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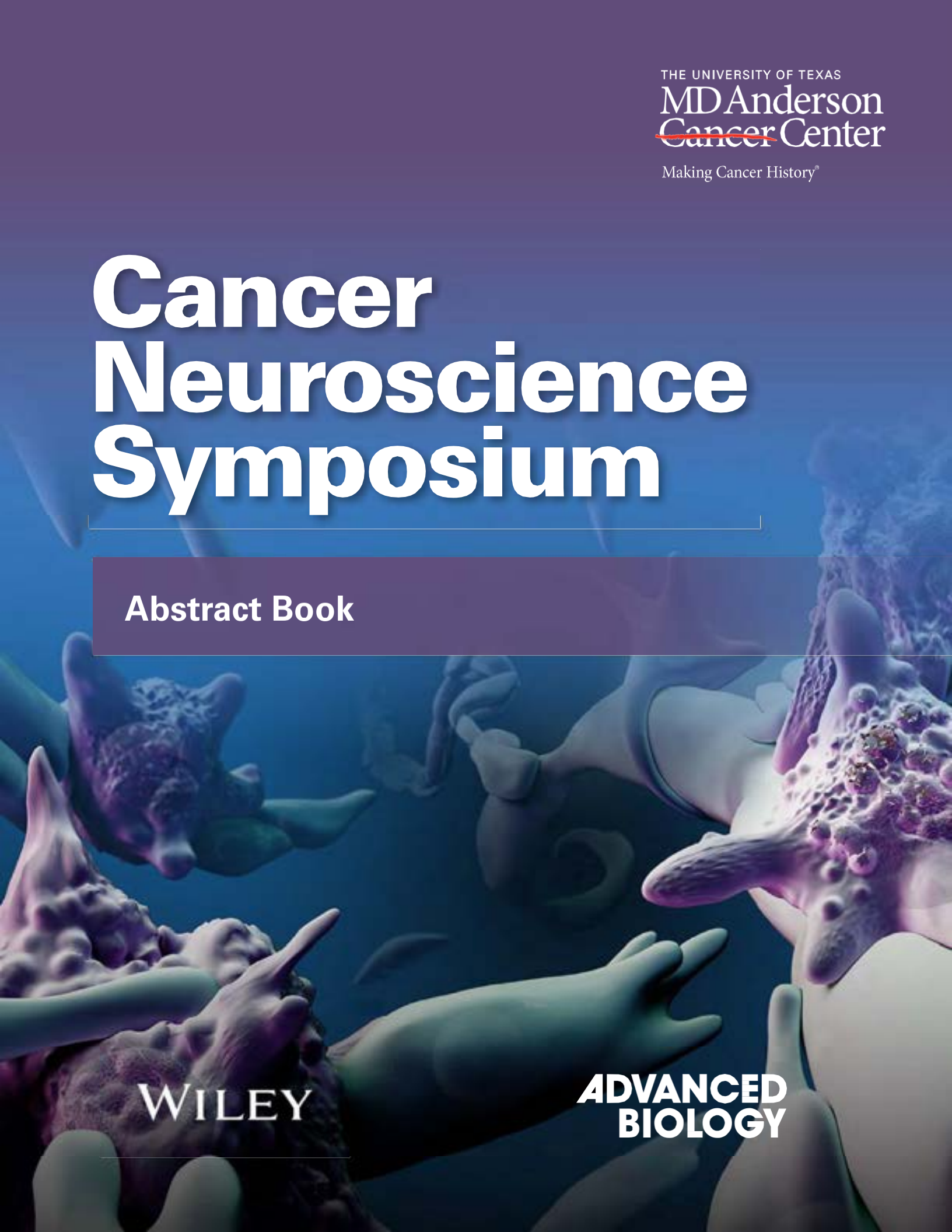
Cancer Neuroscience Symposium

