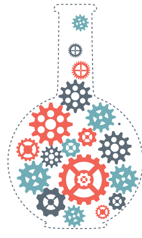
2023
Margie & Robert E. Petersen
NEUROENDOCRINE TUMOR RESEARCH SYMPOSIUM

November 15-17, 2023
Boston, Massachusetts



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WELCOME TO THE 2023 MARGIE AND ROBERT E. PETERSEN NEUROENDOCRINE TUMOR RESEARCH SYMPOSIUM!

Thank you for joining us to learn about and discuss the latest advances in neuroendocrine cancer research, made possible by NETRF funding.

Our Symposium is unique in its focus and format. Our grantees will share their progress through posters and presentations, and they look forward to your questions and comments. There will also be plenty of opportunities for informal networking and collaboration. Our hope is that you take full advantage in the spirit of building community and sharing information.

NETRF is the largest private global funder of NET research. Since 2005, we have invested more than $36 million in neuroendocrine cancer research. NETRF has funded two and a half times more NET investigators than the NIH. We are proud of our impact on the field and our success in recruiting and supporting new researchers in NETs.

Thank you to NETRF's grantees, past and present, who have dedicated their careers to seek greater understanding of NETs, discover improved treatments, and work toward cures for hundreds of thousands of patients and their families. Your willingness to share your work and data is truly appreciated. For those of you who are new to the field and our Symposium, we welcome you to our diverse and thriving scientific community.

Lastly and importantly, on behalf of the Board of Directors, Board of Scientific Advisors, and the NETRF staff, we thank our loyal donors, whose continued support sustains our ambitious research portfolio.

Our amazing staff has worked hard to make this meeting an energizing experience for all, so we hope that you enjoy your time in Boston.

Elyse

Elyse Gellerman
CEO

Todd

Todd Gilman
President, NETRF Board of Directors

NEUROENDOCRINE TUMOR
RESEARCH FOUNDATION
DEDICATED TO CURING NEUROENDOCRINE CANCER

AGENDA

2023
Margie & Robert E. Petersen
NEUROENDOCRINE TUMOR
RESEARCH SYMPOSIUM

DAY 1 – Wednesday, November 15, 5:30 p.m.-8:00 p.m.

- **5:30 p.m. – Registration**
- **6:00 p.m.-8:00 p.m. – Welcome Reception & Poster Session**

DAY 2 – Thursday, November 16, 7:30 a.m.-5:00 p.m.

- **7:30 a.m.–9:00 a.m. – Breakfast & Registration**
- **9:00 a.m.–9:15 a.m. – Welcome**

SESSION 1: PERSONALIZED MEDICINE, 9:15 a.m.-11:00 a.m.

Session Chairs: Chrissie Thirlwell, MBBS, PhD, University of Bristol Medical School
James Bibb, PhD, University of Arizona Medical School - Phoenix

- **9:15 a.m.–9:30 a.m. – Enhancing Drug Development for Neuroendocrine Tumors: A novel radiomic signature to predict survival in patients enrolled in Alliance A021202**
  Emily Bergsland, MD, University of California San Francisco
- **9:30 a.m.–9:45 a.m. – The Detection of Alternative Lengthening of Telomeres via Chromogenic in situ Hybridization (ALT-CISH) for the Prognostication of Pancreatic Neuroendocrine Tumors and Other Neoplasms**
  Christopher Heaphy, PhD, Boston University School of Medicine
- **9:45 a.m.–10:00 a.m. – Neuroendocrine tumor ex-vivo angiogenesis; correlation with overall survival and SUTENT sensitivity**
  Nicholas Skill, PhD, Louisiana State University Health Science Center New Orleans
- **10:00 a.m.–10:15 a.m. – Establishment of novel patient-derived models for neuroendocrine neoplasms**
  Iacovos Michael, PhD, Sunnybrook Research Institute; University of Toronto
- **10:15 a.m.–10:30 a.m. – Reconciling lung carcinoid histopathological and molecular classifications**
  Alexandra Sexton-Oates, PhD, International Agency for Research on Cancer
- **10:30 a.m.–10:45 a.m. – Immunohistochemical identification of clinical subtypes and potential therapeutic vulnerabilities of lung carcinoids based on multi-omic analysis**
  Jules Derks, MD, PhD, Maastricht University Medical Centre
- **10:45 a.m.–11:00 a.m. – Discussion**
- **11:00 a.m.–11:15 a.m. – Coffee Break**

SESSION 2: IMPROVING RADIOTHERANOSTICS, 11:15 a.m.–12:15 p.m.

Session Chairs: Daniel Halperin, MD, University of Texas MD Anderson Cancer Center
Martyn Caplin, MD, University College London & Royal Free Hospital

- **11:15 a.m.–11:30 a.m. – COPPER PET with Cu-61-NODAGA-LM3 for the detection of Neuroendocrine Tumors: establishment of the Cu-61 and Cu-61-NODAGA-LM3 productions**
  Guillaume Nicolas, MD and Melpomeni Fani, PhD, University Hospital Basel
- **11:30 a.m.–11:45 a.m. – Using statins to enhance DOTATATE binding and efficacy in SSTR2-low tumors**
  Patricia Pereira, PhD, Washington University School of Medicine
- **11:45 a.m.–12:00 p.m. – All-Trans Retinoic Acid Radiosensitizes Neuroendocrine Tumors via Peptidyl-prolyl cis-trans isomerase 1 Inhibition**
  Xavier Keutgen, MD, University of Chicago Medicine
- **12:00 p.m.–12:15 p.m. – Discussion**
- **12:15 p.m.–1:30 p.m. – Lunch**
- **1:30 p.m.–1:45 p.m. – Group Photo**

SESSION 3: NET PATHWAYS & TARGETS, 1:45 p.m.-5:00 p.m.

Session Chairs: Dawn Quelle, PhD, University of Iowa
Justin Annes, MD, PhD, Stanford University

PPGLS AND LUNG NETS

- **1:45 p.m.–2:00 p.m. – Preliminary whole exome sequencing analyses of clonal relationships between premalignant and invasive neuroendocrine lesions in Diffuse Idiopathic Pulmonary Neuroendocrine Cell Hyperplasia**
  Dan Merrick, MD, University of Colorado Anschutz Medical Campus
- **2:00 p.m.–2:15 p.m. – TMEM127 exerts a tumor suppressive role in pheochromocytoma by mediating RET ubiquitin-dependent degradation**
  Hector Gonzalez-Cantu, MSc, University of Texas Health Science Center at San Antonio

PANCREATIC NETS

- **2:15 p.m.–2:30 p.m. – Vascular regulation of liver metastasis in PanNET**
  Minah Kim, PhD, Columbia University
- **2:30 p.m.–2:45 p.m. – mTORC1-ATF4 Signaling Drives Amino Acid Biosynthesis in PanNETs**
  Scott Oakes, MD, University of Chicago
- **2:45 p.m.–3:00 p.m. – ARID1A mutations drive metastasis of pancreatic neuroendocrine tumors and pancreatic adenocarcinomas by activating NTN1/UNC5B signaling**
  Chris Harris, PhD, University of Rochester Medical Center
- **3:00 p.m.–3:15 p.m. – Altered splicing programs in PNETs affect the function of neuroendocrine cells**
  Panagiota Kafasla, PhD, Alexander Fleming Biomedical Sciences Research Center
- **3:15 p.m.–3:30 p.m. – Discussion**
- **3:30 p.m.–3:45 p.m. – Coffee Break**

GASTROINTESTINAL NETS

Session Chairs: Ramesh Shivdasani, MD, PhD, Dana-Farber Cancer Institute
Jess Maxwell, MD, MBA, University of Texas MD Anderson Cancer Center

- **3:45 p.m.–4:00 p.m. – Investigating serotonin metabolism in neuroendocrine cancers**
  Po Hien Ear, PhD, University of Iowa
- **4:00 p.m.–4:15 p.m. – Mesenteric fibrosis in small intestinal neuroendocrine tumours (SI-NETs): pathogenesis and therapeutic targets**
  Maria Martins, MSc, University College London
- **4:15 p.m.–4:30 p.m. – Studying the microbiome of small intestinal neuroendocrine tumor patients**
  Eric Nakakura, MD, PhD, University of California San Francisco and Netta Mäkinen, PhD, Dana-Farber Cancer Institute
- **4:30 p.m.–4:45 p.m. – Midgut neuroendocrine tumor patients have a depleted gut microbiome with a discriminative signature**
  Merijn Mulders, MD, Erasmus University Medical Center
- **4:45 p.m.–5:00 p.m. – Discussion and End of Day**

DAY 3 – Friday, November 17, 7:30 a.m.-1:00 p.m.

- **7:30 a.m.–9:00 a.m. – Breakfast**
- **9:00 a.m.–9:15 a.m. – Welcome**

SESSION 4: TUMOR EVOLUTION & MICROENVIRONMENT, 9:15 a.m.-10:30 a.m.

Session Chairs: Minah Kim, PhD, Columbia University
Iacovos Michael, PhD, Sunnybrook Research Institute

- **9:15 a.m.–9:30 a.m. – Early emergence of high grade subclones in pancreatic neuroendocrine tumors**
  Etay Ziv, MD, PhD, Memorial Sloan Kettering Cancer Center
- **9:30 a.m.–9:45 a.m. – Characterizing chemoresistant persister cells in relapsed high-grade neuroendocrine carcinomas**
  Allison Stewart, PhD and Carl Gay, MD, PhD, University of Texas MD Anderson Cancer Center
- **9:45 a.m.–10:00 a.m. – Molecular landscape of pancreatic neuroendocrine tumors at single-cell resolution**
  Jeanna Qiu, AB, and William Hwang, MD, PhD, Harvard Medical School
- **10:00 a.m.–10:15 a.m. – Single-cell transcriptomics identified IGF2 and VEGF as growth factors in PNETs**
  Chang Chan, PhD, Rutgers Cancer Institute
- **10:15 a.m.–10:30 a.m. – Discussion**
- **10:30 a.m.–11:00 a.m. – Coffee Break & Grab Lunch Box**

SESSION 5: IMMUNOTHERAPIES, 11:00 a.m.-12:45 p.m.

Session Chairs: Jennifer Eads, MD, University of Pennsylvania
Carl Gay, MD, PhD, University of Texas MD Anderson Cancer Center

- **11:00 a.m.–11:15 a.m. – Temozolomide treatment induces an MMR-dependent hypermutator phenotype in well differentiated pancreatic neuroendocrine tumors**
  Jérôme Cros, MD, PhD, INSERM, Université Paris Cité
- **11:15 a.m.–11:30 a.m. – TILs from panNET liver metastasis: in search of novel adoptive transfer strategies for the treatment of NETs**
  Mauro Cives, MD, University of Bari “Aldo Moro”
- **11:30 a.m.–11:45 a.m. – The role of the B7x signaling pathway in the development and progression of neuroendocrine tumors**
  Ziqiang Yuan, MD, Rutgers University
- **11:45 a.m.–12:00 p.m. – Therapies targeting CDK4/6 cause regression, immune cell activation, and sensitization to PD-L1 immunotherapy in pancreatic neuroendocrine tumors**
  Dawn Quelle, PhD, University of Iowa
- **12:00 p.m.–12:15 p.m. – A novel hormone based anti-SSTR bispecific T-cell engager for the treatment of neuroendocrine tumors**
  Eleonora Pelle, MD, Moffitt Cancer Center
- **12:15 p.m.–12:30 p.m. – Mutation-Targeted Immunotherapy for Atypical Pulmonary Carcinoids using CRISPR/Cas9**
  Kevin McHugh, PhD, Rice University
- **12:30 p.m.–12:45 p.m. – Discussion**
- **12:45 p.m.–1:00 p.m. – Meeting Wrap Up and Close Out**

ORAL PRESENTATIONS: SESSION 1, PERSONALIZED MEDICINE

Enhancing Drug Development for Neuroendocrine Tumors: A novel radiomic signature to predict survival in patients enrolled in Alliance A021202

Laurent Dercle(1), Susan M. Geyer(2), Timothy R. Asmis(3), Sanja Karovic(4), Michael Knopp(5), Ardaman Shergill(6), Andrew B. Nixon(7), Spencer Behr(8), Eileen Mary O'Reilly(9), Jonathan R. Strosberg(10), Jeffrey A. Meyerhardt(11), Lawrence H. Schwartz(9), Emily K. Bergsland(8)

(1) Columbia University Medical Center, New York, NY, USA; (2) Alliance Statistics and Data Management Center, Mayo Clinic, Rochester, MN, USA; (3) Ottawa Hospital Cancer Centre, Ottawa, ON, Canada; (4) Inova Center for Personalized Health and Schar Cancer Institute, Fairfax, VA, USA; (5) The Ohio State University, Columbus, OH, USA; (6) University of Chicago, Chicago, IL, USA; (7) Duke Cancer Institute, Durham, NC, USA. (8) University of California San Francisco, San Francisco, CA, USA (9) Memorial Sloan Kettering Cancer Center, New York, NY, USA (10) Moffitt Cancer Center, Tampa, FL, USA; (11) Dana Farber Cancer Institute, Boston, MA, USA.

Presenting Author

Emily Bergsland, MD

Background

Efficient drug development for extrapancreatic neuroendocrine tumors (ep-NETs) is hampered by lack of actionable mutations for risk stratification, drug toxicity leading to treatment cessation, and suboptimal monitoring through conventional imaging due to inter-observer variability, slow tumor growth, and cytostatic drugs. We sought to validate, using radiomics and machine learning, the performance of a signature for estimating overall survival (OS) in patients with ep-NETs enrolled on Alliance A021202 (a national randomized phase II trial of pazopanib versus placebo). Pazopanib improved progression-free survival, at the expense of increased toxicity. When comparing local versus central imaging review, 52% of patients experienced a discordant scan result, with discordance observed in both directions (e.g., some patients potentially over- or under-treated because of difficulties establishing the timing of radiographic progression [Geyer et al. ASCO GI 2021; Abstr#361]), highlighting the need for improved NET imaging methods.

Methods

This prognostic study used radiomics and machine learning to retrospectively analyze CT images obtained at baseline and first follow-up and their associated clinical metadata. Data were prospectively collected in A021202 patients (N=171 with low- to intermediate-grade advanced ep-NETs of any primary site, showing radiologic progressive disease within 12 months). For the radiomic analysis, images (baseline and 3 months) and clinical data were available for 123 patients, who were randomly assigned to training and validation sets using 1:1 ratio (60:63 patients). In the training set, machine-learning integrated a radiomics signature derived from imaging features extracted from CT-segmented tumors in a dataset of 3,242 patients with advanced solid tumors with clinical variables, resulting in the creation of an ep-NET signature (epNETSig) that predicted overall survival (OS) landmarked from the first follow-up. Kaplan Meier analysis (Log-rank test) was employed to evaluate the efficacy of epNETSig in estimating OS on the validation set.

Results

In the training set, epNETSig combined the externally validated radiomics signature with 7 clinical variables (primary site, age, disease status-regional/distant, grade, ethnicity, arm, and functional status by order of importance) to optimize the prediction of OS in this heterogeneous dataset. In the validation set, the high-risk group (epNETSig above the median, n=31) had a median [95CI] OS of 26.1 months [15.5- 50.9] with 21 events, while the low-risk group had a longer median OS of 60.8 months [46.3-NA] with 14 events (P=0.013).

Conclusions

The preliminary findings of this prognostic study suggest that a signature combining clinical data with features from conventional CT images at baseline and on first follow-up has potential to provide an early readout of future OS probability in patients with advanced ep-NETs. This could enhance risk stratification in ep-NET drug development trials and identification of clinically significant treatment effects, aiding the evaluation of future therapeutic agents in ep-NETs by targeting potential beneficiaries while reducing exposure and toxicity in non-beneficial cases. Analysis of potentially predictive factors is ongoing. Additional novel radiologic endpoints such as tumor volume and tumor growth rate are also under evaluation.

Support: U10CA180821, U10CA180882, U24CA196171; NETRF Investigator Award; U10CA180863, CCS 707213; U10CA180888; and Novartis. https://acknowledgments.alliancefound.org
Clinical trial information: NCT01841736.

The Detection of Alternative Lengthening of Telomeres via Chromogenic in situ Hybridization (ALT-CISH) for the Prognostication of Pancreatic Neuroendocrine Tumors and Other Neoplasms

Christopher M. Heaphy (1), Simmi Patel (2), Katelyn Smith (2), Anne R. Wondisford (3), Michelle L. Lynskey (3), Roderick J. O'Sullivan (3), Kimberly Fuhrer (2), Xiaoli Han (2), Raja R. Seethala (1), Ta-Chiang Liu (4), Dengfeng Cao (4), Onur Ertunc (5), Qizhi Zheng (5), Marija Stojanova (1), Amer H. Zureikat (6), Alessandro Paniccia (6), Kenneth Lee (6), Melanie C. Ongchin (6), James F. Pingpank (6), Herbert J. Zeh (7), Melissa E. Hogg (8), David Geller (6), J. Wallis Marsh (9), Randall E. Brand (10), Jennifer S. Chennat (10), Rohit Das (10), Kenneth E. Fasanella (10), Charles Gabbert (10), Asif Khalid (10), Kevin McGrath (10), Savreet Sarkaria (10), Harkirat Singh (10), Adam Slivka (10), Dennis Hsu (10), Janie Y. Zhang (10), Benjamin A. Nacev (10), Marina N. Nikiforova (2), Abigail I. Wald (2), Neel Vaddi (11), Angelo De Marzo (5), Phoenix D. Bell (2), and Aatur D. Singhi (2)

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Presenting Author

Christopher Heaphy, PhD

Background

Telomere maintenance and elongation allows cells to gain replicative immortality and evade cellular senescence during cancer development. While most cancers utilize telomerase, a subset of cancers engage the Alternative Lengthening of Telomeres (ALT) pathway for telomere maintenance. ALT is mediated through a homology-directed DNA repair mechanism and is associated with inactivating mutations in the ATRX/DAXX histone chaperone complex. While present in 5-10% of all cancers, the prevalence of ALT is dramatically higher in certain cancer types, including pancreatic neuroendocrine tumors (PanNET) and leiomyosarcomas (LMS). Molecular studies have shown ALT to be an important prognostic biomarker of shorter relapse-free survival (RFS) for patients with PanNETs and other neoplasms. However, the preferred method of detecting ALT in tissue is by fluorescence in situ hybridization (FISH), which has several clinical limitations. These issues necessitate the creation of a novel chromogenic ALT assay that can be easily implemented into routine practice.

Methods

An ALT-CISH assay was developed using 20 normal pancreata, 20 ALT-positive PanNETs, and 20 ALT-negative PanNETs. Thereafter, it was validated on a multi-institutional cohort of 360 surgically resected PanNETs and correlated with multiple clinicopathologic features, RFS, and FISH results. In addition, 109 LMS cases were evaluated by both CISH and FISH and ALT status was assessed for prognostic significance.

