 15 triple negative breast cancer (TNBC), 15 estrogen receptor positive (ER+), and 15 human epidermal growth factor receptor 2 (HER2+) fresh frozen tumour biopsies and their corresponding normal tissue, all from the Nova Scotia Health Breast Tumour Biobank. Using western blotting of these same specimens, we grouped tumours based on expression of two key KP enzymes: indole 2,3 dioxygenase (IDO1) and kynureninase (KYNU) and then correlated metabolite levels with these enzymes. We also tested the effects of significantly different metabolites across KP groups using ***in vitro*** cell proliferation and colony formation assays and determined their putative mechanism of action using target engagement proteomics.

Results

We observed significant elevation of the KP metabolites 3-hydroxyanthranilic acid (3-HAA) and kynurenine, in high-IDO1 TNBC tumours, but not in their normal counterparts. Phenotypic analysis of KP metabolites ***in vitro*** revealed that 3-HAA decreases cell proliferation and colony formation in several breast cancer cell lines. We also identified several proteins of the 40S ribosomal subunit as putative targets of 3-HAA alluding to translation inhibition as a possible mechanism of action.

Conclusions

These data suggest that the KP may be manipulated to elicit 3-HAA-mediated cytotoxicity to target IDO1-high breast cancers as a potential new therapeutic strategy.

Outcome/Impact

This project will improve our fundamental understanding of kynurenine pathway metabolites in breast and other cancer cells to help identify novel drug targets and improve the efficacy of current therapeutics.

KEYWORDS

breast cancer, metabolism, kynurenine pathway, tumours

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73. PREDICTING RESPONSE TO IMMUNE CHECKPOINT BLOCKADE IN SOFT TISSUE SARCOMA

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Despite the immunologically cold tumour microenvironment of most soft tissue sarcomas (STSs), recent studies show a subset of sarcoma patients respond to immune checkpoint blockade (ICB). Here, we develop an approach to predict ICB response in STS patients through multi-cohort whole transcriptome sequencing (WTS) data integration.

Methods

While multiple aspects of the tumour and microenvironment play roles in ICB response, many of these are measurable from the perspective of the transcriptome, including immune cell composition, checkpoint expression, etc. We use a machine learning tool (***mosaicMPI***) to integrate our MOHCCN WTS cohort (98 samples from 86 patients) with several external datasets of STS and normal tissues, including cohorts with known ICB responses (552 sarcomas; 1,267 normal samples). Data integration is based on shared transcriptional programs that are further grouped into communities of biological themes using network analysis. The community themes were characterized using pathway enrichment, and separately validated through estimation of immune cell fractions (***immunedeconv***). These communities were then linked to clinical patient phenotypes (e.g., ICB responses, immune class labels, etc.), enabling label transfer across cohorts and identification of MOHCCN patients that could benefit from ICB.

Results

WTS integration with ***mosaicMPI*** stratified STS patients from multiple cohorts into those with immune-cold/ICB-resistant tumours, and those with immune-hot/ICB-responding tumours. Using ***immunedeconv***, we further annotated patients with high versus low infiltration of immune cell populations that shape ICB response, including CD8+ T cells, B-cells, and macrophages. Together, our analyses revealed that ~9 per cent of MOHCCN STS patients have tumour profiles that are highly similar to those of ICB responders.

Conclusions

We distinguished putative ICB responders using immune cell estimation and multi-cohort data integration, transferring ICB response status (and other clinical and molecular variables) to our MOHCCN cohort from external datasets.

Outcome/Impact

Our integrative WTS-based approach leverages well-annotated, clinically relevant external cohorts to characterize our MOHCCN STS dataset and predict ICB response. This will facilitate stratification of prospectively profiled sarcoma patients likely to benefit from immunotherapies at disease relapse. Because this strategy is disease-agnostic, we anticipate it will be broadly useful to other MOHCCN projects.