Results

Upon optimization, ALT-CISH was identified in 31% (112 of 360) primary PanNETs and was 100% concordant with FISH testing. As expected, ALT correlated with adverse prognostic findings and distant metastasis (all $p<0.004$). The 5-year RFS for patients with a PanNET differed by ALT status (ALT-positive was 35% vs. 94% for ALT-negative). By multivariate analysis, ALT was an independent poor prognostic factor (HR: 4.46, 95% CI: 2.25-8.82, $p<0.001$). Similarly, ALT was detected in 40% of LMS patients, associated with shorter RFS, and analogous to PanNETs, an independent poor prognostic factor (HR: 1.74, 95% CI: 1.01-2.98, p=0.043).

Conclusions

An ALT-CISH assay was developed and validated in 2 tumor types enriched for ALT, PanNETs and LMS. ALT-CISH testing has multiple advantages over FISH that facilitate its widespread clinical use in the detection of ALT and prognostication of patients with these diverse neoplasms. Furthermore, ongoing work is being performed to identify the exact spatial architecture and composition of the tumor and tumor microenvironment in ALT-positive and ALT-negative PanNETs by spatial transcriptomics profiling either via an unbiased spatial sequencing approach (Visium) or a high multiplexed in situ hybridization approach (MERFISH).

Neuroendocrine tumor ex-vivo angiogenesis; correlation with overall survival and SUTENT sensitivity

Nicholas J. Skill (1), Kenneth Avanzino (2), Joseph Mason (2), Yvette Bren Mattison (2), J. Philip Boudreaux (2), Ramcharan Thiagarajan (2) and Mary Maluccio (2)

(1) Louisiana State University Health Science Center, New Orleans, LA 70012. Department of Interdisciplinary Oncology, (2) Louisiana State University Health Science Center, New Orleans, LA 70012. Department of Surgery

Presenting Author

Nicholas J. Skill, PhD

Background

SUTENT, (Sunitinib) is a multiple receptor tyrosine kinases (RTKs) inhibitor that likely impacts tumor growth and metastasis through pathologic angiogenesis. In 2011, based on clinical trial NCT00428597, the FDA approved SUTENT for the treatment of neuroendocrine tumors (NET). NCT00428597 reported 1) 11.4month median progression-free survival in sunitinib group vs 5.5months in controls 2) 9.3% objective response rate in sunitinib group versus 0% in controls, and 3) 9 deaths in sunitinib group (10%) versus 21 deaths in controls (25%). The purpose of this was threefold. 1) Correlate ex-vivo surrogate of NET angiogenesis with overall survival in order to stratify risk and improve patient selection for anti-angiogenesis intervention. 2) Quantify NET SUTENT sensitivity in order to increase objective response by focusing on predicted responders. 3) Identify NETs that are "exceptional responders" (as defined NCI Division of Cancer Treatment and Diagnosis) to SUTENT that can be studied using OMIC analysis to advance new/novel therapies.

Methods

NET tumor samples, collected during surgery were tested using a previously published ex-vivo angiogenesis assays developed in our laboratory. Tumors were dissected into 1mm samples and cultured in thrombin-coated wells for 14days. Endothelial cell outgrowths, which are indicative of angiogenesis, were quantitated for 1) percentage initiation and 2) growth intensity. Ex-vivo angiogenesis and OS: OS was calculated based on dates of diagnosis and dates of death extracted from medical and public records. Patients were stratified into two groups for Kaplan Meier curves. Group 1. Low-angiogenesis. Angiogenesis initiation and/or growth (< average-2SEM). Group 2. High-angiogenesis. Angiogenesis initiation and/or growth (> average-2SEM). NET SUTENT Sensitivity: Matched NET tumor sample were treated with 188nM SUTENT. Initiation and growth were compared to untreated tumors to calculate percentage inhibition. SUTENT exceptional responders were identified by three criteria: 1) Ex-vivo angiogenesis growth and initiation greater than mean-2SEM, 2) SUTENT-induced inhibition of initiation and growth >80%, and 3) SUTENT ex-vivo angiogenesis initiation and growth < mean-2SEM.

Results

Ex-vivo angiogenesis and OS: The 10yr survival for NET patients was 62% (N=58). In non-survivors the ex-vivo NET angiogenesis initiation and growth were greater (58.3 ± 1.4 and 3.4 ± 0.1 $p<0.05$) when compared to surviving patients (50.9 ± 4.9, 2.6 ± 0.36). NET tumors with low ex-vivo angiogenesis were linked to an increased OS when

compared to high ex-vivo angiogenesis. SUTENT Sensitivity: Ex-vivo response to SUTENT was inconsistent. In some samples SUTENT had no effect. In contrast, some tumors, that grew in control conditions, had 100% inhibition of sprouting. SUTENT reduced ex-vivo angiogenesis initiation and growth > 50% in 29% of tumors 3) SUTENT exceptional responders: Four tumors were identified as exceptional responders and subjected to genomic & proteomic analysis.

Conclusions

Quantification of NET tumor ex-vivo angiogenesis activity has the potential to inform and improve treatment stratification and define personalized treatment plans; the appropriateness of anti-angiogenesis treatment in particular. Future studies develop biomarkers to replace our ex-vivo assay. Ultimately it will be important for us to cross-reference predicted SUTENT efficacy against actual SUTENT response in patients.

Establishment of novel patient-derived models for neuroendocrine neoplasms

Nilakshi Kulathunga(1), Zoey Wang(1,2), Sara Mar(1,2), Hubert Tsui(1,3), Julie Hallet(4,5), Calvin Law(4,5), Hon S. Leong(1,2), Iacovos P. Michael(1,2)

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Presenting Author

Iacovos Michael, PhD

Background

Neuroendocrine cancers arise in the endocrine cells localized throughout the body. Most are found in the pancreas, stomach, and small and large intestines – referred to as gastroenteropancreatic-neuroendocrine tumors (GEP-NETs). GEP-NETs are heterogenous in their etiology, morphology, and phenotypes with limited treatment options. Thus, there is an urgent need to develop better models to understand the underpinning pathways involved in the progression of the disease and identify new therapies. Patient-derived xenografts (PDX) are cancer models that use fresh patient tumor specimens transplanted into a host organism. This approach enables precision oncology, for example, by assessing the response to various drug treatments and combinations thereof.

Methods

In this study, we focus on establishing a patient-derived xenograft ex-ovo (PDXovo) model for GEP-NETs to broaden our understanding and screen for therapeutic candidates. In the PDXovo model, patient-derived fresh tumor tissue is transplanted into a highly vascularized extra-embryonic membrane, a chicken embryo's chorioallantoic membrane (CAM). The PDXovo technology has many advantages: (a) take rates of fresh tumor specimens are high, (b) the implantation site of the chick embryo, e.g., CAM, is highly accessible, allowing for easy manipulation, (c) highly powered experiments are possible due to the low cost of chick embryos, (d) animal ethics approval are not needed as embryos do not fall under animal board jurisdiction, and (e) permits drug paneling with various agents.

Results

To optimize the conditions of NET samples' engraftment, we first used mouse and human cell lines and tumor fragments from the prototypical mouse RIP1-Tag2 model of PanNETs to recapitulate the clinical scenario. We demonstrated successful derivation of PDXovo both from NET cell lines and mouse PanNET tumor fragments transplanted into the CAM. Subsequently, we established PDXovos using fresh, surgically excised samples from patients with NETs. We showed we can derive PDXovos from primary tumors and lymph node and liver metastasis biospecimens. So far, we have used biospecimens from four patients, three with small-intestine NETs and one with colon NET. We derived 76 PDXovos with > 80% take rate (33 primary tumors, 85% take rate; 18 lymph node metastasis tumors, 76% take rate; 25 liver metastasis tumors, 78% take rate). To characterize the growth properties and vascularity of the PDXovos, we use high-frequency ultrasound (HF-US). We currently use immunostaining approaches to characterize the derived PDXovos, such as staining for Ki-67, synaptophysin, and SSTR2, and markers for tumor microenvironment cells, such as immune cells. We plan to examine the response of the PDXovos to various treatments, such as the antiangiogenic inhibitor sunitinib, the mTOR inhibitor everolimus, and the immune checkpoint inhibitor anti-PD-1 antibody, and combinations thereof.

Conclusions

Overall, this study showed that we can use a novel approach to derive PDX models from biospecimens from NETs successfully. To our knowledge, this is the first approach to allow for a reproducible and large-in-scale establishment of NET PDX models. Utilizing the PDXovo system as a pre-clinical model for NETs will result in new findings that might result in novel approaches to treating patients with NETs.

Reconciling lung carcinoid histopathological and molecular classifications

Alexandra Sexton-Oates (1), Emilie Mathian (1), Nicolas Alcala (1), Ricardo Blazquez-Encinas (2), Alejandro Ibanez-Costa (2), Catherine Voegele (1), Francesca Damiola (3), Justo P. Castaño (2), David Taïeb (4), Liming Chen (5), Sylvie Lantuejoul (3), Thomas Walter (6), Lynnette Fernandez-Cuesta (1), Matthieu Foll (1)

(1) Rare Cancers Genomics Team, International Agency for Research on Cancer, Lyon, France, (2) Department of Cell Biology, Physiology, and Immunology, University of Córdoba, Córdoba, Spain, (3) Biopathology Department and Pathology Platform, Centre Léon Bérard Hospital, Lyon, France, (4) Department of Nuclear Medicine, Aix-Marseille University, Marseille, France, (5) Department of Mathematics and Computer Science, Ecole Centrale de Lyon, Lyon, France, (6) Department of Hepato-Gastroenterology and Digestive Oncology, Hôpital Edouard Herriot-Hospices Civils de Lyon, Lyon, France

Presenting Author

Alexandra Sexton-Oates, PhD

Background

Lung neuroendocrine neoplasms (LNENs) are rare diseases encompassing low-grade, well-differentiated tumours (LNETs) and high-grade, poorly differentiated carcinomas. The current WHO Classification of Thoracic Tumours subdivides LNETs into low-grade (typical) and intermediate-grade (atypical) carcinoids, primarily by mitotic count and presence or absence of necrosis. Typical carcinoids exhibit better prognosis, with a 10-year overall survival rate of 89%, whilst the prognosis is worse for atypical carcinoids which have a 10-year overall survival rate of 51%. Recently, several different analysis techniques applied to carcinoids have identified clinically and biologically meaningful subgroups which do not correspond directly to the typical/atypical WHO classification system. Unsupervised multi-omics analysis has identified three molecular groups of carcinoids, named A1, A2, and B, and uncovered a new entity of LNETs, the supra-carcinoids. These molecular groups contain both typical and atypical tumours and display unique clinical and genomic characteristics. Additionally, the use of immunohistochemistry (IHC) markers, such as Ki-67, OTP, and TTF-1, has shown potential in better stratifying patients into prognostically relevant categories than the current typical/atypical system. Finally, neuroendocrine neoplasms in other body sites have benefited from advances in nuclear medicine for diagnosis and treatment, including the proposal of a nuclear imaging-based grading system, which has yet to be fully explored in LNETs. Despite these observations, there is no consensus on the optimal approach for LNET differential diagnosis and treatment. There is therefore a strong need to reconcile carcinoid classifications to improve diagnostic practice and clinical management of patients.

Methods

This work takes advantage of the LNET biorepository established as part of the ongoing lungNENomics project. Using a selection of pulmonary carcinoids with known molecular groups we will first perform IHC staining on previously proposed markers (Ki-67, SSTRs, CD44, OTP, TTF-1, HNF1A, and PD-L1) to establish the relationship between the molecular classification and protein markers. IHC will then be used to classify patients who have undergone nuclear imaging for both metabolic reprogramming and key receptor targets into their respective molecular groups, thus analysing concordance between the three diagnostic techniques. Subsequently artificial intelligence (AI) algorithms will be trained to determine whether there are distinct morphological features associated with molecular groups. Lastly, we will use spatial proteomics to understand the relationship between morphological and molecular features.

Results

To date we have (i) identified and retrieved the tissues and data required for the study from the lungNENomics biorepository, (ii) examined the concordance between the expression of genes corresponding to IHC and nuclear imaging panel proteins and the carcinoid molecular groups, and (iii) developed an AI-based computer vision algorithm for anomaly detection that is specifically applicable to LNETs.

Conclusions

Many challenges exist in the implementation of molecular findings to tumour classification and diagnosis. In this study we aim to provide the knowledge required to overcome these by using a combined approach to LNET classification that includes multi-omic data, IHC, nuclear imaging, and morphological features identified through AI. Our results will allow for the reconciling of the current and proposed classification systems, and for the implementation of robust and cost-effective diagnostic practices in LNET clinical management.

Immunohistochemical identification of clinical subtypes and potential therapeutic vulnerabilities of lung carcinoids based on multi-omic analysis

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Presenting Author

Jules Derks, MD, PhD

Background

Multi-omic studies have identified three lung carcinoid subtypes (A1, A2, B) with unique expression for OTP, ASCL1 & HNF1a genes. We developed an immunohistochemical (IHC) panel for lung carcinoid subtype identification and clinical-pathological correlation.

Methods

IHC was evaluated in a blinded fashion in mRNA-profiled lung carcinoid (n=5 per A1/A2/B). IHC H-score was assessed for OTP/ASCL1/HNF1a. Then, surgically resected pathology revised lung carcinoids (2003-2012) from a Dutch cancer/pathology registry (n=478) were evaluated including matching metastasis of 20 patients with recurrent lung carcinoid. Principal component clustering (PCA) was applied for H-score evaluated markers and correlated to RNA based established H-score IHC cut-offs. Additionally, potential therapeutic targets (SSRT2a/DLL3) were assessed. Normal lung tissue distant to the primary lung carcinoid tumor (≈30/type) was screened for neuroendocrine cell hyperplasia (NECH) using OTP/ChrA IHC. Disease free survival (DFS) was calculated, chi-square and log rank applied.

Results

IHC and mRNA expression of matched primary samples were near-identical. IHC identified A1 in 50% classified as OTP(high/low) - ASCL1(high) - HNF1a (low), A2 (36%) as OTP(high) - ASCL1(low) - HNF1a (high) and B (14%) as OTP(low) - ASCL1(low) - HNF1a (high/low) generally matching clusters established by PCA analysis. Patients with A1 were middle-aged/elderly females (81%) with a peripheral tumour (54%). By contrast, in A2 were primarily young patients with endobronchial tumours (86%). In B, the rate of recurrence was highest (21%, vs. 12% (A1) and 7% (A2)). Patients with A2 had significantly longer DFS compared to A1 and B. Matching primary-metastatic tumours showed similar IHC expression patterns. SSRT2a was highest in A2-B (median H-score 160 and 147 vs. 7 (A1)) while DLL3 showed unique expression in A1 (median H-score 51, vs. 0 (A2/B). NECH was enriched in A1 (26% vs 5% (A2) and 2% (B) also in lung tissue at distance).

Conclusions

An OTP/ASCL1/HNF1a IHC panel enables separation of molecular LC types into clinically different groups with distinct therapeutic vulnerabilities. Potentially these subtypes are also relevant for metastatic lung carcinoid; a retrospective multicentre NETRF funded study is currently ongoing to address this question.

During the NETRF symposium updated data will be presented, also including PCA analysis and previously identified TTF1/S100 markers.

ORAL PRESENTATIONS: SESSION 2, IMPROVING RADIOTHERANOSTICS

COPPER PET with Cu-61-NODAGA-LM3 for the detection of Neuroendocrine Tumors: establishment of the Cu-61 and Cu-61-NODAGA-LM3 productions

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Presenting Author

Guillaume P. Nicolas, MD

Background

Gallium-68 (Ga-68) labeled DOTATATE (NETSPOT®) and DOTATOC (SOMAKIT TOC®), or copper-64 (Cu-64) labeled DOTATATE (Detectnet TM) are FDA approved somatostatin receptor (SST) agonists for positron emission tomography (PET) imaging of gastroenteropancreatic NET patients. However, there are still significant limitations due to high costs, production capacity and physical properties of the radioisotopes. We propose the use of Copper-61 ($t_{1/2}$ = 3.33 h, 61% β+-fraction, Emax = 1.2 MeV), a still unexplored radioisotope with much more favorable physical properties compared to Cu-64 ($t_{1/2}$ = 12.7 h and low β+-fraction (18%)), longer half-life and lower energy than Ga-68 ($t_{1/2}$ = 68 min, Emax = 1.9 MeV), and far more cost-effective production and scale up capacity. Given the superior imaging properties showed by Ga-68-labeled SST antagonists over the agonists, we combine Cu-61 with the SST antagonist NODAGA-LM3. We aim to: 1) establish Cu-61 production at 2 sites (Zurich, Switzerland and Munich, Germany) and delivery to a clinical site (Basel, Switzerland) to show feasibility of satellite distribution and 2) show safety, biodistribution, dosimetry and diagnostic efficacy of Cu-61-NODAGA-LM3 PET/CT in NET patients.

Methods

The production of Cu-61 was established at a GE cyclotron using Ni-electroplated silver coins and irradiation at 45-50 µA through the solid target. The radiochemical separation was performed on a cassette-based FASTlab (GE) platform. The production of Cu-61-NODAGA-LM3 was established in a GMP environment, using the Modular-Lab PharmTracer synthesizer (EZAG) and own labeling procedures. Quality control methods were developed and validated.

We plan a first-in-human, prospective, open-label, randomized, controlled, single center PET/CT study with Cu-61-NODAGA-LM3 at the University Hospital Basel in patients with well-differentiated gastroenteropancreatic and bronchopulmonary NET. Tumor and organ uptake, image contrast and sensitivity of Cu-61-NODAGA-LM3 PET/CT will be compared head to-head with standard-of-care Ga-68-DOTATOC PET/CT, in the same patients. Scans will be reviewed by 2 independent blinded readers. Standard of truth will be the best imaging for a given patient (including liver MRI, contrast-enhanced CT and/or SST PET/CT) at 2- to 7-month follow-up.

Results

So far, the following has been achieved: i) optimization of the Cu-61 production process by using enriched solid targets (Ni-61) and of the developmental steps for Cu-61 purification using an automated module in Zurich, ii) reproducible production of high-quality Cu-61, iii) know-how transfer and set up of Cu-61 production at the second production site in Munich and iv) development of the production and quality control methods for Cu-61-NODAGA-LM3, including validation, in Basel.

We are currently finalizing the study documentation for submission to regulatory authorities in Switzerland and to the ethics committee of Northwestern Switzerland.

Conclusions

Since the beginning of the study (Q1 2023) we have established Cu-61 production at 2 different sites (Zurich and Munich) and have prepared the product and study documentations including patient facing information. We are currently setting up the Good Manufacturing Practice (GMP) production of Cu-61-NODAGA-LM3 for human use at the University Hospital Basel. The required documentations will be submitted to the Swiss regulatory authorities and Ethics in Q3 2023 for approval. The first patient enrolled in the trial is foreseen during Q1 2024.

Using statins to enhance DOTATATE binding and efficacy in SSTR2-low tumors

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Presenting Author

Patricia Pereira, PhD

Background

Somatostatin receptor 2 (SSTR2)-targeting radiolabeled peptides are clinically approved for imaging and systemic radiotherapy of neuroendocrine tumors. However, tumor heterogeneity and low SSTR2 contribute to inadequate tumor targeting and low drug delivery that leads to disease progression. In this work, we determined modulation of SSTR2 membrane levels with pharmacologic modulators of endocytosis to enhance DOTATATE accumulation in tumors.

Methods

SSTR2-high (IMR32), SSTR2-low (FTC133), and SSTR2-negative (H292) cancer cells were used in in vitro and preclinical studies. 64Cu-DOTATATE was prepared by reacting copper-64 with DOTATATE (RCP>98%). Nu/nu nude mice bearing SSTR2-low FTC133 tumors (n=4 mice/group) where divided into three groups: Group 1) 2 doses of lovastatin given 12 h apart from each other, Group 2) 1 dose of lovastatin, and 3) no statin. Mice were given an oral administration of saline or statin (8.3 mg/kg/day). Mice were injected with 64Cu-DOTATATE and PET imaging and biodistribution were performed at two time points: 1 hour and 24 hours after injection.

Results

Given our previous studies showing that the cholesterol-depleting drug lovastatin improves the availability of receptors at the cell membrane by modulating caveolae-mediated endocytosis, we sought to determine whether the use of lovastatin would result in improved binding of the peptide DOTATATE to SSTR2 in tumors. The IMR32 cells express high levels of SSTR2, but they do not form caveolae as they do not express caveolin-1 or cavin-1 proteins, which are the major structural proteins of caveolae-mediated endocytosis. SSTR2-low FTC133 cells and SSTR2-negative H292 cancer cells express high levels of caveolin-1. IMR32 cells showed a 1.5-fold increase in SSTR2 expression at the cell membrane after statin treatment. The statin did not impact SSTR2 levels in total protein extracts. Cellular fractionation of lovastatin-treated IMR32 or FTC133 cancer cells incubated with 64Cu-DOTATATE revealed a significant increase ($P < 0.05$, Student t test) in membrane-bound radioactivity. We observed a significant decrease ($P < 0.05$, Student t test) in internalized 64Cu-DOTATATE after treatment with lovastatin. Further Western blot analyses demonstrated that lovastatin induces major depletion in endophilin, without major alterations in proteins associated with non-caveolae-mediated endocytosis.

In vivo experiments revealed a positive correlation between DOTATATE-tumor binding and SSTR2 protein levels in tumors. PET imaging of mice with SSTR2-low FTC-133 tumors showed higher tumor accumulation at 2 hours post-injection of 64Cu-DOTATATE in mice that were treated twice with statin when compared with saline. Biodistribution studies showed that both one and two doses of statin increase DOTATATE tumor accumulation compared with the control.

Conclusions

Our data suggest that statin treatment with appropriate pharmacokinetics is a potential adjuvant for radiotheranostics. Lovastatin enhanced membrane-bound DOTATATE, while also temporarily modulating proteins of the endocytic trafficking systems and enhancing target density at the cell membrane. Our ongoing studies are exploring statin/DOTATATE radiotheranostic approaches in p53 positive and negative cell lines that are transfected with varying levels of SSTR2, as well as in patient-derived xenografts. Additionally, since we have shown

that statins enhance 64Cu-DOTATATE tumor uptake, ongoing therapeutic studies are using statins in combination with Lutathera.

All-Trans Retinoic Acid Radiosensitizes Neuroendocrine Tumors via Peptidyl-prolyl cis-trans isomerase 1 Inhibition

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Presenting Author

Xavier Keutgen, MD

Background

Peptide receptor radionuclide therapy (PRRT) is a promising radiation based therapy for metastatic neuroendocrine tumors (NETs) but remains palliative. Peptidyl-prolyl cis-trans isomerase (Pin1) is an evolutionally conserved enzyme that catalyzes the cis-trans isomerization of phosphorylated serine/threonine-proline motifs of its substrates and has recently been involved in DNA double strand break (DSB) repair in BRCA-proficient breast cancer cells. Here we study whether Pin1-inhibition with All-Trans Retinoic Acid (ATRA) radiosensitizes NET cells, which could improve PRRT response.

Methods

Immunohistochemistry using Pin1 antibody was performed to evaluate Pin1 protein expression in human pancreatic and small bowel NET tissue. The pancreatic and lung NET cell lines QGP1, BON1 and NCI-H727 were treated with 4Gy of radiation (IR) and 100nM of ATRA and cell proliferation was measured. Pin1 knockdown using siRNA was also performed. To determine mechanistic effects, BRCA1 and gH2AX western blot were completed and Pin1, BRCA1, and BRCA2 mRNA expression was determined in cell lines using RT-PCR. The poly (ADP-ribose) polymerase 1 inhibitor (PARPi) Talazoparib (10nM) was added to QGP1 cells to evaluate the additive vs. synergistic effects with ATRA and IR. In vivo experiments were performed using mouse xenografts with QGP1 cell tumors. Mice were treated with 1.5 mg/kg ATRA via intraperitoneal injection, 20Gy IR, or both; tumor volume and survival metrics were calculated.

Results

Pancreatic, small bowel and lung human NET tissue expressed Pin1 protein, with 90%, 50% and 85% of pancreatic, small bowel an lung samples, respectively, expressing intermediate/high levels of Pin1. Combining ATRA + IR yielded significant decrease in cell viability vs. IR alone (QGP1 ($p = 0.0001$), BON1 ($p = 0.0001$), NCI-H727 ($p = 0.0003$)). Pin1 knockdown with siRNA + IR further decreased cell viability in QGP1 ($p = 0.0002$) and BON-1 ($p = 0.015$) cells when compared to IR alone, suggesting that ATRA radiosensitizes NET cells through Pin1 inhibition. Addition of ATRA also decreased BRCA1 ($p = 0.004$) and BRCA2 ($p = 0.016$) mRNA levels in QGP1 cells after IR as well as increased DNA double strand breaks as evidenced by increased gH2AX protein expression after treatment. ATRA synergized with Talazoparib and IR in QGP1 cells ($p < 0.0001$). In vivo, time required for tumor volume to reach 1000mm3 was 26.7 days in the no treatment, 27.5 days in the ATRA alone, 41.3 days in the IR and 73.25 days in the ATRA+IR group (ANOVA $p=0.015$). Mean tumor volume was significantly smaller at days 15, 20, 22, 26 and 40 when comparing mice xenografts in the ATRA+ IR and IR groups. Lastly, survival in the ATRA + IR group was prolonged when compared to IR alone (78 vs. 41 days $p=0.09$).

Conclusions

ATRA radiosensitizes pancreas and lung NET cells through Pin1-inhibition, likely through decreases in homologous recombination repair genes, like BRCA1, following radiation, thereby allowing proliferation of DNA damage. This ATRA-induced BRCA1-deficient phenotype synergizes with PARP1 inhibition in vitro. Additionally, initial studies have replicated ATRA's radiosensitization effect in vivo. Further studies will focus on validating these results in other cells lines, optimizing dosing of ATRA, and incorporating PRRT.

ORAL PRESENTATIONS: SESSION 3, NET PATHWAYS & TARGETS

Preliminary whole exome sequencing analyses of clonal relationships between premalignant and invasive neuroendocrine lesions in Diffuse Idiopathic Pulmonary Neuroendocrine Cell Hyperplasia

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Presenting Author

Daniel T. Merrick, MD

Background

Diffuse Idiopathic Pulmonary Neuroendocrine Cell Hyperplasia (DIPNECH) is a condition characterized by multiple bilateral foci of pre-invasive neuroendocrine cell hyperplasia (NECH) involving the medium or small airways. Clinical presentation includes chronic cough and progressive dyspnea, along with multiple nodules and mosaic air trapping radiographically. We hypothesize that DIPNECH results from a somatic mutation affecting pulmonary neuroendocrine cells resulting in proliferation and wide dispersal within the bronchial epithelium.

Methods

A cohort of 60 patients with the terms "NECH" and/or "DIPNECH" were identified in the University of Colorado Cancer Center pathology archive. Histologic review to classify lesions as NECH, carcinoid tumorlets and carcinoid tumors as well as enumeration of lesions and quantification of lesion size were performed. Clinical chart review was performed to document the presence of DIPNECH related symptoms, abnormal pulmonary function testing results, and DIPNECH related CT results, and cases were classified as consistent with DIPNECH, possible DIPNECH or non-DIPNECH. For all cases, two or more foci of NECH, the associated carcinoid tumor and/or tumorlet, if present, and normal lymph node or lung tissue were micro-dissected from formalin fixed paraffin embedded (FFPE) tissue. DNA extraction with carrier RNA (cRNA) was performed on these micro-dissected lesions. Following Qubit quantification, samples from the first 16 cases have been selected for WES to identify somatic variants using matched normal lung tissue or lymph node as reference germline sequence.

Results

All histologic reviews, full clinical and radiographic reviews on 60 potential DIPNECH cases have been completed. Of the 52 cases with complete tissue and clinic-radiologic review, 31 cases show strong histologic and clinic-radiologic features of DIPNECH. Twelve cases are classified as non-DIPNECH and 9 are indeterminant. With the establishment of a standard protocol, the NECH lesions have been assessed for each case to decide whether to combine small lesions when the tissue is very limited or to collect more robust lesions as a single lesion for subsequent DNA extraction. We have micro-dissected and extracted DNA for 16 cases thus far. A total of 61 DNA samples were generated for WES including NECH lesions, tumorlets, carcinoid tumor and normal lung tissue or lymph nodes. DNA yields were low (<50 ng) for 6, intermediate (50-100 ng) for 9, and high (>100 ng) for 46 samples. Replacement specimens for eight of the low/intermediate samples are available and undergoing re-extraction.

Conclusions

Classification of DIPNECH cases has been completed by histological and clinico-radiologic review. For DIPNECH cases, the micro-dissection of NECH, tumorlet, and carcinoid tumor, are in progress. Pre-extraction tissue requirements and optimized cRNA based DNA extraction protocols have been established. Via recent consultation with our Genomics core and Bioinformatics personnel a schedule for starting WES analyses in 2-4 weeks has been planned. Identification of 24 additional cases of sporadic carcinoid tumor to complement aim 2 studies is underway. All the WES data will be reviewed to identify potential somatic alterations underlying the development of DIPNECH and its progression to invasive carcinoid tumors.

TMEM127 exerts a tumor suppressive role in pheochromocytoma by mediating RET ubiquitin-dependent degradation

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Presenting Author

Hector Gonzalez-Cantu, M.Sc.

Background

TMEM127 encodes for a ubiquitously expressed transmembrane protein with limited knowledge into its role. TMEM127 germline loss-of-function is a driver of pheochromocytoma and paraganglioma (PPGLs), tumors derived from the adrenal medulla and extra-adrenal paraganglia, respectively. Molecularly, TMEM127 mutant PPGLs belong to the kinase cluster, characterized by kinase signaling transcriptional programs. Receptor tyrosine kinase RET, a driver of PPGLs via germline or somatic gain-of-function mutations similarly belongs to the kinase cluster. Previously, we reported that TMEM127 loss led to mTOR signaling activation, suggesting that TMEM127 loss had an impact on kinase signaling pathways associated with mTOR, such as RET. Compellingly, and in line with the shared kinase signaling pathway signatures, TMEM127 mutant PPGLs display high levels of RET at the protein level, a phenomenon which is conserved in murine and cell line models. Here we sought to mechanistically interrogate the impact of TMEM127 loss on RET in an oncogenic context.

Methods

Primary tumor samples harboring different mutations, engineered Tmem127 KO mice, and engineered TMEM127 KO cell lines were analyzed for the abundance, localization, turnover, and signaling of RET as impacted by TMEM127 loss. Additionally, we interrogated the impact of TMEM127 functional motifs in targeting RET for degradation via recruitment of an E3 ligase. Lastly, we interrogated the RET-dependent oncogenic impact of TMEM127 loss on RET by investigating viability, proliferation, and transformation features in vitro and in vivo

Results

Compellingly, and in line with the shared kinase signaling pathway signatures, TMEM127 mutant PPGLs display high levels of RET at the protein level, a phenomenon which is conserved in murine and cell line models. Further investigation revealed that TMEM127 impacted RET degradation. Mechanistically, we showed that TMEM127 was critical for the recruitment of NEDD4, an E3 ligase, to ubiquitinate RET, targeting it for endosomal trafficking and lysosomal degradation, with TMEM127 loss impacting RET localization and abundance. Our experiments with functional TMEM127 mutants determined that its C-terminal PxxY motifs were necessary to recruit NEDD4 to target RET for degradation. Lastly, in vitro and in vivo models of TMEM127 loss were found to be sensitive to RET clinical grade inhibitors.

Conclusions

Our data supports a novel tumor suppressive role of TMEM127 in PPGLs by targeting RET for degradation via recruitment of NEDD4, establishing the RET accumulation in TMEM127 mutant PPGLs as a dysregulation of this mechanism. Translationally, our work supports the clinical benefit of RET targeted therapy in TMEM127 mutant PPGLs.

Vascular regulation of liver metastasis in PanNET

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Presenting Author

Minah Kim, PhD

Improving the management of metastasis in pancreatic neuroendocrine tumors (PanNET) is critical as nearly half of PanNET patients present with liver metastases, which account for the majority of patient mortality. We identified angiopoietin-2 (ANGPT2) as one of the most upregulated angiogenic factors in the RNA-seq data from liver metastases of human PanNETs and found that higher ANGPT2 expression correlated with poor survival rates. Immunohistochemical staining revealed that ANGPT2 was localized to the endothelial cells of blood vessels in PanNET liver metastases. We observed an association between the upregulation of endothelial ANGPT2 and liver metastatic progression in both patients and transgenic mouse models of PanNETs. In human and mouse PanNET liver metastases, ANGPT2 upregulation coincided with poor T-cell infiltration, indicative of an immunosuppressive tumor microenvironment. Notably, both pharmacologic inhibition and genetic deletion of ANGPT2 in PanNET mouse models slowed the growth of PanNET liver metastases. Furthermore, pharmacologic inhibition of ANGPT2 promoted T-cell infiltration and activation in liver metastases, improving the survival of mice with metastatic PanNETs. These changes were accompanied by reduced plasma leakage and improved vascular integrity in metastases. Together, these findings suggest that ANGPT2 blockade may be an effective strategy for promoting T-cell infiltration and immunostimulatory reprogramming to reduce the growth of liver metastases in PanNETs.

mTORC1-ATF4 Signaling Drives Amino Acid Biosynthesis in PanNETs

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Presenting Author

Scott A. Oakes, MD

Background

Based on its efficacy in clinical studies, the mTORC1 inhibitor everolimus (Afinitor®) was FDA approved in 2011 for patients with advanced pancreatic neuroendocrine tumors (PanNETs); however, its benefits are relatively modest with a ~6-month average increase in progression-free survival and no profound impact on disease-free survival. To more effectively utilize this treatment, it is critical to elucidate the key signaling outputs of the mTOR pathway on PanNET tumorigenesis and the escape mechanisms that allow tumor cells to resist mTORC1 inhibition.

We previously discovered that PanNETs often upregulate the transcription factor ATF4, which is best known as the primary effector of the integrated stress response (ISR) but was also recently shown to be downstream of mTORC1 signaling in some cancers. Under conditions where amino acids are limiting, ATF4 transcriptionally upregulates genes that promote amino acid transport and biosynthesis, including serine glycine one-carbon (SGOC) metabolic enzymes. However, the connection (if any) between mTORC1, ATF4 and SGOC has not been studied in PanNETs.

Methods

In order to elucidate the potential mechanisms behind everolimus resistance, we have extensively analyzed ATF4 expression and target genes in BON-1 and QGP-1 human PanNET cell lines before and after a timecourse of everolimus treatment. In addition, we have generated everolimus resistant BON-1 and QGP-1 cell lines by

culturing them in the presence of clinically relevant concentrations of everolimus over several months and have subjected the resistant QGP-1 cell line to RNA-Seq analysis to identify differentially expressed genes and potentially modulated pathways.

Results

Confirming prior studies, we have convincingly shown that baseline ATF4 expression is elevated in both panNET cell lines as well as panNET patient samples compared to a healthy pancreas. Moreover, while we find everolimus treatment rapidly reduces ATF4 levels (mRNA and protein) along with known downstream serine-glycine biosynthesis genes (PHGDH, PSPH, PSAT1, SHMT2), the everolimus resistant cell line demonstrates an upregulation of ATF4 and ISR pathway genes. In addition, RNA sequencing indicates ~59 genes potentially controlled by ATF4 that are upregulated in everolimus resistant cells, further alluding to ATF4's critical role in conferring resistance. Finally, pharmacologic inhibition (via NCT-503) of phosphoglycerate dehydrogenase (PHGDH), the first committed enzyme in the SGOC pathway, reduces panNET cell growth in culture. We are currently testing whether genetic or pharmacologic inhibition of the ISR will re-sensitize these cells to everolimus.

Conclusions

While inhibition of mTORC1 leads to rapid loss of ATF4 expression and SGOC genes, sustained treatment ultimately confers resistance through upregulation of the transcription factor ATF4, which is necessary for expression of SGOC target genes and amino acid biosynthesis, all contributing to the survival of the panNET cells. This has been shown through our everolimus resistant PanNET cell lines that demonstrate upregulation of ATF4 in the absence of mTORC1 signaling, likely due to activation of the integrated stress response (ISR). Studies are ongoing to test the role of ISR, ATF4 and amino acid biosynthesis in mTORC1 inhibitor resistance in PanNET cell lines and mouse models.

ARID1A mutations drive metastasis of pancreatic neuroendocrine tumors and pancreatic adenocarcinomas by activating NTN1/UNC5B signaling

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Presenting Author

Chris Harris, PhD

ARID1A, a component of the SWI/SNF DNA chromatin remodeling complex, is recurrently mutated in many types of cancer. In pancreatic ductal adenocarcinomas (PDACs), mutations in ARID1A are found in 6-15% of cases, and ARID1A expression has been shown to associate with poor patient outcome. The effect of ARID1A mutations on pancreatic neuroendocrine tumors (PNETs) has not been previously studied, but we observed that ARID1A mutations were highly enriched in patients with metastatic PNETs than in patients with localized disease, indicating the importance of ARID1A mutations in driving the progression of what might otherwise be an indolent disease. We have studied ARID1A mutations in models of both PDACs and PNETs and observe that in both diseases ARID1A mutations associated with several properties common to metastatic cells including epithelial-mesenchymal transition, in vitro invasiveness, and insensitivity to anoikis. Mutation of ARID1A activated expression of the axonal guidance protein NTN1 in both PDACs and PNETs, and interaction between the NTN1 ligand and its dependence receptor UNC5b were required for the metastatic properties of ARID1A mutant cells. Treatment with a NTN1 inhibitor, NP137, overcame insensitivity to anoikis, while deletion or knockdown of UNC5b caused ARID1A mutant cells to undergo a mesenchymal-epithelial transition. In vivo, metastasis of PDACs was blocked by NP137 treatment, or by deletion of UNC5b. NP137 is currently in clinical trials for many tumor types including PDACs, and our data strongly indicate that NP137 should also be tested in patients with metastatic PNETs.