KEYWORDS

data integration, sarcoma, patient stratification, (immunotherapeutic) response prediction

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74. PROFILING THE TRANSCRIPTOME TO IDENTIFY NOVEL GENETIC MEDIATORS OF BREAST TUMOUR IMMUNE SUPPRESSION

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Breast cancer progression and response to immune checkpoint inhibitor (ICI) treatment are influenced by T cell proportions within the tumour microenvironment. Higher levels of T cells are associated with enhanced efficacy of ICIs in breast cancer treatment; however, estrogen receptor positive (ER+) tumours exhibit lower T cell levels, limiting the effectiveness of ICIs in this subtype. Investigating genetic factors contributing to immune suppression holds promise for enhancing ICI treatment efficacy in ER+ breast cancers and other subtypes.

Methods

Tumour biopsies from 16 ER+ breast cancer patients in the MOH-ACC cohort were profiled by RNA-sequencing and corresponding formalin fixed tissue sections were stained using multiplex immunofluorescence for CD8+ (CD3+/CD8+) T cells. Analyses aimed to identify genes negatively correlated (Spearman coefficient < -0.8, p-value < 0.01) with this T cell subset. Additionally, expression and clinical data from The Cancer Genome Atlas Breast Adenocarcinoma cohort (TCGA-BRCA) was leveraged to analyze the role of candidate genes in patient survival and their differential expression in tumour compared to normal-adjacent tissue.

Results

Among the 12 genes meeting the Spearman coefficient threshold, the long non-coding RNA (lncRNA) LINC00536 exhibited notably strong correlations with CD8+ T cells (Spearman coefficient of -0.853, p-value = 0.0016). Moreover, patients from the TCGA-BRCA cohort with high expression of LINC00536 had significantly worse survival compared to those with low expression of the lncRNA (hazard ratio = 1.721, p-value = 0.0031). Additionally, LINC00536 showed enrichment in breast tumours compared to normal-adjacent samples in the TCGA-BRCA cohort ($\log_2$(fold-change) = 1.98, adjusted p-value = 4.263e-12).

Conclusions

The integration of transcriptome and immunofluorescence analyses unveiled genetic factors within breast cancer cells potentially involved in tumour immune suppression. Notably, the lncRNA LINC00536 has emerged as a promising candidate warranting further investigation.

Outcome/Impact

LINC00536 may hold potential as a therapeutic target to enhance the effectiveness of immune checkpoint inhibitors for ER+ breast cancer patients.

KEYWORDS

ER+ breast cancer, CD8+ t cells, immune checkpoint inhibitors, long non-coding RNA

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75. INTEGRATIVE BIOMARKER DISCOVERY IN IMMUNOTHERAPY AND DEVELOPMENT OF AN INFORMATIC TOOL

Farnoosh Abbas-Aghababazadeh[1], Mayra Carneiro[3], Sisira Nair[1], Sogol Bondar Sahebi[1], Philippe Bedard[2], John Stagg[3] and Benjamin Haibe-Kains[1]

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Although immunotherapies provide substantial benefits for various cancers, 60 to 80 per cent of patients do not respond, highlighting the need to identify non-responders to understand resistance mechanisms and improve treatment decisions. A recent meta-analysis of clinical genomic data aimed to validate biomarkers but faced limitations due to shallow molecular profiling and small sample sizes across different cancer types.

Methods

A literature review sourced studies with clinical datasets of patients treated with immune checkpoint blockers, such as anti-PD-1/anti-PD-L1 and anti-CTLA-4, along with their associated sequencing data. Public immunotherapy trial datasets were standardized to MOHCCN data standards. We analyzed the associations of published biomarkers predictive of immunotherapy response individually and through a meta-analysis, employing federated learning techniques to ensure data confidentiality and securely leverage data across institutions.

Results

Our comparative meta-analysis of genomic and transcriptomic biomarkers across 14 tumour types found that 25 out of 55 gene signatures were predictive of immunotherapy response. Our pipeline was also integrated into the MOHCCN Digital Health and Discovery Platform to enhance biomarker discovery for immunotherapy using a federated learning network.

Conclusions

Our computational framework will integrate clinical genomic data from diverse sources, including the MOHCCN database and public datasets, effectively identifying biomarkers predictive of immunotherapy responses.

Outcome/Impact

The biomarker discovery tool will utilize robust genomic predictors to enhance trial designs and potentially increase response rates in cancer patients undergoing targeted and immune therapies.