Altered splicing programs in PNETs affect the function of neuroendocrine cells

Myrto Potiri (1), Jonas Juan Mateu (2), Anastasia Kotsoni (1), Margarita Andreadou (1), Zoi Erpapazoglou (1), Malgorzata Ewa Rogalska (2), Panagiota Kafasla (1)

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Presenting Author

Panagiota Kafasla, PhD

Background

Alternative Splicing (AS) of the pre-mRNA generates diverse mRNA and protein isoforms from a single gene. AS is performed by the spliceosome, a large multimeric ribonucleoprotein (RNP) complex. In almost all cancers, the expression of spliceosome components is altered, contributing to the generation of advantageous for tumor development AS isoforms, with impact in almost every hallmark of tumor progression, including cell invasion, angiogenesis, metabolism. We will present our data on how altered splicing programs drive Pancreatic Neuroendocrine Tumors (PNETs).

Methods

Under the aegis of an NETRF pilot grant, we explored 129 human RNAseq datasets (62 from PNET patients and 67 from normal tissue samples). Our bioinformatic strategy was dual. First, we compared the expression levels of splicing factors in PNETs with those in normal samples. Second, we analyzed the expression levels of all mRNA isoforms produced by AS in both PNET and normal samples.

For validations we performed qPCR, RT-PCR/gel electrophoresis, using the human PNET cell lines BON1, QGP-1 cells, NT-3 and the PNET mouse model RIP1-Tag2. To alter the levels of selected splicing factors or AS events, we used siRNA/shRNA or ASOs (antisense oligonucleotides) in cell lines. To link the identified AS changes to PNET characteristics we used cellular assays (cell proliferation, migration, invasion, xenograft mouse models and insulin secretion, vesicle mediated secretion and calcium assays).

Results

Altered splicing factor expression: In our detailed examination we pinpointed 26 splicing factors that exhibited significant overexpression in PNETs when compared to normal islets and isolated pancreatic cell populations. Remarkably, six of these overexpressed factors are neuron-specific, known for their influential roles in AS impacting axon guidance, synaptogenesis, or synaptic transmission. Most notably, our exploration of The Cancer Genome Atlas (TCGA) demonstrated a unique upregulation of these six neuronal splicing factors specifically in PNET.

Altered isoform expression: We identified a significant number of deregulated AS events in PNETs compared to normal datasets. Gene Ontology (GO) term enrichment analyses of the genes affected by AS changes in PNETs, underscored vesicle mediated transport and pre-synapse formation as the primary cellular components largely affected by changes at the AS level only. These AS changes resulted mainly in the production of isoforms with altered functional potential. A group of the deregulated neuronal splicing factors mentioned above orchestrates a substantial portion of these changes. We will present our data on the validation of the dependency of selected AS event changes on this group of splicing factors, their importance for PNET cell growth and their implication in insulin secretion and vesicle mediated transport.

Conclusions

We have pinpointed a group of pre-mRNAs, regulated at the level of AS, that affect hormone secretion and vesicle mediated transport in PNET cells.

We have identified splicing factors that are responsible for regulating these AS events in PNETs.

Our findings suggest the activation of a neuronal phenotype in PNETs that could modify the neuronal character of pancreatic neuroendocrine cells, potentially driving tumor growth and invasion as it is increasingly appreciated that neuronal activity robustly promotes PNET progression.

Investigating serotonin metabolism in neuroendocrine cancers

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Presenting Author

Po Hien Ear, PhD

Background

Small bowel neuroendocrine tumors (SBNETs) originate from enterochromaffin cells in the intestine which synthesize and secrete serotonin. Other NETs and other cancers may also produce serotonin but do not store them in vesicles. The rate limiting enzyme of serotonin biosynthesis is tryptophan hydroxylase 1 (Tph1). Patients with high serotonin level could develop carcinoid syndrome, which can be treated with somatostatin analogues and the Tph1 inhibitor telotristat ethyl (TE) in severe cases. Little is known about the effect of serotonin on tumor cells during the dynamic process of neuroendocrine cancer growth. Here, we determined the effect of serotonin inhibition on tumor growth in vitro and in vivo using genetic and pharmacologic approaches. We identified improved tumor inhibition by combining TE with sunitinib, a tyrosine kinase inhibitor (TKI). In addition, we engineered a serotonin biosensor to track changes in serotonin levels in real-time.

Methods

The levels of Tph1 in various cancer cell lines were determined. The biological effects of Tph1 inhibition using shRNAs targeting TPH1 stable knockdown and TE +/- sunitinib treatment were tested. Control and knockdown lines were assessed for their growth rates, angiogenesis potential, serotonin levels, endothelial cell tube formation, tumor weight, and tumor vascularity. To create a biosensor to detect endogenous serotonin levels in live cells, we fused the serotonin binding domain and Renilla luciferase reporter. Mass spectroscopy, immuno-fluorescence, and western blotting were used to study serotonin metabolism under different conditions.

Results

TPH1 is highly expressed in SBNETs and several other cancer types. TPH1 knockdown cells and TE treated cells showed similar growth rates as control cells in vitro. However, TPH1 knockdown cells formed smaller tumors in vivo and tumors were less vascularized. The combination of TE and sunitinib led to a further decrease in tumor growth and lower serotonin levels in both tumor and blood samples. Moreover, we detected the dynamic changes in serotonin levels in tumor cells undergoing anchorage-independent growth and during serum starvation.

Conclusions

Although Tph1 inhibition with TE showed no effect on tumor cell growth in vitro, Tph1 inhibition reduced tumor formation in vivo and is potentiated in the presence of a TKI. Our serotonin biosensor enables real-time detection of alterations in serotonin synthesis in living cells under various growth conditions and has the potential to provide greater insight into serotonin metabolism in different stages of tumor progression and to identify therapeutic strategies to target cancer metastases and carcinoid crisis.

Mesenteric fibrosis in small intestinal neuroendocrine tumours (SI-NETs): pathogenesis and therapeutic targets

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Presenting Author

Maria C. Martins, MSc

Background

Mesenteric fibrosis (MF) can affect up to 50% of patients with small intestine neuroendocrine tumours (SI-NETs), causing significant morbidity and mortality. Our group has previously shown that patients with MF have a significantly shorter overall survival compared to patients without mesenteric lymphadenopathy. MF pathophysiology is poorly understood, limiting the development of effective treatments as well as the identification of biomarkers. The research conducted under a collaborative project from UCL/Royal Free London and Erasmus MC Rotterdam aims to improve the understanding of MF and identify useful molecular markers with diagnostic and predictive value.

Methods

Tissue was collected from a cohort of 46 SI-NET patients, including normal SI (n=46), primary SI-NET (n=40) and mesenteric mass (n=38). Patients were classified into 4 groups according to severity of mesenteric fibrosis (none, minimal, mild, severe), based on radiological, surgical, and histological assessments. Genome-wide DNA methylation was assessed using Illumina EPIC microarrays and RNA sequencing was performed on the Illumina NovaSeq instrument.

Fresh tissue collected from SI-NET patients was used for isolation of primary fibroblasts from normal intestine tissue, normal mesentery, primary tumour, and mesenteric metastasis. To characterize the primary fibroblasts, cytopsins were prepared and immunohistochemistry was performed for inclusion (alpha-smooth muscle actin, vimentin) and exclusion (synaptophysin, CD31, E-cadherin) markers.

A new decellularization protocol was optimized to obtain acellular intestinal 3D structures of normal intestine collected from SI-NET patients undergoing surgery. Quality control was performed using histology and DNA quantification of native and decellularized samples. Decellularized tissues were lyophilised and milled to obtain an extracellular matrix (ECM) powder. ECM powders were solubilised and a cell suspension containing SI-NET primary fibroblasts and/or GOT1 cells (SI-NET cell line) were mixed into the ECM solution together with nano-cellulose to obtain an ECM gel and cells were cultured up to 14 days. PrestoBlue assays were performed to analyse cell viability.

Results

Epigenetic and transcriptomic analysis has highlighted several genes involved in inflammation and fibrosis pathways including ACOT7, SFRS4 and GNG4. There were significant differences in methylation patterns and gene expression between mesenteric mass and primary SI-NET (adjusted p-value <0.05).

Decellularised human intestine showed significant decrease in DNA content compared to the native tissue. Furthermore, histological analysis showed no remaining cellular content and preservation of collagen fibres. GOT1 cells cultured in human intestine ECM gels showed good viability at 7 and 14 days. Co-cultures of GOT1 and SI-NET primary fibroblasts also showed good viability in ECM gels with formation and growth of GOT1 clusters over 14 days.

Conclusions

Epigenetic and transcriptomic differences highlighted pathways with relevance to disease pathophysiology which provide insight into development of MF. SI-NET cells and SI-NET primary fibroblasts can be cultured in a 3D model using human ECM intestine collected from SI-NET patients. This model will be used as a more suitable and physiological platform mimicking tumour cell - CAF interactions to better understand MF pathophysiology in SI-NET patients and to test candidate anti-fibrotic drugs targeting MF.

This research into mesenteric fibrosis is funded by NETRF Accelerator Grant 720627 (UCL/Exeter/Erasmus).

Studying the microbiome of small intestinal neuroendocrine tumor patients

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Presenting Author

Eric Nakakura, MD, PhD

Background

Small intestinal neuroendocrine tumor (SI-NET) is one of the major cancer subtypes of the small bowel. Most SI-NETs are located in the distal ileum with a high incidence of multiple synchronous primary tumors. We have shown that synchronous primary tumors from the same SINET patient display distinct somatic mutational profiles, suggesting these tumors originate independently and their tumorigenesis is unlikely driven by genomic alterations alone. We now hypothesize that environmental factors, such as the gut microbiota, could play a role in the growth and development of SI-NETs. A deeper understanding of the molecular mechanisms that underlie the formation of SI-NETs is important for the optimal treatment of the patients.

Methods

Our sample cohort consisted of 144 well-annotated fresh-frozen tissue specimens from 23 de-identified SI-NET patients, including 85 ileal NETs, 21 metastases, and 38 patient-matched normal ileum and/or whole blood specimens. Thirteen SI-NET patients had been diagnosed with multiple synchronous primary tumors. Whole-genome sequencing data of these samples were analyzed using PathSeq to study the potential role of gut microbiome in SI-NET tumorigenesis. In brief, PathSeq subtracts sequence reads derived from the human reference genome and aligns the remaining non-human reads to microbial reference sequences (viral, fungal, bacterial, eukaryotic) to determine the presence and abundance of microbial organisms in the samples.

Results

We identified microbial reads in all four tissue types: whole blood, normal ileum, ileal NET, and metastasis. Most of these microbial reads were derived from bacteria. Intestinal tissue samples and metastases had significantly more bacterial reads compared to whole blood specimens. The five most prevalent bacterial phyla in our sample cohort were Actinobacteria, Proteobacteria, Firmicutes, Bacteroidetes and Fusobacteria. Firmicutes were significantly more prevalent in intestinal tissue samples than whole blood specimens or metastases. Additionally, the composition of these five bacterial phyla varied among the SI-NET patients.

Conclusion

The five most prevalent bacterial phyla identified in our sample cohort are consistent with bacterial phyla present in the human gut. The two dominant phyla of the human gut are Firmicutes and Bacteroidetes. We will next assess the presence and abundance of bacteria on lower taxonomic ranks, such as genus and species. We will also carefully check our data for potential contaminants on species level that can be derived from laboratory reagents and experimental environments to exclude them from further analysis. Finally, we will examine if we can identify bacterial sequences enriched in SI-NETs compared to the patient-matched normal ileum suggestive of a causative role in SI-NET tumorigenesis. Finding the cause(s) of SI-NETs is essential for decisions regarding prevention, treatment, surgery, and patient outcome.

Midgut neuroendocrine tumor patients have a depleted gut microbiome with a discriminative signature

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Presenting Author

MCF Mulders, MD

Background

The prevalence of midgut neuroendocrine tumors (NET) has disproportionally increased over the past decades, while little progress has been made in the discovery of (epi)genetic drivers and treatment options of these tumors. Recent microbiome research has discovered novel driving factors and treatment targets among various cancer subtypes. However, the role of the microbiome in midgut NET and its associated carcinoid syndrome (CS) has not been studied. Hence, our aim was to analyze the role of the gut microbiome in midgut NET patients.

Methods
Fecal samples, collected from midgut NET patients and matched healthy controls, were analyzed with next generation 16S as well as whole metagenome sequencing. Relevant variables were extracted from questionnaires and electronic health records. Microbial composition and abundance of microbial pathways were compared between patients and controls as well as between patients with and without CS.

Results
182 participants were included: 87 patients, of whom 53 with the CS, and 95 controls. The 16S analysis revealed that midgut NET patients have a less rich and diverse gut microbiome compared to controls ($p < 0.001$). A total of 31 differentially abundant species were found, 6 of them being more abundant in patients. A microbial signature consisting of 17 species was identified, which predicted the presence of a midgut NET with an area under the receiver operating characteristic curve (AUROC) of 0.87. No differences in microbial richness or composition were found between midgut NET patients with or without CS ($p > 0.05$). These differences in gut microbiome diversity were confirmed with the use of whole metagenome sequencing in a subset of 30 CS patients, 30 non-CS patients and 20 controls ($p < 0.001$ for patient vs. control and $p > 0.05$ for CS vs. non-CS). Again, a microbial signature predictive of the presence of a midgut NET had an AUROC of 0.88. In addition, 13 differentially abundant microbial pathways, all enriched in patients, and 233 species contributing to these pathways were identified. Adding these pathways to our microbial signature did not increase accuracy. While no microbial species or pathways were individually differentially abundant between patients with and without CS, a signature consisting of 18 microbial pathways was able to distinguish CS patients from non-CS patients with an AUROC of 0.80.

Conclusions
Midgut NET patients have an altered gut microbiome, independent of the presence of the CS, which could suggest a role in NET development and provide novel targets for microbiome-based therapeutics. Furthermore, a fecal microbial signature could constitute a novel biomarker for the diagnosis of a midgut NET or CS.

ORAL PRESENTATIONS: SESSION 4, TUMOR EVOLUTION & MICROENVIRONMENT

Early emergence of high grade subclones in pancreatic neuroendocrine tumors

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Presenting Author
Etay Ziv, MD, PhD

Background
Well-differentiated pancreatic neuroendocrine tumors (pNET) are heterogeneous tumors with variable degree of aggressiveness. Responses to treatment and clinical outcomes are largely determined by tumor grade which is defined by the proliferation index. Some high-grade tumors arise de novo, but others evolve over time as tumors transform from low grade to high grade. In this study, we sought to define the clonal relationship between spatially and temporally distinct low grade and high regions.

Methods
We retrospectively identified a cohort of pNET patients that had mutation panel testing in our institution and that had multiple specimens obtained from different sites over the course of their treatment including surgical resection and needle biopsy. Patients were categorized based on grade changes. A subset of patients with preserved specimens that passed quality control (QC) steps were selected for downstream analyses including immunostaining, micro-dissection, DNA extraction and library generation. Tumor grade was established by pathologist review and proliferation index. Whole exome sequencing (WES) was performed on tumor specimens (250X) and matched normals (100X). Paired-end sequencing data from exome capture libraries were aligned to hg19

reference genome provided in GATKv4.2 with BWA aligner. BAM files were processed using Picard-v2.18 CleanSam and MarkDuplicates. For variant calls, tumor and normal BAMS were input to Mutect2. Generated variant calls were further refined using FilterMutectCalls. Treeomics v1.9.2 was used for phylogeny inference including variants with at least 5% frequency and coverage of at least 20x in at least one sample.

Results

We identified 141 of pNET patients that had mutation panel testing and multiple specimens sampled over time. Patients were categorized into 5 groups: Group A (60/141 [42%]) transformed to high grade; Group B (27/141 [19%]) increased from grade 1 to grade 2, group C (23/141 [16%]) remained grade 1 or grade 2, group D (20/141 [14%]) had de novo high grade components and group E (11/141 [8%]) decreased grade over time. We selected 19 patients that were either group A or group D and that passed QC for WES. Tumor specimens included primary site (pancreas) and metastases from liver, lymph node, bowel, and ovary. Proliferative index ranged from 1% to 90% across all samples. Exonic TP53 mutations were identified in 10% samples. DAXX and ATRX alterations arose early across multiple subclones. Tumor phylogenies demonstrated highly branched patterns. High grade regions arose early in the tumor evolution from progenitors of the primary tumor rather than late descendants of low grade metastases.

Conclusions

We found pNETs commonly transform to higher grade. Subclones that transform to high grade appear to arise early in tumor evolution from progenitors of the primary tumor. Ongoing analysis includes tracking driver mutations across the phylogenies and correlating with treatment history.

Characterizing chemoresistant persister cells in relapsed high-grade neuroendocrine carcinomas

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Presenting Author

C. Allison Stewart, PhD

Background

High-grade neuroendocrine carcinomas (hgNECs) are aggressive malignancies that arise, most commonly, from the aerodigestive tracts, though, more rarely can claim gynecologic, genitourinary, or cutaneous origin, among others. hgNECs are typically classified as small cell carcinoma (SCC), large cell neuroendocrine carcinoma (LCNEC), or mixed histology. While small cell lung cancer (SCLC) is the most common of these malignancies, other hgNECs represent a significant source of morbidity and mortality due to their early dissemination and propensity for rapid growth and relapse, with median overall survival ranging from just 12 to 22 months. Frustratingly, hgNECs are, initially, exquisitely sensitive to chemotherapy and/or radiation, but these responses are short-lived and inevitable relapses are more resilient. We hypothesize that these refractory relapses are driven by a population of chemoresistant persister cells that evade initial cytotoxic treatments and repopulate more resistant tumors in the recurrent setting.

Methods

We have assembled a cohort of tissue (both fresh and archival) and plasma samples from more than 50 patients with rare hgNECs (i.e. those other than SCLC) treated at MD Anderson Cancer Center. These samples were subjected to analyses including immunohistochemistry, whole exome sequencing (of tissue or plasma circulating tumor DNA), bulk RNAseq, and single-cell RNAseq to assemble a molecular atlas of both treatment-naïve and, especially, relapsed disease. Fresh tissue and plasma circulating tumor cells were also transplanted into the flank of immunocompromised mice for generation of patient-derived xenograft models, while disassociated cells were placed in media to culture immortalized cancer cell lines. Cell line and patient-derived xenograft tumors were infected with the Watermelon barcode library for lineage tracing experiments with and without chemotherapy to map the origin of, and characterize the features of, chemoresistant persister cells.

Results

We demonstrate that hgNEC tumors, even in the treatment-naïve setting, can be subclassified into two subsets. One subset, defined by high expression of the transcription factor YAP1, is mesenchymal, neuroendocrine-low, chemoresistant, and more inflamed. The other subset, defined by a dearth of YAP1 expression, but presence of other neuroendocrine-associated transcription factors (e.g. ASCL1, NEUROD1, POU2F3), is epithelial, neuroendocrine-high, chemosensitive, and exhibits an immune desert phenotype. Under therapeutic stress, even among initially YAP1-low tumors, emerging persister cell populations identified by single-cell RNAseq resemble YAP1-high tumors. While both YAP1-high tumors and emerging YAP1-high persister populations are resistant to conventional therapies for hgNEC, they possess unique vulnerabilities ranging from therapies targeting their unique genomic features (e.g. SMARCA2 degraders for SMARCA4-deficient tumor cells) to those targeting their unique cell surface proteome (e.g. chimeric-antigen receptor (CAR) T-cells targeting AXL).

Conclusions

YAP1 expression defines both a significant subset of treatment-naïve hgNECs, as well as emergent populations in relapsed disease from tumors that were initially YAP1-high or -low. These emergent populations exhibit features (plasticity, chemoresistance) that suggest a role as persister cells. The emergence of these populations offers an explanation for the remarkable shift from chemosensitivity to chemoresistance following hgNEC recurrence. Thus, YAP1-high tumors and models represent a discovery set for therapies to eradicate persister cells, including both targeted- and immune-based therapeutics.

Molecular landscape of pancreatic neuroendocrine tumors at single-cell resolution

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Presenting Author

Jeanna Qiu, AB

Background

Pancreatic neuroendocrine tumors (PNETs) are a diverse tumor type that derive from neuroendocrine cells of the pancreatic islets. About 10% of PNETs are described as "functional" due to their ability to produce hormones, while 90% are termed "non-functional." Non-functional PNETs are often found later in the course of disease due to their ability to grow asymptomatically until they reach a significant tumor burden. While some PNETs are indolent, approximately half are aggressive and metastasize quickly. A clinically-relevant molecular classification for PNETs to predict patient outcomes and guide therapeutic decision-making has been elusive, in part because prior studies have primarily looked at the entire tumor in aggregate, leading to an unknown mixture of cancer cells, immune cells, and other stromal components.

Methods

We applied single-nucleus RNA-seq (snRNA-seq) to 20 surgically-resected, untreated, primary PNETs of different grades and stages from our biobank. Following quality control, we separated cells into malignant and non-malignant subsets. We focused our downstream analyses on the 14 nonfunctional PNETs that passed quality control. We performed de novo molecular classification using consensus non-negative matrix factorization (cNMF) on the malignant compartment to identify gene expression programs shared across multiple patients. We compared these gene expression programs to previously identified, bulk derived proteo-transcriptomic signatures of PNETs and single-cell derived signatures of physiologic pancreatic neuroendocrine cell subtypes. Finally, we performed gene ontology term enrichment analysis to create a preliminary annotation of these gene programs.

Results

We recovered 9,472 non-malignant and 82,267 malignant single-nucleus profiles after quality control and doublet identification and removal. We used established literature marker genes to identify 17 broad cell type categories, including malignant neuroendocrine cells, epithelial cells, cancer-associated fibroblasts, endothelial cells, and various immune cell types. Consensus NMF yielded 25 gene expression programs across the malignant cells of nonfunctional PNETs and we focused our analysis on the 7 programs that were distributed across multiple patients and robust to 90% downsampling. Comparison to known gene signatures and gene ontology term analysis revealed previously established alpha cell-like, beta cell-like, ribosomal/proliferative, cell-cycling, and mitochondrial gene programs. The remaining two programs included genes related to neuronal and synaptic signaling, such as genes involved in synaptic scaffolding and signaling (HOMER1, SNAP25, NRXN3, ERBB4), glutamate receptor signaling (GRIA1, DLG2); and axonal guidance (SEMA3A, SEMA4A, NTRK3, NTNG1).

Conclusions

Leveraging snRNA-seq, we have created a refined molecular taxonomy of PNET that includes previously established malignant cell states such as alpha cell-like and beta cell-like, as well as two novel programs enriched in gene expression programs related to neuronal and synaptic signaling, which suggests that a subset of malignant neuroendocrine cells may interact with nerves, similar to physiologic neuroendocrine cells. Further validation of these novel programs is ongoing.

Single-cell transcriptomics identified IGF2 and VEGF as growth factors in PNETs

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Presenting Author

Chang Chan, PhD

Background

Pancreatic neuroendocrine tumors (PNETs) are a heterogeneous group of neoplasms arising from the endocrine cells of the pancreas which exhibit a wide range of clinical behaviors, including variable growth rates and treatment responses, necessitating a deeper understanding of the underlying molecular mechanisms governing their growth. Dysregulated growth factor (GF) signaling pathways have been implicated in various cancers, highlighting their potential significance in PNET pathogenesis. However, the specific GFs influencing PNET growth, and the cellular heterogeneity remain poorly understood. This study employs single-cell RNA sequencing (scRNAseq) to comprehensively profile the transcriptomes of individual cells within PNETs, and by characterizing the molecular diversity of tumor cells and their neighboring microenvironment, this research explores distinct subpopulations of cells expressing specific growth factors and receptors.

Methods

The scRNAseq data were acquired using the 10X Genomics platform; the sequencing data was mapped to the reference GRCh38-2020-A using CellRanger v6.0.1. Seurat V4.3.0.1 was used for data preprocessing. SingleR v2.2.0, cell-type biomarkers, and Azimuth web application were used for identifying distinct cell types within the PNETs. MAST was used for differential gene expression analysis. Gene Set Enrichment Analysis was used to identify biological pathways. CellphoneDB v4.1.0 was used to identify ligand–receptor interactions.

Results

We have performed scRNAseq analysis on 7 PNETs (5 non-functional PNETs and 2 insulinomas). We annotated the cell types found in the tumor microenvironment (TME) and identified the normal neuroendocrine cell types that were the closest match to the tumor cells by gene expression. We analyzed the heterogeneity within the tumors and identified subpopulations of tumor cells in three non-functional PNETs that had increased expression of IGF2. Differential gene expression and pathway analyses between the tumor cells and their respective normal neuroendocrine cell types found enrichment for epithelial to mesenchymal transition, TNFA via NFKB, and hypoxia pathways in the tumor cells. Cell-cell communication analysis using ligand and receptor pairs identified GF IGF2 with receptors IGF1R and IGF2R in the three tumors that had subpopulations over-expressing IGF2 in autocrine and paracrine signaling. GFs VEGFA and VEGFB with receptors KDR, NRP1, NRP2, and FLT1 were identified in all tumors and signaled to endothelial cells. We analyzed the RNA sequencing data of 33 non-functional PNETs (Chan et.al. Nature Comm 2018) and found IGF2 expression in 72% of samples.

Conclusions

The identification of GF-enriched cell clusters, coupled with pathway enrichment and ligand-receptor interaction analyses, suggests that IGF2 and VEGF mediated signaling may be a critical driver of tumor growth and angiogenesis in PNETs. These findings provide a foundation for further mechanistic studies and potential therapeutic interventions targeting GF-related pathways to mitigate the growth and progression of PNETs.

ORAL PRESENTATIONS: SESSION 5, IMMUNOTHERAPIES

Temozolomide treatment induces an MMR-dependent hypermutator phenotype in well differentiated pancreatic neuroendocrine tumors

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Presenting Author

Jérôme Cros MD, PhD

Background

Temozolomide (TMZ), an alkylating agent, is an effective treatment used in multiple tumor types. In glioblastomas, TMZ induces a hypermutator and hyperprogressor phenotype in a subset of patients through a specific MSH6-related MMR deficiency that does not seem to trigger an immune response. In contrast, in advanced colon cancer, preliminary studies suggest that TMZ priming increases the tumor mutational burden (TMB), favoring immunotherapy efficacy. TMZ treatment has also been shown to be effective in patients with advanced pancreatic neuroendocrine tumors (PanNET). The aim of this study was to assess the potential of TMB-associated immunotherapy efficacy in TMZ-treated PanNET.

Methods

Out of 107 patients with PanNET treated by TMZ, we selected 57 patients with low grade G1/G2 PanNET and without early progression to TMZ+/- capecitabine, treated in one expert center between 2009 and 2020. Sequencing of 500 cancer genes was performed on 23 pre-TMZ and 23 matched post-TMZ samples, without intercurrent DNA-damaging treatment. Results were validated in an independent cohort of 1079 PanNETs, not selected by the treatment received, profiled by Foundation Medicine. TMB >30mut/Mb was considered TMB-high and only alterations at variant allele frequency ≥10% were included in this analysis.

Results

After TMZ, 29/57 (51%) PanNET became high grade G3. They showed no difference in their preTEM clinical or molecular profile nor in the TMZ dose received. In the profiled samples, 14/23 (61%) post-TMZ samples became G3 and 6/23 (5 G3) were TMB-high. TMB-high post-TMZ samples had various alterations in the MMR genes (88% vs. 26%, p=0.003). In the validation cohort, 25/1079 PanNETs were TMB-high and 16/898 displayed the alkylating-related signature. 67% of TMB-high samples displayed the predominant alkylating signature and 88% of samples with a high alkylating signature had a TMB high suggesting a key role of the treatment. 44% of TMB-high samples had various MMR alterations. In both cohorts, none of the TMB-high samples showed the glioblastoma-specific MSH6 mutation, suggesting an immunogenic potential.

Conclusions

These results suggest that TMZ induces an hyperprogressor and hypermutator phenotype in a subset of PanNETs, but through a potentially immunogenic mechanism, thereby opening the path to immunotherapy, a treatment not otherwise effective in patients with treatment-naive PanNET.

TILs from panNET liver metastasis: in search of novel adoptive transfer strategies for the treatment of NETs

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Presenting Author

Mauro Cives, MD

Background

It is currently unknown whether tumor infiltrating lymphocytes (TILs) may exert antitumor activity against NETs.

Methods

We have collected matched i) blood, ii) FFPE and iii) cryopreserved or fresh samples of liver metastases from 22 patients with sporadic well-differentiated panNETs (G1: 4; G2: 17; G3: 1). Tumor samples have been subjected to whole-exome sequencing and RNAseq, and bioinformatic analysis of sequencing data is currently underway to decipher the tumor neoantigen landscape. Multi-region analysis of individual tumor samples has been carried out to evaluate the spatial heterogeneity of TIL distribution and TIL outgrowth. In particular, fragments from different tumor regions have been subjected to transversal section, with one half undergoing H/E and IHC staining and the other being further dissected in small numbered areas (approximately 2 mm3 each) and then subjected to different protocols of TILs isolation/expansion. The mapped TIL outgrowth was then compared with the intratumor regional characteristics. TILs were expanded for up to 105 days and were enumerated and phenotyped weekly by flow cytometry to gain insights on the lymphocyte composition (B cells, NK cells, total T cells and T cell subsets), differentiation (TN, TSCM, TCM, TEM, TE) and exhaustion status (PD-1, CD39, TIGIT, TIM3 and CTLA4). Similar analyses were conducted on autologous PBMCs. The Seahorse technology was used to evaluate the metabolism of TILs at different culture time-points to determine their functional exhaustion.

Results

TILs were successfully grown from 15/22 patients (68%), and a mean of 2975x106 and 23x106 T cells were obtained at the peak of the expansion phase when fresh and cryopreserved samples were used respectively. Overall, a number of TILs sufficient for adoptive cell therapies in humans (pre-REP) was obtained in 12/22 samples (55%; 77% and 33% when using fresh or cryopreserved tumors respectively). There was no difference in the growth rate of TILs on the basis of the tumor grading, Ki-67, or vascular/perineural invasion, prior therapies, whereas a significant correlation was found between T cell yield and T cell density as assessed by IHC ($p<0.05$). In particular, the T cell yield peaked in samples harboring tertiary lymphoid structures. Wide differences were observed in terms of T cell yield according to the different tumor regions analyzed, with a positive correlation between stromal rather than tumor areas and TIL outgrowth ($p<0.05$). Immediately after tumor digestion, the immune cell infiltration of tumors appeared minimal (approximately 1% of total cell population, range 0.25-2.5%). T lymphocytes were the predominant population to grow in the TIL cultures at the time of cryopreservation (median CD3+: 80%), followed by NK cells (median: 2%) and NKT cells (median: 1%). Among T cells in these TIL cultures, CD4+ was the main subset (median CD4+: 70%), exceeding CD8+ T cells by a median ratio of 7:1. Infiltrating Treg cells represented 5% and 6% of CD4+ and CD8+ T cells respectively. The percentage of circulating Treg cells was higher in patients with NETs (n=6) rather than in healthy controls (n=3), with an almost doubled percentage of CD8+ Treg cells in tumors rather than peripheral blood. Among exhaustion markers, CD39 and TIGIT were mostly expressed by both CD4+ and CD8+ TILs. The differentiation

of CD8+ T cells changed over time in culture, with the emergence of TEM cell clones and the disappearance of the originally present TE after 2 weeks of culture. Long-term cultured TILs exhibited a significantly reduced maximal respiratory and spare respiratory capacity as well as a significantly reduced glycolytic capacity and glycolytic reserve ($p<0.05$).

Conclusions

A number of TILs sufficient for adoptive cell transfer in humans can be obtained in more than half of cases. Metabolic exhaustion of TILs takes place during long-term in vitro expansion.

The role of the B7x signaling pathway in the development and progression of neuroendocrine tumors

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Presenting Author

Ziqiang Yuan, MD

Background

Cancer immunotherapy is rapidly becoming an important component of treatment for patients with a variety of tumor types. A newly characterized member of the B7/CD28 family, B7x, is expressed in a number of tumors and can modulate cancer development and progression by inhibiting T-cell function, thus making it an appealing target for immunotherapy. We demonstrated that B7x is upregulated in human and murine pancreatic neuroendocrine tumors (PNETs). Furthermore, we demonstrated that blockade of B7x with an anti-B7x antibody can inhibit tumor cell proliferation and induce apoptosis, both of which improve the tumor immune microenvironment and result in increased survival in the preclinical setting. In this Accelerator Award, we will investigate: (1) The molecular mechanisms leading to B7x upregulation in PNET tumorigenesis; (2) The role of immune checkpoint B7x activation on PNET tumorigenesis; and (3) Whether loss of MEN1 drives an immunogenic phenotype in islet cells that is suppressed by B7x. During this reporting period, we further investigated the molecular mechanism of B7x regulation in the tumor microenvironment (TME) and the role of B7x activation on PNET tumorigenesis in our in vitro and in vivo models.

Materials and Methods

We assessed the role of immune checkpoint B7x activation on PNET tumorigenesis by crossing our Men1 KO mice with B7x KO mice and assessed the efficacy of novel and high-affinity antibodies targeting the immune checkpoint B7x on the growth of PNETs using our Men1 KO mice. In addition, we identified the immune cell populations present in the tumor microenvironment as well as underlying immune regulatory mechanisms by complementary FACS and multiparameter IHC as well as single cell RNA-seq. Finally, we performed T cell Receptor repertoire (TCR) determinations from human PNET tissues by TCR-seq.

Results

We demonstrated that Men1/B7x double KO mice exhibited decreased islet β-cell proliferation and tumor transformation accompanied by increased T-cell infiltration compared with Men1 single knockout mice. Furthermore, we have shown that systemic administration of a B7x mAb to our Men1 KO mice with PNETs promotes an antitumor response mediated by increased T-cell infiltration. In addition, we demonstrated that some T cell and NK cell immune regulators (T cell immune checkpoints: PD-1, LAG3, and Tim-3; NK cell immune checkpoints: KIR2DL2, KIR2DL3, and KIR2DL4) were upregulated in the immune populations of TME by single cell RNA-seq. Finally, in our preliminary analysis from TCR-seq data, we found that some novel peptides may drive an immunogenic phenotype and manifest in the immune tumor microenvironment of PNETs.

Conclusions

Our results suggest that B7x and other immune checkpoints may be the critical mediators of tumor immunity in the tumor microenvironment of NETs. Therefore, targeting B7x and immune related pathways will offer an attractive strategy for the immunotherapy of patients suffering from NETs.

Therapies targeting CDK4/6 cause regression, immune cell activation, and sensitization to PD-L1 immunotherapy in pancreatic neuroendocrine tumors

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Presenting Author

Dawn Quelle, PhD

Background

New effective therapies are needed to improve the survival of patients with metastatic pancreatic NETs (pNETs). RABL6A is a novel oncogenic driver of pNET pathogenesis. Kinome and phosphoproteome analyses of proliferating (RABL6A-positive) pNET cells, versus arrested (RABL6A-knockdown) controls, demonstrated that cyclin-dependent kinase 4 and 6 (CDK4/6) and MEK kinases are actionable drug targets in growing pNET cells. In agreement, published studies of patient pNETs by immunohistochemistry (IHC) and RNAseq identified robust activation of CDK4/6 and MEK in tumors. In other tumor types, CDK4/6 and MEK inhibitors display synergistic antitumor activity linked with heightened CD8 T cell, plasma cell and/or NK cell activation. This drug combination has not yet been evaluated in pNETs.

Methods

Synergistic effects of MEK inhibitor (Mirdametinib) and CDK4/6 inhibitor (Palbociclib) were measured by cell proliferation, survival, colony formation, and immunoblotting assays. Tumor suppressive effects were measured in vivo using 3 pNET mouse models: 1) flank xenografts in immunodeficient mice, 2) tail vein metastasis xenografts in immunodeficient mice, and 3) immune competent, Pdx1-Cre;Men1fl/fl;Ptenfl/fl knockout mice that develop insulinoma by 5-6 months of age. Single cell RNAseq and multiplex IHC were performed on human pNETs.

Results

In vitro, CDK4/6-MEK inhibitor therapy caused synergistic pNET cell death for cell lines as well as patient-derived tumor spheroids and organoids. In vivo, the CDK4/6-MEK combination significantly slowed growth of flank pNET xenografts, yielding a 6-fold extension of average survival (~120 days versus 20 days for control). This combination likewise suppressed (but did not eliminate) pNET growth in a bioluminescence metastasis model and reduced tissue colonization relative to monotherapy controls. By comparison, dual CDK4/6-MEK inhibition in immunocompetent Pdx1-Cre;Men1fl/fl;Ptenfl/fl insulinomas caused dramatic tumor regression associated with B/plasma cell infiltration. Pilot analyses demonstrate the presence of B-lineage cells in human pNETs, which in other tumors prognose better survival and response to immunotherapy. Indeed, further tumor regression was achieved in Pdx1-Cre;Men1fl/fl;Ptenfl/fl insulinomas by combining CDK4/6 inhibitors with anti-PD-L1 blockade.