KEYWORDS

immunotherapy, biomarker, meta-analysis, federated learning

**This poster will not be judged.*

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76. INTEGRATING CANCER GENETICS AND IMMUNOLOGY TO INFORM PRECISION THERAPIES FOR PANCREATIC CANCER

Riley J. Arseneau[1,3], Jorge A. Mejia[2,3], Sarah Nersesian[2,3], Stacey N. Lee[2], Carley Bekkers[4], Boris L. Gala-Lopez[1,2,3,5], Ravi Ramjeesingh[1,6], Thomas Arnason[1,4], Daniel Gaston[1,3,4], Jeanette E. Boudreau[1,2,3]

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Pancreatic ductal adenocarcinoma (PDAC) is highly lethal, and usually only diagnosed after it has accumulated multiple genetic mutations. Genetic mutations have consequences on the biology of cancer cells and can influence the tumour's response to therapy and its interaction with the immune system. Against PDAC, natural killer (NK) cells may be ideal effectors because they target tumours using a diverse array of germline-encoded receptors and are triggered for cytotoxicity and inflammation by signs of stress and DNA damage. We hypothesize that genetic features of the tumour will predict immunogenicity in the tumour microenvironment and inform targets for NK cell-based therapies.

Methods

We compared gene expression data from a PDAC TCGA cohort in patients either wild type or mutated for PDAC driver mutations. To assess the genomic landscape of patients with PDAC treated in Nova Scotia, we used a targeted solid tumour sequencing panel, the TruSight Oncology 500 (n=27). To ascertain immune contexture, we used Opal multiplex immunohistochemistry (mIHC), spatial analysis, and machine learning (n=59). Univariate and multivariate analyses are ongoing to query relationships between immune cells, localization, genetics and patient outcomes.

Results

Mutations in *KRAS*, *TP53*, *CDKN2A,* and *SMAD4* were associated with unique landscapes of ligands targetable by NK cells. Notably, *TP53* and *CDKN2A* mutated tumours exhibited an upregulation of the NK cell activating ligand, *ULBP2* ($p<0.05$). Early analysis of our Atlantic Canada cohort confirmed genomic alterations consistent with TCGA data. Concurrently, we developed and optimized a spatial Opal multiplex panel for these PDAC tissues which reveals five distinct patterns of immune, tumour, and stromal cell co-localization, which we termed "neighbourhoods".

Conclusions

Genetic analysis of the first 27 samples from Nova Scotia has been completed, with sequencing of further samples underway. By conducting Opal mIHC and sequencing on the same patient cohort, we expect to be able to test predictions made from the public datasets and define the relationship between genomic features and immune cell infiltration.

Outcome/Impact

Profiling the genetic landscape of PDAC may inform NK cell-based immunotherapies.

KEYWORDS

pancreatic cancer, genomics, spatial biology, precision therapy

**This poster will not be judged.*

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77. INTEGRATING GENOMIC AND MEDICAL IMAGING DATA FOR PRECISION ONCOLOGY

Caryn Geady, Katy Scott, Stephenie Prokopec, Jeffrey Bruce, Celeste Yu, Phillipe Bedard, Trevor Pugh, Lillian Siu, Benjamin Haibe-Kains

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Radiogenomics explores the link between disease imaging characteristics and genetic features, offering insights into cancer risk stratification and management. Combining radiomic and genomic features promises the discovery of imaging biomarkers for various endpoints. However, standardized processes are needed to address limitations like small sample sizes and lack of prospective validation in radiogenomic analysis.

Methods

An open-source radiogenomic analysis pipeline is currently under development, utilizing publicly available datasets as its foundation. This comprehensive pipeline aims to offer an end-to-end solution, encompassing preprocessing, quality control of both imaging and genomics data, feature extraction and reduction, correlation assessment between different data types, and inference. The pipeline is programmed in Python, so far utilizing the Med-Imagetools and READII packages for image handling. The development process leverages publicly available datasets sourced from The Cancer Imaging Archive (TCIA). The ultimate objective is to apply this pipeline to MOHCCN datasets, with a particular focus on INSPIRE.