Conclusions

Combination therapy targeting CDK4/6 and MEK inhibits pNET growth and metastatic colonization. Monotherapies were not effective, in agreement with lack of response to CDK4/6 monotherapy in pNET patient trials. In immune competent Pdx1-Cre;Men1fl/fl;Ptenfl/fl mice, CDK4/6-MEK inhibition causes significant tumor regression linked with immune activation while CDK4/6 inhibition alone sensitized insulinomas to anti-PD-L1 immunotherapy. These data reveal activation of anti-tumor immunity in pNETs following CDK4/6 and MEK or PD-L1 inhibition. Such data provide compelling pre-clinical rationale for pNET clinical trials testing these combination therapies.

A novel hormone based anti-SSTR bispecific T-cell engager for the treatment of neuroendocrine tumors

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Presenting Author

Eleonora Pelle, MD

Background

Somatostatin receptor 2 (SSTR2) is overexpressed in well-differentiated NETs. We designed a novel bispecific T-cell engager targeting SSTR2 using Somatostatin-14 (SST14), the hormone that physiologically binds the SSTRs. SST14 was linked with a scFV-based anti-CD3, to efficiently engage and activate tumor infiltrating lymphocytes against NET cells.

Methods

The optimized sequence of the engager was subcloned into a vector (pAcGP67a) designed for protein expression in insect cells using Baculovirus, and the recombinant protein was subsequently expressed in Trichoplusia-ni (High Five) cells, isolated and characterized by chromatography. Flow cytometry and confocal microscopy were used to determine the interaction of the molecule with CD3 and SSTR2. Effector CD3+ T cells were isolated from the peripheral blood of healthy donors and target 293T cells were stably transduced to concurrently express SSTR2 and GFP. Effector and target cells were co-incubated in the absence or presence of the engager. The SSTR2- parental 293T cell line was used as negative control, while anti-CD3/CD28 beads were added in positive control preparations. The engager-induced T cell activation was evaluated measuring the secretion of IFNγ and TNF-alpha by ELISA, and the engager-induced cytotoxicity was assessed by real-time quantitative live-cell imaging using the Incucyte system.

Results

The T-cell engager was detected by flow cytometry on approximately 85% of T cells at a concentration of 100 nM. At the same concentration, the molecule was found to coat SSTR2+ 293T cells by confocal microscopy. IFN-γ secretion was significantly higher when the T cells were co-cultured with SSTR+ 293T cells in the presence of the engager as compared with conditions using SSTR- 293T cells or in absence of the molecule, suggesting that the molecule-induced T-cell activation is specific. When added to SSTR+ 293T cell cultures, the engager exerted antiproliferative activity per se ($p < 0.0001$), possibly as result of an agonist activity on the SSTR2. Such a cytotoxic effect was significantly more pronounced when T cells were also present in the cultures ($p<0.0001$).

Conclusions

To our knowledge, this is the first T-cell engager to incorporate a hormone in one binding site, exerting antiproliferative activity against SSTR2-expressing cells.

Mutation-Targeted Immunotherapy for Atypical Pulmonary Carcinoids using CRISPR/Cas9

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Presenting Author

Kevin J. McHugh, PhD

Background

Pulmonary neuroendocrine tumors (NETs) account for approximately 25% of all lung cancers. Atypical pulmonary carcinoids are particularly aggressive tumors with much higher rates of proliferation and metastasis, leading to increased mortality. The current standard of care for pulmonary atypical carcinoids is the surgical removal of the tumor; however, this approach is inadequate for the nearly half of tumors that have already spread to the lymphatic system or systemically, resulting in five-year survival rates of just 18% for regional disease and 5% for distal disease. In addition, surgical interventions are prone to relatively high rates of recurrence

(25%) and can leave individuals with reduced lung function. As a result, there is a clear clinical need for an alternative treatment that improves survival and avoids damage to healthy tissues. In this research, we describe the development of an immunotherapeutic strategy that uses mutations in DNA itself to identify and destroy atypical carcinoids. More specifically, we use clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 genome editing technology to selectively make cuts in the genome of cancer cells that permits the insertion of donor DNA encoding a suicide gene that kills the edited cell and generates an anti-cancer immune response against other atypical carcinoid cells based on the presence of neoantigens.

Methods

We first sequenced proto-oncogenes in three cell lines derived from atypical carcinoids (NCI-H720, NCI-H727, and NCI-H510A) to identify the presence of hotspot mutations commonly found in NETs. Next, we created promoter-less donor DNA plasmids featuring left and right homology arms, a self-cleaving peptide sequence and either diphtheria toxin subunit A or green fluorescent protein using standard microbiological techniques. In parallel, we recombinantly produced HiFi Cas9 and VQR Cas9 and confirmed their cutting activity. Then, after optimizing the transfection reagents and protocols used, we evaluated pre-integration insertion—which is necessary to achieve killing specificity in this approach—by treating cells with the promoter-less plasmid without Cas9 or any Cas9 variants.

Results

Sequencing results revealed mutational heterogeneity among the three cell lines tested, which is to be expected. This set of mutations included mutations in genes known to be commonly altered in NETs. HiFi Cas9 and VQR produced recombinantly were also shown to be active, cutting at their “NGG” and “NGAN,” respectively, when complexed to appropriate guide RNA. Lastly, the promotor-less donor DNA was successful in suppressing the pre-integration expression of protein, as measured by flow cytometry and fluorescence microscopy, suggesting the ability to prevent cell death in normal cells lacking the target oncogenic mutation.

Conclusions

Genomic targeting of atypical pulmonary carcinoids and other NETs using CRISPR/Cas9 shows potential as a personalized and precise method for destroying NETs. Future work will focus on the demonstration of selective NET cell killing in vitro and ablation in vivo via directly induced cell death from diphtheria toxin subunit A and indirect cell killing from the immune response generated by dying cancer cells.

POSTER PRESENTATIONS

Increasing the Therapeutic Window in Peptide Receptor Radionuclide Therapy with Long-acting Somatostatin Analogues: Study Protocol

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Presenting Author

Else A. Aalbersberg, PhD

Background

Treatment for patients with neuro-endocrine tumors (NET) with both peptide receptor radionuclide therapy (PRRT) and long-acting somatostatin analogues (LA-SSA) utilize the somatostatin receptor. To avoid possible competitive binding, current guidelines recommend withdrawing LA-SSAs 4-6 weeks prior to PRRT in order to ensure effective receptor occupation. However, recent retrospective research has shown that LA-SSA use during PRRT leads to a decreased uptake measured 24 hours after injection of [177Lu]Lu-HA-DOTATATE in the liver and spleen whilst tumor uptake remains unchanged.* We therefore hypothesize that continuous use

of LA-SSAs during PRRT does not negatively affect the absorbed dose in tumor lesions due to an absence of competitive binding and could increase the therapeutic window of PRRT.

Study Protocol

The main objective of the study is to determine the effect of LA-SSAs (i.e. octreotide LAR and lanreotide) on the absorbed dose in tumor lesions during PRRT with [177Lu]Lu-HA-DOTATATE. Secondary objectives include the effect of LA-SSAs on the absorbed dose in normal tissues, tumor-to-background ratio, and pharmacokinetic parameters of [177Lu]Lu-HA-DOTATATE. As an exploratory objective health-related quality of life assessments (HRQoL) are performed.

Patients ≥18 years with a NET and a clinical indication for PRRT will be included. The study is a randomized, prospective non-inferiority trial with 1 control arm and 2 interventional arms: (1) patients without LA-SSA for at least 3 months prior to PRRT (control arm), (2A) patients with LA-SSA, discontinued 4-6 weeks prior to PRRT, (2B) patients with LA-SSA, administered within 1 week prior to PRRT. Only the first cycle of PRRT will be included since the absorbed dose in subsequent cycles could be decreased due to response to treatment. Following the administration of [177Lu]Lu-HA-DOTATATE, SPECT/CT imaging of the thorax and abdomen is performed after 4h, 24h, and 5-7d to image the biodistribution and to enable dosimetric analysis. Blood samples will be drawn after 2h and after each SPECT/CT for pharmacokinetic analysis and to estimate the dose to the bone marrow. HRQoL questionnaires are provided prior to PRRT and after 3 weeks.

The primary endpoint (difference absorbed tumor dose) was used for the sample size calculation. 39 patients will be included in the clinical trial, resulting in 13 patients in each arm. With this number of patients, a power of 80% and a significance level of 0.05 can be achieved to show a 15% non-inferiority of both intervention arms compared to the control arm. This non-inferiority margin is considered to be clinically relevant based on normal variation between patients.

Conclusions

This prospective clinical trial has been designed to study the effect of LA-SSAs on the uptake of [177Lu]Lu-HA-DOTATATE in tumors and normal tissues. We hypothesize that LA-SSAs do not negatively affect the tumor or healthy tissue absorbed dose when continued during PRRT. If confirmed, this would negate the need for patients to discontinue LA-SSAs multiple times during PRRT and to switch from LA-SSAs to short-acting SSAs. Furthermore, potentially this approach could increase the therapeutic window, i.e. increase the delivered dose in tumor lesions without increasing toxicity.

* Veerman et al. EJNMMI 2023.

Identifying Biomarkers for Prognosis and Treatment Selection in Metastatic Neuroendocrine Tumors

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Presenting Author

Daniel Ackerman, PhD

Background

Liver metastases are common in neuroendocrine neoplasms (NEN) and are often treat-ed using locoregional therapies such as transarterial chemoembolization (TACE). While effective in many patients, treatments are rarely selected based on molecular tumor features and clinical out-comes are highly variable. Our study aims to identify urgently needed biomarkers of disease progres-sion and treatment response in order to improve treatment selection and support the development of new therapies for metastatic NEN. As with most cancers, short-read sequencing has been the predom-inant approach taken in molecular profiling studies of NEN to date. Because this technology is better suited for the identification of small alterations, structural variants (SVs) remain understudied despite their recognized role as oncogenic drivers. Our study seeks to address this shortfall by combining complementary approaches to molecular profiling in a cohort of NEN liver metastases.

Methods

We have identified genetic changes affecting cancer genes in liver metastases obtained from 30 NEN patients undergoing TACE. We combined targeted short-read sequencing data with optical genome mapping (OGM), a novel technique that leverages ultra-high molecular weight DNA to maximize sensitivity for structural variants. In order to associate molecular features with clinically relevant features of the treated lesions, we extracted measures of tumor growth and TACE response from MRI or CT imaging studies of patients in our cohort and associated these measures with clinical and molecular datasets.

Results

These data have revealed previously underappreciated relationships between NEN subtypes and measures of local progression, including growth rates and TACE response. We have also identified strong evidence for subtype specific differences in several genomic changes detected by OGM, including chromothripsis and genomic instability. Structural variants identified in our dataset affect numerous known oncogenes and tumor suppressor genes, including several known to correlate with clinical outcomes, and affect several known drug targets that are currently the focus of clinical trials. These findings have significant treatment implications and are currently being investigated further in an expanded sample set.

Conclusions

Our study is designed to further our understanding of the molecular basis of neuroendocrine neo-plasms and to identify novel molecular subgroups associated with clinically relevant outcomes. Our results demonstrate that integrating complementary profiling methods and developing carefully curated datasets of clinical response measures are critical for the discovery of novel biomarkers. Our findings show significant potential for tailoring treatment approaches based on molecular signatures and pave the way for more personalized therapeutic strategies in managing advanced NEN.

Single-Cell Transcriptome Analysis Reveals Mechanisms of Tumor Immune Escape in NETs

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Presenting Author

Alexandra Adams, MD

Background

Immunotherapy is rapidly becoming a mainstay of cancer treatment. However, little is known about the role of these therapies in pancreatic neuroendocrine tumors (PNETs). Using single cell RNA sequencing of 7 human PNETs we sought to (1) identify the immune cell populations present in the tumor microenvironment (TME) (2) explore expression patterns of immunoregulatory genes in immune cells in the TME and (3) explore immunoregulatory gene expression of tumor cell populations.

Methods

Single cell RNA-sequencing (scRNAseq) data were acquired using 10X Chromium platform to analyze tumor cells as well as immune cells in 7 PNETs (5 non-functional PNETs, 2 insulinomas). CellRanger v.6.0.1 with the built-in library (reference) GRCh38-2020-A was used to obtain the UMI count matrix and Seurat v4.3.0.1 was used for scRNAseq analysis. Frozen tissue sections were stained with CD45 antibody (1:100 dilution, Invitrogen 14-0451-82) followed by incubation with an appropriate conjugated secondary antibody.

Results

Tumor tissues were infiltrated by CD45+ cells on IHC. From single-cell data, CD8+ T cells were identified in 5/7 tumor samples. This subpopulation of T cells expressed PD-1 (5/5 samples), LAG-3 (4/5 samples) and CTLA-4 (1/5 samples). Natural killer cells (NK) were identified in 5/7 tumor samples. The inhibitory NK receptors KIR2DL1 (4/5 samples), KIR2DL3 (5/5 samples), KIR2DL4 (4/5 tumor samples), KIR3DL1(5/5 samples), and KIR3DL2 (5/5 samples) were expressed. A small population of tumor cells within each sample (~20%) were found to express the immune checkpoints B7-H3 (5/7 samples) and Adora2a (2/7 samples).

Conclusions

Using scRNAseq, we have identified populations of immune cells within tumor samples from human PNETs. These data suggest a suppressive immune phenotype. We will continue to study the mechanisms of immune regulation in PNETs.

Multi-institutional landscape of somatic genetic variants in Pancreatic Neuroendocrine Tumors

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Presenting Author

Leanne Brown, MD

Background

Pancreatic neuroendocrine tumors (PNETs) comprise a diverse group of rare neoplasms with heterogeneous behavior and presentation, resulting in a paucity of generalizable molecular data. Existing studies of somatic mutations in PNETs are limited by single-institutional analyses and small sample size. This study sought to employ larger and more diverse publicly-available repositories of genomic data to define a comprehensive landscape of PNET somatic mutations and prognostic findings for future study.

Methods

The following publicly-available genomic datasets were queried via cBioPortal: MSK-IMPACT, MSK-MET, PCAWG, ARC-NET, and MET500. All documented primary and metastatic PNET tumors were included. Somatic mutations and available clinical data were analyzed. A 5% mutational frequency was employed to exclude infrequently mutated genes. Significance testing was done by Mann-Whitney U for central tendency, Chi-squared for association, Wald for logistic regression, and logrank for survival.

Results

402 unique PNET cases were analyzed; of these, 281 (69.9%) were primary tumors and 121 (30.1%) were metastatic tumors. WHO grade and differentiation were not available. 98.8% and 29.4% patients had analyzable survival data and staging information, respectively. For all samples, nonsynonymous tumor mutational burden (TMB) was noted to be 0.98 mutations/megabase, with significantly higher TMB noted in metastases (2.59 mutations/megabase) than primaries (0.86 mutations/megabase) ($p<0.01$). For all tumors, 6,208 nonsynonymous variants were identified with median of 7 variants/tumor (IQR 2-18) of which 33.4%, 32.5%, 21.1%, and 8.6% were missense, frameshift, nonsense, and splice site mutation, respectively. Frequently mutated genes amongst all lesions were MEN1 (41.3%), DAXX (23.4%), ATRX (15.2%), SETD2 (8.5%), PTEN (6.7%), without significant difference between primary and metastatic tumors. Metastases displayed increased incidence of somatic variations in TP53 (19.0% vs 4.9%), TSC2 (16.5% vs 5.34%), ARID1A (9.9% vs 3.2%), KRAS (9.1% vs 0.36%), ATM (8.3% vs 2.8%), and FAT1 (5.8% vs 0.7%) compared to primaries. Logistic regression revealed TP53, TSC2, and ARID1A correlated with metastasis (OR=4.07, $p<0.01$; OR=3.163, $p<0.01$; OR=2.940, $p=0.03$, respectively). Gene-specific survival analyses demonstrated worse prognoses associated with TP53 and KRAS mutations ($p<0.01$, both), whereas ATRX and DAXX mutations were associated with improved survival ($p<0.01$, both). Nodal metastasis correlated with DAXX and ATRX mutations on logistic regression (OR=3.585, $p=0.03$; OR=6.024, $p<0.05$, respectively). DAXX and ATRX are mutually exclusive in primaries (z-score=4.16) but not metastases (z-score=0.6). Within ATRX or DAXX mutant tumors, metastases demonstrated an increased frequency of DAXX mutations compared to primary tumors ($p<0.01$), similar findings were not observed for ATRX mutations.

Conclusions

This study provides the largest aggregate landscape of somatic mutations present in PNET utilizing multiple publicly-available datasets. MEN1 remains the most commonly mutated gene in PNET and the prognostic importance of TP53, KRAS, TSC2 and ARID1A in metastatic disease was demonstrated. Mutual exclusivity of ATRX and DAXX variants demonstrates the importance of chromatin remodeling in primary tumors. Intriguingly, exclusivity was lost in metastases, meriting further study. Future analysis of somatic variants in PNETs would benefit from careful correlation with clinicopathologic data, including segregation of disease grade and differentiation status, to characterize the prognostic implications and biomarker potential of specific mutations.

C-terminal phosphorylation of Sstr2 reveals a paradigm for controlling GPCR interactions with PDZ domain proteins

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Presenting Author

Jeffrey A. Frost, PhD

Background

Many GPCRs interact with PDZ domain proteins to regulate aspects of GPCR function. However, little is known of how these interactions are regulated. We have shown previously that the Wnt pathway protein Dvl1 interacts with Sstr2 via its PDZ domain to target Sstr2 for lysosomal degradation. Dvl1 preferentially interacts with Sstr2 phosphorylated within its C-terminal PDZ domain binding site. In the present work we examine the full spectrum GPCRs that contain type I PDZ domain binding sites and demonstrate that the ability of many of GPCRs to interact with PDZ domains may be regulated by phosphorylation. We also characterize Sstr2 phosphorylation within its PDZ domain binding site and screen for kinases that phosphorylate this site. As Dvl1 controls Sstr2 expression, these studies have important implications for Sstr2 expression in neuroendocrine tumor cells.

Methods

We used bioinformatic approaches to identify GPCRs with type I PDZ domain binding sites and screened representative peptides for interaction with recombinant PDZ domains in vitro. We created a pS368-Sstr2 antibody and used it to characterize Sstr2 phosphorylation. Kinases capable of phosphorylating Sstr2 peptides in vitro were identified, and positive hits were examined for effects on Sstr2 in cells.

Results

We show that the interaction of many GPCRs with PDZ domain proteins is likely to be regulated by phosphorylation. We also show that Sstr2 phosphorylation within its PDZ domain binding site is regulated by agonist, and identify IKKβ as a Sstr2 S368 kinase in actively growing cells.

Conclusions

These studies demonstrate that phosphorylation of residues within type I PDZ domain binding sites of GPCRs may be a common mechanism to regulate interactions with PDZ domain proteins. We also show that Sstr2 is phosphorylated within this site in response to agonist. Moreover, we show that IKKβ contributes to Sstr2 phosphorylation on S368 in actively growing cells. Future studies will be aimed at determining whether inhibition of IKKβ or other S368 kinases represents a way to boost Sstr2 levels in neuroendocrine tumor cells.

Canine adrenomedullary and pheochromocytoma organoids: an in vitro animal model

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Presenting Author

Sara Galac, PhD, DVM

Background

Currently, there is a lack of appropriate experimental models for pheochromocytoma (PCC). The spontaneous nature, relatively high prevalence, and close resemblance of canine PCC to human PCC make it an attractive animal model. Organoids are self-organizing, self-renewing, three-dimensional cellular structures that closely resemble the organ or tumour they originate from. Organoid cultures can constitute a valuable disease modelling and drug screening platform. We aimed to establish and characterize organoid cultures of canine normal adrenal medullas and PCCs.

Methods

Normal adrenal tissues were obtained from healthy dogs euthanized for reasons unrelated to the present study. Adrenal glands were cut longitudinally and separated into cortex and medulla. Pooled adrenal medullas were used to develop organoid cultures. PCC tumour tissue was obtained from client-owned dogs with PCC following surgical removal. The primary cell suspension was seeded in a 3D matrix (basement membrane extract), and cells were cultured with a medium containing specific growth factors. Organoids were characterized using histopathology, immunohistochemistry for adrenomedullary markers, and qPCR analysis.

Results

We established four adrenomedullary organoid lines, which could be passaged every 3-4 weeks for an extended period (up to passage (P) 2 to 5). Two PCC tissue samples were used to develop PCC organoid cultures, which could be passaged after 4-6 weeks and are at P1 and P2 at the time of writing. Primary adrenomedullary and PCC cell suspensions showed mRNA expression of adrenomedullary markers tyrosine hydroxylase and chromogranin A, while their mRNA expression was very low in organoids. Similarly, organoids did not stain for chromogranin A and synaptophysin, except for PCC organoids at P0. Organoids had increased mRNA expression of adreno-medullary progenitor markers SOX10, nestin, and vimentin. Likewise, organoids showed immunolabeling for SOX10 and vimentin.

Conclusions

This study has demonstrated the feasibility of establishing canine adrenomedullary and PCC organoid lines. Currently, the organoids are in a progenitor state, and research efforts toward differentiation are ongoing. Canine adrenomedullary and PCC organoid lines have great potential as an in vitro research tool, paving the way towards developing an experimental model that faithfully recapitulates the phenotype of human PCCs.

Establishment and validation of pediatric and adult pheochromocytoma and paraganglioma organoids for high-throughput drug screening

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Presenting Author

Hector Gonzalez-Cantu, M.Sc.

Background

Pheochromocytomas and paragangliomas (PPGL) are rare catecholamine-secreting neuroendocrine tumors known for their genetic diversity and high heritability rates. About two-thirds of PPGLs have an identifiable driver event and can be part of hereditary syndromes including von Hippel Lindau (VHL) disease, multiple endocrine neoplasia, neurofibromatosis type 1, and familial PPGL syndromes. Metastatic and recurrent PPGLs cannot be diagnosed at earlier stages, and these patients have few therapeutic options, in part due to the lack of appropriate study models. Here, we report the establishment of viable organoids from PPGLs, and their histological, molecular, biochemical, and functional characterization.

Methods

PPGLs were obtained from patients of different age range (15-77y) with distinct clinical history and diverse ethnic and genetic backgrounds. We generated 12 PPGL organoids from fresh and/or frozen tissue that uses few cells with no need for expansion, and in a format compatible with histologic characterization and high-throughput studies including catecholamine measurements (by LC-MS/MS) and drug screening (mini-rings). PPGL growth in culture can be quantified using a machine learning-based pipeline.

Results

All PPGL organoids for which sufficient number of cells could be obtained grew, regardless of the original tumor location, patient age, sex, genotype or molecular subtype. PPGL organoids could be cultured short term (6 days) as well as long term (4 weeks). We detected several viable cell types including neuroendocrine markers (chromogranin A and tyrosine hydroxylase), the sustentacular cell marker S100, the vascular marker CD34. We confirmed that secreted catecholamines matched the primary tumor pattern and estimated organoid growth using a machine learning image analysis pipeline. Sensitivity profiles of 35 drugs or drug combinations revealed tumor-specific responses.

Conclusions

We can establish PPGL organoids that largely recapitulate properties of the tumor of origin. These models will provide the ability to investigate tumor initiation and progression for various genetic backgrounds and may reveal novel patterns of drug sensitivity and resistance.

Enhanced functionality of 3rd generation CAR-T cells mediated by activation of the IL23 cytokine pathway

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Presenting Author

Annapurna Haran, PhD

Background

Neuroendocrine tumors (NETs) pose a colossal burden owing to the development of resistance to the existing therapy modalities. Currently the 5-year survival rates are poor for metastatic NETs. Immunotherapy using CAR-T cells have proven very efficient in the treatment of many blood malignancies but still remain mostly ineffective for the solid tumors in general and the NETs in particular. Our studies so far resulted in development of novel nanobody-directed CDH17 CAR T cells to treat NETs in preclinical models. Data from preclinical studies demonstrated that the 3rd generation CAR-T therapy surpassed the 2nd generation CARs in eradicating the NETs. Here, we further explore the mechanisms that power the 3rd generation CAR-Ts by using broad range sequencing approaches. These massive sequencing approaches have been beneficial in identification of potential signaling pathways that can further improve the efficacy of CAR-T therapy for pancreatic NETs.

Methods

NSG mouse models were used to study the success of the 2nd and 3rd generation CAR-T cells by analyzing tumor specific xenografts. Sc-RNA sequencing was performed using sorted CAR-T cells that were obtained from the tumors itself. In addition, bulk RNA sequencing was done from the ex-vivo cocultures of 2nd or 3rd generation CAR-Ts with the CDH17-expressing tumor cells (appropriate controls analyzed). Bioinformatic analysis of the scRNA sequencing and bulk RNA-sequencing were used to obtain answers about the cell lineage as well as information about key signaling pathways that are altered in these conditions.

Results

Results from the sequencing analyses suggest the up-regulation of multiple T cell receptor signaling pathways including that of the IL23 cytokine pathway. Furthermore, the CD226 a cell adhesion molecule that is important for the activation and migration of the CD8 T cells was also identified. Experiments were designed to study the effects of IL23 signaling on the performance of 3rd generation CDH17 CAR-T. Studies from IL23 overexpression in the CAR-T cells resulted in proving the better performance of these CARs as evident from our ex-vivo studies that measure cytokine release and cytotoxicity. In vivo studies are being carried out to further confirm our findings. These results are promising and pave the way for evaluation of more targets such as CD226 that were obtained from these sequencing approaches.

Conclusions

3rd generation CAR-Ts have proven to perform better in preclinical studies in comparison to the 2nd generation CAR-Ts. Using sequencing approaches we were able to identify signaling pathways such as IL23 that can further improve the functionality of 3rd generation CAR-Ts. Ex vivo results indicate that the activation of IL23 could be beneficial for better CDH17-CART function. Next steps include confirmation of these results using in vivo mice models.

The Interaction between Small Intestinal Neuroendocrine Tumor Cells and Cancer Associated Fibroblasts

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Presenting Author

Leo J. Hofland, PhD

Background

The pathophysiology of mesenteric fibrosis (MF) in patients with a small intestinal neuroendocrine tumor (SI-NET) is still not completely elucidated. To date, surgery is the only therapeutic option for MF, therefore it is urgent to further examine the origin of SI-NET-associated MF. The tumor microenvironment, especially the interaction between tumor cells and cancer-associated fibroblasts (CAFs), is postulated to play an important role in the development of MF. By investigating this interaction in more detail we hope to further clarify the evolution of MF and hopefully uncover new therapeutic options for this condition in SI-NET patients.

Methods

As a model for studying the interaction between CAFs and SI-NET cells, fibroblasts from SI-NET tissue originating from the primary tumor, the mesenteric metastasis and adjacent mesenterium are isolated and cultured. The degree of mesenteric fibrosis in these SI-NET patients is scored radiologically and pathologically. Isolated cells are stained with different identification markers (FAP, aSMA, vimentin) and exclusion markers (CD31, E-CAD, synaptophysin) for characterization. Additionally, mRNA expression of genes associated with different types of CAFs will be measured using RT-qPCR.

To study the paracrine effects of SI-NET cells on fibroblasts, isolated CAFs are incubated with either conditioned medium from GOT1 cells, a cell line derived from a SI-NET, or control medium. Proliferation is measured using BrdU incorporation (n>3 per experiment).

In order to investigate direct cell-cell interaction next to the paracrine effects, GOT1 spheroids, CAF spheroids and combined spheroids consisting of both CAFs and GOT1 cells are generated and immunohistochemically stained with proliferation markers, activation markers and markers for identification (vimentin and synaptophysin). Proliferation within the spheroids is further quantified using flow cytometry. To evaluate which specific factors are involved in the interaction between tumor cells and CAFs, secretome analysis will be performed of supernatant derived from spheroids (co-cultured versus separately cultured) and monolayer CAF cultures using high-throughput antibody microarrays.

Results

Preliminary data show a predominantly higher proliferation rate of CAFs cultured in GOT1 conditioned medium compared to CAFs cultured in control medium (in 3 out of 4 CAF cultures). Within the combined spheroids we observed that the patient derived CAFs and GOT1 cells mainly formed clusters instead of mixing. Preliminary immunohistochemical data show an increased αSMA (2 out of 2) and COL1A1 (1 out of 2) staining in the co-cultured spheroids compared to the separate spheroids, especially located at sites were GOT1 cells and CAFs co-localize.

Conclusions

Our data suggest that mediators produced by the neuroendocrine tumor cells are able to stimulate the proliferation of CAFs. The increase in activation markers within the co-cultured spheroids indicates that GOT1 cells are able to activate CAFs. Therefore we propose that the 3D spheroids are a valuable model to study SI-NET-CAF interactions. Next steps include replication of the above experiments with different CAF cultures, further quantification of proliferation using flow cytometry and secretome analysis.

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Accelerating the Development of Peptide-Based NET Tracers with 'Next-Gen' 18F Chemistry

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Presenting Author

James A.H. Inkster, PhD

Despite the "best-in-class" PET imaging characteristics of 18F and a sustained interest in dual-isotope radiohybrid radiopharmaceuticals for theragnostic applications, the development of 18F-labeled peptides as NET imaging agents has languished in recent years in favor of radio-metalated versions. The main challenges are associated with radiosynthetic simplicity, with the first significant bottleneck being a perceived need of scrupulously dry [18F]F-. This is typically achieved through repeated azeotropic evaporations to remove [18O]H2O (aka 'drydown' steps). Secondly, while chelator-modified peptides can usually be labeled directly, under mild conditions, and in such small quantities as to afford high molar activity doses without the need to remove precursor by HPLC, most peptide ligands will not tolerate the high temperatures and carbonate base usually employed to incorporate [18F]F- by nucleophilic substitution. A common solution is to synthesize a small prosthetic group (PG) first, then couple it to the peptide in a subsequent step. In most cases, the 18F-labeled peptide must be separated from precursor peptide by HPLC, a practice made difficult when the peptide is large. Overall, labelings with 18F PGs are challenging to translate to automated synthesis devices, which is a requirement for clinical development.

Innovative new radiochemical methods have emerged in recent years designed to obviate both the [18F]fluoride 'drydown' and prosthetic group approaches. One such strategy is based on the observation that small quantities of water were less detrimental to many nucleophilic 18F-fluorination reactions than previously assumed; thus, by trapping [18F]F- on smaller-than-usual anion exchange cartridges and eluting with non-basic anions (e.g. tosylate, triflate), good radiochemical conversions can be achieved in 'damp' (<5% water), mild reaction mixtures (aka NAMB chemistry). Another approach, dubbed Silicon-Fluoride Acceptor (SiFA) technology, utilizes isotopic exchange reactions to directly 18F-label small quantities of peptides modified with fluorodi-t-butylphenylsilane groups. However, the pH of such reaction mixtures must be carefully controlled, a process made difficult when basic eluents are used, and manual titration is impossible (e.g. automated synthesizers).

We recently discovered that the NAMB approach can be applied to the direct synthesis of [18F]SiFA-ylated peptides. Thus, as little as 50 nmol of Tyr3-octreotide derivative [19F]SiFAlin-TATE in MeCN/DMSO mixtures containing 3% water was radiolabeled at room temp. (60-84% RCC), in volumes amenable to automation (1 mL). Simple solid phase extraction on C18 cartridges afforded [18F]SiFAlin-TATE in NDC-RCYs of 37-52%. Next efforts will involve translation of this merged NAMB/SiFA protocol to a FastLab synthesizer. We then intend to leverage these learnings towards the discovery of novel insulinoma imaging agents. The clinical recommendation for such cancers is imaging prior to partial pancreatic resection or enucleation, but the 111In- and 68Ga-exendin-4 agents invented thus far have the drawback of substantial renal accumulation, a phenomenon we hypothesize less likely to arise from lipophilic 18F pendant groups. Workflow will start with the synthesis of candidate excendin-4 analogues with pendant SiFA moieties varying in bioconjugate linker and aromatic ring, followed by assessment of parameters important to tracer distribution (i.e. lipophilicity, plasma stability), in vitro receptor binding studies (INS-1 cells), and biodistribution/µPET experiments in a mouse model of insulinoma.

Prolonged inhibition of VEGF signaling promotes liver metastases in pancreatic neuroendocrine tumors

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Presenting Author

Sophie O'Keefe, BS

Background

Pancreatic neuroendocrine tumors (PanNETs) with liver metastases have poor survival outcomes due to the limited treatment options available. One of the few treatment options for patients with PanNET liver metastases is anti-angiogenic therapy, such the multi-target tyrosine kinase inhibitor (TKI) sunitinib. However, anti-angiogenic treatment is limited by the development of therapeutic resistance and provides minimal benefit to patients' overall survival. Current research into the mechanisms of resistance to anti-angiogenic therapy has been limited to primary PanNETs and fails to examine the role of prolonged anti-angiogenic therapy on metastatic PanNETs. Consequently, our study aims to investigate the effects of prolonged inhibition of VEGF signaling on the development of PanNET liver metastases using the spontaneous PanNET mouse model RT2;AB6F1.

Methods

Mouse Model of PanNETs: Transgenic mice, RIP1-Tag2;C57BL6, were kindly provided by D. McDonald (UCSF, CA). RIP1-Tag2;AB6F1 (RT2;AB6F1) mice were generated by mating female A/J mice with male RIP1-Tag2;C57BL6 mice. Using RT2;AB6F1 mice, tumor progression and liver metastases were studied from 15 to 16 and 15 to 20 weeks of age. At the experimental endpoint, liver and pancreatic tissues were collected after cardiac perfusion. Treatments: RT2;AB6F1 mice were treated with a VEGF trap (REGN3, 12.5 mg/kg, ip) or IgG (REGN1945, 12.5 mg/kg, ip) twice a week for one or five weeks beginning at 15 weeks of age.

Results

We found that prolonged inhibition of VEGF signaling for 5 weeks results in a liver metastatic burden similar to control mice, along with increased vascular leakage, despite an initial decrease in liver metastatic burden observed after 1 week of treatment.

Conclusions

Our findings suggest that extended inhibition of VEGF signaling induces changes to the vascular endothelium that promote the metastatic progression of PanNETs. The exact changes that occur in the tumor vasculature to promote metastatic progression after prolonged inhibition of VEGF signaling are unknow. Future work aims to identify these changes at the transcriptional level and begin to elucidate the mechanism of resistance to long term VEGF inhibition in PanNET liver metastases.

Targeting MUC1-C in pancreatic neuroendocrine tumor

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Presenting Author

Hiroki Ozawa, MD, PhD

Background

The MUC1 gene evolved in mammals for adaptation of barrier tissues to loss of homeostasis. Dependence on the oncogenic MUC1-C subunit for the cancer stem cell (CSC) state, self-renewal capacity and tumorigenicity has been uncovered across pan-cancers, including neuroendocrine prostate cancer (NEPC) (Yasumizu Y, et al. Nature Communications. 2020), small cell lung cancer (SCLC) (Fushimi A, et al. Molecular Cancer Res. 2022) and Merkel Cell Cancer (MCC) (Morimoto Y, et al. Oncogene. 2022). However, there is no known involvement of MUC1-C in the pathogenesis of pancreatic neuroendocrine tumors (pNETs).

Methods

Publicly available datasets were analyzed for MUC1 gene expression in metastatic and localized patient pNET tissue samples. Targeting MUC1-C genetically and pharmacologically in pNET cell lines was performed to assess effects on intracellular signaling pathways, clonogenic survival and self-renewal capacity as determined by tumorsphere formation. Metastatic and localized patient pNET tissue samples were analyzed for MUC1-C expression by immunohistochemistry (IHC).

Results

Analysis of a pNET dataset (GSE73338) demonstrated that the MUC1 gene is significantly upregulated in metastatic as compared to localized tumors, indicating that the MUC1-C subunit may contribute to pNET progression. In addressing this notion, we found that the widely studied BON-1 and QGP-1 pNET cell lines express MUC1-C transcripts and protein. We therefore established BON-1 and QGP-1 cells expressing a tet-inducible control CshRNA or a tet-MUC1shRNA. DOX treatment of BON-1/tet-MUCshRNA and QGP-1/tet-MUC1shRNA cells, but not those transfected with the tet-CshRNA vector, resulted in downregulation of MUC1-C expression. Silencing MUC1-C decreased mTOR and MYC, which have been associated with pNET cell progression. Silencing MUC1-C also suppressed colony and tumorsphere formation, which was rescued by expressing the MUC1-C cytoplasmic domain. In extending these results, treatment with the GO-203 inhibitor, which blocks MUC1-C function, similarly suppressed (i) mTOR and MYC expression, and (ii) colony and tumorsphere formation. These findings demonstrate that BON-1 and QGP-1 cells are dependent on MUC1-C for clonogenic survival and self-renewal capacity. We therefore analyzed MUC1-C expression in surgically resected pNETs by IHC and found upregulation of MUC1-C staining in metastatic vs localized tissue samples.

Conclusions

Our results demonstrate that (i) pNET cell lines are addicted to MUC1-C, and (ii) MUC1-C expression is increased in metastatic pNET tissues. These findings indicate that MUC1-C represents a potential target for advancing the treatment of patients with metastatic pNETs with the anti-MUC1-C agents that are under clinical and preclinical development.