Results

Processed datasets include publicly available NSCLC-Radiogenomics (n=144) and CPTAC-HNSCC (n=59), along with the MOHCCN dataset INSPIRE (n=25), with anticipated increases in size as trial data becomes available. Each sample comprises a CT image, tumour segmentation, and RNA-Seq data. Pre-processing, quality control, and feature extraction have been applied to all datasets, resulting in 1016 PyRadiomics features per contoured image and gene expression data for approximately 60,000 genes (INSPIRE and CPTAC-HNSCC) and 22,126 genes (NSCLC-Radiogenomics). Next steps involve feature reduction, including selection of protein coding genes, and correlation assessment, projected for completion by May 2024.

Outcome/Impact

Upon completion of this project, we anticipate contributing to the MOHCCN community via our radiogenomic analysis pipeline – a necessary step in the standardization of Methods. This will support the development of robust multi-modal predictors of patient outcomes and comprehensive evaluation of tumour heterogeneity at both genomic and radiographic levels and will ideally catalyze an interest in the application of radiogenomics across PM2C and beyond.

KEYWORDS

radiogenomics, correlative analysis, survival, methods standardization

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78. CLINICAL AND PHENOTYPIC CONSEQUENCES OF HLA MEDIATED ANTIGEN PRESENTATION DEFICIENCY IN PANCREATIC ADENOCARCINOMA AT BULK AND SINGLE-CELL RESOLUTION

Michael Geuenich[1,2], B. Grünwald[3], C. Yu[1,2], T.T. Ju[1,2], A. Zhang[4], O. Hamza[4], GH. Jang[4], G. O'Kane[3], F. Notta[4], S. Gallinger[4], KR. Campbell[1,2,4,5,6,7]*

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Pancreatic ductal adenocarcinoma (PDAC) is a lethal neoplasm of the pancreas characterized by a low survival rate and limited treatment options. Despite the success of immunotherapy in various cancers, its efficacy in PDAC remains low. The underlying reasons for this discrepancy are not fully understood. A possible determinant could be the loss of heterozygosity (LOH) at the human leukocyte antigen (HLA) loci, resulting in compromised antigen presentation. In this work, we aim to identify and validate HLA LOH events to gain insights into their prevalence and clinical and phenotypic impact on PDAC.

Methods

We applied LOHHLA, a specialized pipeline to identify HLA LOH events from paired whole genome sequencing to two Marathon of Hope cohorts comprising over 650 patients. We contextualized these results with paired transcriptome-wide gene expression data and immunohistochemistry stains. In addition, we developed a machine learning classifier to predict HLA LOH from transcriptomic data. We transfer this classifier to single cell RNA sequencing data to identify the impact of (subclonal) HLA LOH on the tumour microenvironment.

Results

HLA LOH events occur in approximately 30 per cent of PDAC patients, with a small number of these deletions being focal deletions. We find evidence that focal but not non-focal LOH is a driver of immune escape. Overall, we find that HLA LOH events are early genetic alterations and observe a significant association between HLA LOH and the Basal PDAC expression subtype. In addition, we find that HLA expression is the most important determinant of lymphocyte infiltration followed by an intact HLA locus. Moreover, our transcriptomic classifier allowed us to accurately identify HLA LOH events from RNA sequencing data, which we validated in an independent cohort. Finally, we applied this classifier to single cell sequencing data and identified phenotypic differences in multiple compartments of the tumour microenvironment between samples with intact HLA and HLA LOH.

Conclusions

Our study provides the most in depth characterization of the consequences of altered antigen presentation in PDAC to date.

Outcome/Impact

These findings contribute to a better understanding of the immune landscape in PDAC and may have implications for the development of immunotherapeutic strategies tailored to this challenging cancer type.

KEYWORDS

pancreatic cancer, immunotherapy

**This poster will not be judged.*

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79. DECIPHERING THE PAN-CANCER FUNCTIONAL LANDSCAPE OF CIRCULAR RNAS IN PRIMARY VERSUS ACQUIRED RESISTANCE FOR PRECISION ONCOLOGY

Peter Hyunwuk Her[1,2], Housheng Hansen He[1,2], Benjamin Haibe-Kains[1,2]

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Cancer immunotherapy, especially through immune checkpoint inhibitors, has revolutionized oncology but faces challenges due to primary and acquired resistance. While research in this area has focused on the dynamic changes of protein coding genes, emerging as key players in cancer and as potential biomarkers are circular RNAs (circRNA), a unique and understudied class of noncoding RNAs that form covalently closed loop structures. Previous work from our group utilizing the Canadian Prostate Cancer Genome Project uncovered both global circRNA abundance and the abundances of specific circRNAs to be associated with clinical outcome, distinct from their parental linear genes. However, the roles of circRNAs and their capacity as biomarkers in immune resistance remains unknown.