Somatic variants in primary and metastatic pheochromocytomas and paragangliomas

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Presenting Author

Andrew M. Pregnall, MSc, MPhil

Background

Pheochromocytomas (PCC) and paragangliomas (PGL) are neuroendocrine tumors with the highest heritability of all human tumors. Recent studies suggest that familial pathogenic variants in genes encoding Krebs cycle enzymes such as succinate dehydrogenase (SDHx) confer a homologous recombination deficiency phenotype through inhibition of KDM4B. The goal of this study was to characterize genomic markers of DNA damage repair and chromatin remodeling in PCC and PGL.

Methods

We performed whole exome sequencing and variant discovery using MUTECT2, STRELKA, VARDICT, LANCET, and VARSCAN2. Somatic variants were called using Varlociraptor with a statistical model to account for formalin fixed paraffin embedded tissue artifacts and a false discovery rate of 5%. We limited subsequent analysis to variants with a read depth of ≥ eight reads. To identify loss of function (LOF) driver mutations, we excluded somatic variants with a gnomAD frequency ≥ 0.01 and then filtered for (1) frameshift mutations, (2) nonsense mutations, (3) missense mutations with a REVEL score > 0.5, and (4) splice acceptor or splice donor variants. We then examined variants which met these criteria in genes documented in the Catalog of Somatic Mutations in Cancer (COSMIC) v98 Tier 1 or Tier 2 Cancer Gene Census. Germline pathogenic variants were abstracted from clinical genetic testing.

Results

We analyzed 46 tumors from 27 patients. 20 were PCC (4 primary; 16 metastases) and 26 were PGL (8 primary; 18 metastases). A total of 14 patients had germline pathogenic variants in SDHx (SDHA: 2, SDHB: 11, SDHC: 1, SDHD: 0) and one patient had a germline pathogenic variant in RET. On somatic variant analysis, we found frequent LOF mutations in chromatin remodeling and DNA damage repair related genes. Within chromatin remodeling genes, 30% of patients (n=8) had mutations in ATRX; 26% (n=7) had mutations in FOXO3; 22%

(n=6) had mutations in KMT2A; 33% (n=9) had mutations in KMT2C; and 33% (n=9) had mutations in KMT2D. Within DNA damage response genes, 26% of patients (n=7) had mutations in BRCA1; 26% (n=7) had mutations in BRCA2; 22% (n=6) had mutations in ATM; and 22% (n=6) had mutations in ATR. In total, 59% (n=16) had LOF mutations in either chromatin remodeling or DNA damage repair genes. Rates of LOF mutations were not significantly different in patients with known germline pathogenic variants in SDHx (67% versus 53%, p=0.484).

Conclusions

PCC and PGL tumors demonstrated significant LOF variants in known oncogenes. Our data suggest that LOF mutations in homologous recombination related chromatin remodeling and DNA damage response genes may contribute to somatic tumor progression both in the absence and in the presence of germline variants. These genetic mutations may confer susceptibility to targeted therapies against DNA damage repair pathways, including PARP inhibitors.

Loss of DAXX protein expression results in increased radiosensitivity of BON1 cells

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Presenting Author

Jessica C. Puzzuoli, PhD

Background

Well-differentiated pancreatic neuroendocrine tumors (PNETs) often present late with unresectable liver metastases. These are often treated with liver-directed therapies including ischemia-based transarterial embolization (TAE), or radioembolization (TARE). Preliminary data has shown poor response to TAE in DAXX-mutated PNETs. The purpose of this study was to evaluate the effect of loss of DAXX protein expression on radiosensitivity and ischemia sensitivity of BON1 cells.

Methods

BON-1 wild type and CRISPR/Cas9-generated DAXX knockout C16 cells were plated on 6-well and 96-well plates for trypan blue staining for cell counts and cell viability assays using Cell Titer-Glo, respectively. Cells were plated in normal (high glucose media 37°C at 5% CO_2) and ischemia conditions (low glucose media, hypoxia chamber at 0.5% O_2) and analyzed at days 1, 3, 5, and 7. Data analyses were performed using GraphPad Prism and viability and cell counts compared using Student's t-test. Annexin V/propidium iodide flow cytometry was performed using the Cytoflex LX and analyzed with FlowJo software. Cells were treated with radiation on XRAD 320 with varying doses: 0, 2, 4, 6, 8, and 10Gy. Cells were plated in 6-well plates and incubated in normal conditions for 12 days. Plates were fixed and stained with crystal violet. Imaging and colony counting were performed using the ZEN microscope and ImageJ with the Fiji package. Two-way ANOVA and F-test were performed using Graphpad Prism. All experiments were performed in triplicate and results analyzed at a 95% confidence interval.

Results

C16 cells demonstrated increased percent viability by cell count at day 3 of ischemia (51.3% +/-0.03) compared with BON-1 cells (22.3%±0.02, p<0.001). Flow cytometry at day 3 [4] showed 78% [49%] viability, 3% [3%] apoptotic, 17% [6%] necrotic and 2% [42%] apoptotic/necrotic in C16 cells compared with 29% [25%] viability, 7% [4%] apoptotic, 50% [60%] necrotic, and 14% [11%] apoptotic/necrotic in BON-1 cells. Conversely, C16 cells showed increased radiosensitivity with colony formation and survival fraction percentages at 2, 4, 6, 8, and 10Gy (25.3%, 16%, 2%, 2%, 0.3% and BON-1 at 51%, 43.6%, 15.3%, 12.3%, and 2.6%, respectively at p<0.001).

Conclusions

Loss of DAXX protein expression resulted in transient ischemic resistance and increased radiosensitivity compared with wild type BON-1 cells. Additional studies are ongoing, including downstream analysis of ischemia and radiation stress and correlation with clinical data.

PET imaging of statin-mediated SSTR2 modulation of tumors

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Presenting Author

Shayla Shmuel

Background

The somatostatin receptor 2 (SSTR2)-targeting radiolabeled peptide DOTATATE is clinically approved for imaging and systemic radiotherapy of neuroendocrine tumors. However, tumor heterogeneity caused by endocytosis of SSTR2 contributes to inadequate tumor targeting and low drug delivery that leads to disease progression. In our previous work, we demonstrated that cholesterol-depleting drugs (statins) enhance drug delivery in tumors. In this work, we used positron emission tomography (PET) to optimize the dose of statin needed to enhance DOTATATE accumulation in SSTR2-low tumors.

Methods

Radiolabeling: 64Cu-DOTATATE was prepared by reacting 64Cu with DOTATATE (RCP>98%). Biodistribution study: Nu/nu nude mice bearing SSTR2-low FTC133 tumors (n=4 mice/group) were injected with 64Cu-DOTATATE. SSTR2-negative H292 cancer cells were used as controls. PET imaging study: *Nu/nu* nude mice bearing SSTR2-low FTC133 tumors received 2 doses of lovastatin given 12 h apart (8.3 mg/kg/day). Mice were injected with 64Cu-DOTATATE and PET imaging was performed at two time points: 1 h and 24 h after injection, followed by biodistribution studies. Statin dose escalation study: *Nu/nu* mice bearing SSTR2-low FTC133 tumors (n=4 mice/group) were given 2 doses of statin, 12 hours apart, followed by 64Cu-DOTATATE and 24 h after biodistribution studies were performed. The doses of statin that were given were 2.1, 4.15, 8.3, 16.6 mg/kg/day or no statin.

Results

Given our previous studies showing that the cholesterol-depleting drug lovastatin improves receptor availability by modulating receptor endocytosis, here we show the use of lovastatin to improve binding of DOTATATE to tumors expressing low SSTR2. Lovastatin was shown to increase membrane SSTR2 in vitro as well as 64Cu-DOTATATE uptake in vivo. Tumor uptake values were higher at 1-4 h post DOTATATE injection when compared to uptake values at later time points (24-72 h). When analyzing the dose of statin needed to obtain higher tumor accumulation of 64Cu-DOTATATE in vivo, we observed that 2.1 mg/kg/day resulted in the highest tumor uptake.

Conclusions

Our data suggest that statin treatment with appropriate pharmacokinetics is a potential adjuvant for radiotheranostics. Lovastatin enhances membrane-bound DOTATATE, while temporarily modulating proteins of the endocytic trafficking systems and enhancing target density at the cell membrane. Our ongoing therapeutic studies are testing statins in combination with 177Lu-DOTATATE.

Cancer testis antigen expression correlates with high type-I interferon signal and improved overall survival in small bowel neuroendocrine tumors

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Presenting Author

Y. David Seo, MD

Background

Patients with small bowel neuroendocrine tumors (SBNETs) frequently present with metastatic disease, and efficacy of available therapies is limited. Immune checkpoint inhibitors alone have shown minimal effect in SBNETs. Toward a goal of developing novel immunomodulatory strategies, we sought to evaluate the SBNET tumor immune microenvironment utilizing Nanostring bulk transcriptional and digital spatial profiling (DSP).

Methods

SBNET patients who underwent surgical resection at MD Anderson Cancer Center from 2003 to 2016 were retrospectively analyzed for clinicopathologic data. Overall survival (OS) from date of resection was assessed using the Kaplan-Meier method with log-rank test. Multivariate analysis (MVA) was performed with Cox proportional hazards model. Transcription profiling from bulk RNA was performed using the Nanostring PanCancer-Immune Panel. The DSP experiment was performed with whole human transcriptome on 8 tumors using PanCK and CD45 as segmentation markers; 88 distinct regions (39 with PanCK+ tumor cells and 49 with CD45+ immune cells) were analyzed using differential gene expression and Reactome pathway enrichment pipelines.

Results

Resected SBNETs from 42 patients were selected for transcriptional analysis. Unsupervised clustering of RNA expression data revealed separation into high and low expression groups in the cancer testis antigens (CTA) panel. CTA-high patients (n=12) had significantly improved overall survival compared to CTA-low patients (n=30; median OS not reached vs 1975 days, p=0.0084). MVA controlling for age, sex, metastatic disease, and grade by Ki-67% confirmed CTA-high status as an independent predictor of improved OS (hazard ratio [HR] for death 0.2, 95% confidence interval [CI] 0.05-0.83, p=0.014). CTA-high patients had significantly elevated expression of CTAs (such as PRAME, GAGE1 and MAGEA3) and type-I interferons (such as IFNA1 and IFNB1). High normalized expression levels of these single genes were independent predictors of improved OS in MV analysis (e.g. PRAME with HR 0.045 [95% CI 0.003-0.64, p=0.0062], GAGE1 with HR 0.073 [95% CI 0.0056-0.53, p=0.008]).

A pilot DSP experiment with 4 CTA-high and 4 CTA-low tumor specimens revealed higher expression of genes involved in epigenetic modification (e.g. H2BC8 and H3C1, p<0.001) and DCAF12 (known regulator of MAGEA3 expression) in CTA-high tumors; there was also significant enrichment of epigenetic pathways (e.g. Reactome pathways for epigenetic regulation of gene expression, DNA methylation, and histone acetylation; adjusted p-value<0.05). Within the intra-tumoral immune cells, CTA-high patients demonstrated lower levels of adenosylhomocysteinase, a central mediator of DNA methylation (p=0.0001).

Conclusions

High expression of CTA and type-I interferons in resected SBNETs identified patients with improved survival, agnostic of stage or grade. Abnormal CTA expression in cancers is known to be mediated by epigenetic modification and have garnered interest in their potential for therapeutic targeting; however, this is the first work to identify such a clinically relevant signal in SBNET. The concurrent increase of type-I interferons (known to mediate anti-tumor activity) among CTA-high patients suggests an immune-mediated component to their improved survival. Spatially-resolved transcriptional signatures suggest epigenetic differences in both the tumor and immune compartments. Work is ongoing to validate these findings in the larger cohort, which will potentially provide rationale for combination epigenetic modifiers and immunotherapy in future trials.

Post-Embolization Transcription Changes in Pancreatic Neuroendocrine Tumors and their Microenvironment

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Presenting Author

Himanshu Narayan Singh, PhD

Background

Pancreatic neuroendocrine tumors (pNETs) pose a formidable challenge for treatment due to their heterogeneity. pNET liver metastases are often treated with trans-arterial embolization (TAE), but responses are variable. Few studies have evaluated the effects of TAE on pNETs and their microenvironment. This study was performed to assess the early transcription changes in pNETs and the tumor microenvironment (TME) after TAE.

Methods

We prospectively enrolled patients with pNET liver metastases undergoing TAE. Paired biopsies were performed immediately before and after TAE. Tumor grade was assessed by pathologist using Ki67. We stratified patients to high-grade (Ki67>20%) and low-grade (Ki67<20%). Samples were flash-frozen and RNA extraction was performed, libraries constructed and sequenced as 101-bp paired-end reads. The bioinformatics pipeline included Star aligner facilitated alignment via a 2-pass mapping strategy, HTSeq generated raw counts and finally DESeq2 for gene expression. For TME analysis, we used xCell to quantify cell enrichments patterns and "easier" package to identify ligand-receptor pair cell-cell interactions between pNET and TME. We compared pre- and post-treatment as well as high-grade and low-grade using Wilcoxon Rank Sum and Signed Rank Tests. For overall ligand-receptor score comparison paired t-test was used.

Results

Of the initial 34 paired samples, there were 3 paired samples and 7 non-paired samples that failed quality control, resulting in a refined set of 24 successfully paired samples and 7 non-paired samples for further analysis. There were 32 low-grade and 23 high-grade samples. A total of 174 genes exhibited differential expression in the post-TAE condition, with 125 genes upregulated and 49 genes downregulated. Further analysis revealed activation of differentially expressed genes in pathways including metabolism, inflammation, and angiogenesis. Comparison of TME between high- and low-grade tumors revealed significant differences in cell composition in both pre- and post-TAE conditions. In pre-TAE condition, low-grade tumors exhibited significantly elevated levels of CD8+ naive T-cells (p=0.029), whereas high-grade tumors demonstrated significantly higher levels of megakaryocyte-erythroid progenitors (p=0.035), pericytes (p=0.016), and preadipocytes (p=0.041). In the post-TAE condition, a significantly higher level of lymphoid endothelial cells was observed in low-grade tumors compared to high-grade tumors (p=0.016). Analysis of a comprehensive set of 675 ligand-receptor pairs identified distinct patterns of cell-cell interactions in high-grade and low-grade tumors. The 111-pairs exhibited activation specifically within low-grade tumors prior to TAE, while 71-pairs demonstrated activity in low-grade tumors after TAE. The 17-pairs were shared between both the pre-TAE and post-TAE. Low-grade tumors had overall increased ligand-receptor scores (pvalue<0.001). One notable illustration is the COL1A1_CD44 and COL1A2_CD44 ligand-receptor pairs, demonstrating sustained activity in both pre-TAE and post-TAE settings within low-grade tumors, with diminished presence in high-grade counterparts.

Conclusions

Early global transcriptional changes were observed in post-TAE liver metastatic pNETs including differential expression of genes in multiple pathways including metabolism, inflammation, and angiogenesis, reflecting dynamic shifts within the TME triggered by TAE. Additionally, we observed significant differences in cell-composition and cell-cell interactions within the TME between high grade and low-grade tumors. Next steps include correlation of these observations with tumor response to TAE and recurrence after TAE.

Glutamate dehydrogenase a potential treatment target for SDHB-mutated pheochromocytomas and paragangliomas

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Presenting Author

Mouna Tababi, PhD

Background

Mitochondrial Complex II or SDH (succinate dehydrogenase) plays a central role in mitochondria as the only enzyme of two fundamental metabolic pathways. SDH catalyzes the oxidation of succinate to fumarate in the Krebs cycle and feeds electrons to the respiratory chain. SDH is formed by four subunits, A, B, C and D. SDHB mutations cause pheochromocytomas and paragangliomas (PPGLs) with a high risk of metastasis (29-73.8%), making SDHB functions relevant and an interesting area to study.

Methods

CRISPR-cas9 technology had been used to knocked down SDHB gene in the human pheochromocytoma cell line (hPheo1). Microarray and qPCR had been performed to analyze gene expression. Seahorse Extracellular analyzer XF24 had been used to test cellular metabolic activities.

Results

Microarray data analysis showed the upregulation of genes involved in glycolysis, hypoxia, cell proliferation, and cell differentiation and downregulation of genes involved in oxidative phosphorylation (OXPHOS) in response to SDHB knock down. In vitro studies showed that KD-SDHB hPheo1 survive by shifting their metabolism to aerobic glycolysis on dependence of glutamate and promote OXPHOS activity. TCGA data confirms the enhancement of glutamine dehydrogenase (GDH) gene expression in PPGLs with low expression of SDHB. Using GDH inhibitor (purpurin analogue, R162) which is a potent inhibitor in cancer cells, the enzymatic activity decreases dramatically. The treatment results in accumulation of cells in the G1/G0 phase and coincides with the occurrence of Sub-G1 population which refers to apoptotic cells.

Conclusions

Our data suggest that GDH may represent a potential biomarker and correspondent inhibitor can be a potentially therapeutic target in SDHB-mutated PPGLs.

Validation of radiomics model to predict symptoms complications from small intestinal net mesenteric metastases – preliminary report

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Presenting Author

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Background

The development of mesenteric metastases and associated mesenteric fibrosis (MF) in small intestinal neuroendocrine tumours (SI-NET) can cause significant complications and have implications in patients' survival. Conventional computed tomography (CT) seems suboptimal for accurate assessment of the degree of fibrosis and prediction of complications. A better model is needed, therefore, in order to select patients who may benefit from a prophylactic surgical approach in the mesentery. Blazevic et al., from Erasmus NET Unit demonstrated in 2021 the promising role of a "radiomics model", as a predictive "tool" for development of complications of mesenteric metastases and fibrosis in 68 patients. The aim of this study is to validate that "radiomics model" through a different patients' cohort from Royal Free, NET Unit.

Methods

Forty patients with SI-NET have been included in this preliminary study. Twenty of them were asymptomatic and did not proceed to surgery (Group A), whilst the remaining had symptoms of intestinal ischaemia and/or obstruction (Group B). Demographic parameters (age, gender), clinical parameters (tumour grade and tumour stage at diagnosis), biomarkers' (CgA and 5-HIAA) levels for all patients as well as surgical indication (obstruction, pain, ischemia, perforation) and histopathology fibrosis score for Group B patients' have been collected. The mesenteric cuff around the metastatic node has been segmented (including desmoplasia), using a semi-automated segmentation program ITK-SNAP. Images will be transferred to Erasmus NET Unit for application of "radiomics model".

Conclusions

In this study the predictive value of radiomics on the clinical outcome of mesenterial fibrosis associated with SI-NET will be validated.

This research into mesenteric fibrosis is funded by NETRF Accelerator Grant 702627 (UCL/Exeter/Erasmus)

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