Methods

Leveraging patient-derived whole transcriptome data obtained through the Immune Resistance Interrogation Study (IRIS) cohort at the Princess Margaret Cancer Centre, we have streamlined and implemented a pipeline to identify and quantify circRNAs. This will be followed by unsupervised clustering to categorize patient groups linked to immunotherapy resistance. To determine the functionality and regulatory roles of circRNAs in primary versus acquired resistance, we will perform differential and multi-omic analyses. Longitudinal samples will be utilized to track circRNAs over time and establish potential biomarkers. Additionally, we will integrate circRNA information for the development and validation of predictive models.

Results

By applying the identification and quantification pipeline, we identified thousands of circRNAs across patient samples. Further work will be done to elucidate the precise roles of these circRNAs and their potential as biomarkers for resistance in cancer immunotherapy.

Conclusions

The preliminary findings confirm the presence of circRNAs in the IRIS cohort, underscoring their potential for further in-depth functional analyses.

Outcome/Impact

Insights from circRNAs have the potential to improve treatment planning and reveal novel therapeutic targets, thus enhancing the survival and quality of life of patients.

KEYWORDS

circular RNAs, immunotherapy resistance, noncoding RNA biomarkers, transcriptomics

**This poster will not be judged.*

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80. CHARACTERIZING THE MOLECULAR LANDSCAPE OF NON-V600 BRAF MUTANT COLORECTAL CANCER: TOWARD THE IDENTIFICATION OF NOVEL THERAPEUTIC VULNERABILITIES

Emmanuelle Rousselle[1,2], Suzanne Kazandijan[2], Gerry Batist[1,2], April Rose[1,2]

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The BRAF kinase is mutated (V600 mutants known as Class 1 and non-V600 as Class 2 & 3) in 10 to 15 per cent of metastatic colorectal cancer (CRC). We aim to identify therapeutic vulnerabilities to improve treatment options for this patient population which are currently effective for less than 25 per cent of V600 BRAF CRC.

Methods

We obtained RNA counts for BRAF mutant CRC from public databases. We performed Gene Set Enrichment Analysis (GSEA) and differential gene expression analysis. We leveraged CIBERSORTx to identify differences in immune cell populations between V600 and non-V600 BRAF CRC. We applied a modified technique to assign microsatellite stable (MSS) and instable (MSI) status to samples lacking this information.

Results

We identified 70 V600 (n=54 MSI, n=16 MSS) and 10 non-V600 (n=1 MSI, n=9 MSS) BRAF mutant CRC samples. 517 genes were significantly differentially expressed between the BRAF classes, including important regulators of immune responses. Our previous work showed genetic alterations in non-V600 BRAF CRC were enriched for Wnt beta-catenin signalling pathway and GSEA reflected this at the transcriptional level as well (padj<0.05). Furthermore, GSEA revealed an enrichment for the interferon gamma signalling in the V600 BRAF CRC samples (padj<0.05). Interestingly, using CIBERSORTx we found that V600 BRAF CRC samples have higher proportions of CD8 T cells vs. non-V600 samples, and this remained true when restricted to MSS samples (p=0.0002 & p=0.0299 respectively). Conversely, non-V600 BRAF CRC samples have higher proportions of CD4 T cells (p<0.0001).

Conclusions

We identified transcriptomic differences and inferred differences in the tumour immune microenvironment of V600 vs. non-V600 BRAF mutant CRC. This warrants further validation at the protein level and may have important implications for immunotherapy development.

Outcome/Impact

CRC is the second leading cause of cancer-related deaths worldwide and BRAF-mutant CRC patients have worse outcomes. Identifying therapeutically vulnerable drivers of cancer progression will help define better treatment strategies.

KEYWORDS

colorectal cancer, BRAF mutations, transcriptomic analysis, immune cell populations

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