Abstract Book



WILEY

ADVANCED BIOLOGY

Thursday, February 29th 2024, 12:00-1:00pm; 5:00 – 6:00pm			
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4	Spatial transcriptomic analysis of sensory neurons in murine pancreatic cancer reveals a potential role for neuron-mediated immune suppression in the tumor microenvironment	Kaan Çifcibaşı, Carmen Mota Reyes, Rouzanna Istvanffy,…,Ihsan Ekin Demir	Heidelberg University
5	Sympathetic-sensory nerve coupling in oral squamous cell carcinoma drives tumor progression	Andre Martel Matos, Lisa A. McIlvried, Marci L. Nilsen,…,Nicole N. Scheff	University of Pittsburgh
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14	NKCC1-dependent GABAergic signaling drives neuronal hyperexcitability and tumor progression in glioblastoma	Saritha Krishna, Cesar Nava Gonzales, Andy Daniel,…,Shawn Hervey-Jumper	University of California San Francisco
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Late-Breaking Abstract Talks | Feb. 28th

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ADVANCED BIOLOGY

*__NOVOCURE AWARD – BEST OVERALL CANCER NEUROSCIENCE ABSTRACT__

Electrophysiologic and spatially resolved genomic signatures of glioma-infiltrated cortex

Andy G. S. Daniel, Alexander Aabedi, Gray Umbach, Mikias Negussie, Sanjeev Herr, Saritha Krishna, Jasleen Kaur, Elisa Fazzari, Vidhya Ravi, Aparna Bhaduri, David Brang, Shawn L.Hervey-Jumper
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Introduction
Neocortical circuits selectively organize neuronal signals encoding features of cognitive processing, and route them to specialized short and long-range circuits. Since glioma- infiltrated cortex is demonstrably excitable and can participate in cognitive processing, the underlying cortical laminar structure and functionality may still be preserved despite tumor infiltration. Moreover, as glioma cells remodel existing neural circuits and microenvironmentalfactor drive cellular invasion and proliferation, neuron-glioma interactions may be layer specific. As the electrophysiologic, structural, and genomic landscape of glioma-infiltrated cortex remains poorly understood, we sought to investigate these regions using a multimodal approach.

Methods
The power spectra of normal-appearing and glioma-infiltrated cortex were recorded using subdural high-density electrode arrays at testing and validation sites across the US and Europe. Immunohistochemistry of formalin-fixed paraffin-embedded (FFPE) samples of infiltrated cortexenabled protein-level neuronal and glioma identification to assess laminar preservation and spatial patterns of invasion. Spatial transcriptomics profiling of FFPE tissues, single-cell and single nuclei RNA-sequencing were used to identify cell populations, location-matched genomic alterations, and cell-cell communication within and across samples.

Results
Increased delta range (1-4 Hz) power and decreased power in the beta range (12-20 Hz) was identified as a robust feature of glioma-infiltrated cortex which was maintained across glioma subtypes and preserved in a validation cohort encompassing magnetoencephalography (n= 140patients) and subdural electrocorticography (n= 12 patients). Tissue proteomics and spatial genomics analyses was performed in 8 test set and 32 validation set cortical samples which revealed tumor burden greatest within infragranular cortical lamina regardless of glioma subtype. Genomic analyses confirmed preservation of cortical laminar structure as well as layerspecific differences in glioma-related expression programs such as hypoxia, inflammation, and synaptogenesis compared with control conditions. Cell-cell communication analyses demonstrated greater layer specific interactions in glioma-infiltrated cortex across layers.

Conclusion
These findings suggest that cortical laminar structure may be preserved in glioma-infiltrated cortex supporting glioma specific spectral frequency alterations. Cortical remodeling followingglioma infiltration alters spatiotemporal activity and cell-cell interactions.

Significance
This is the first known study to investigate glioma-infiltrated cortex using a multimodal approach of state-of-the-art techniques. These findings may serve as an atlas for probing thespecific interactions and roles of cells in glioma-infiltrated cortex across subtypes.

AWARDEE BEST EARLY CAREER ABSTRACT

Chemotherapy disrupts neural architecture in the metastatic microenvironment

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Affiliations

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Introduction
Chemotherapy resistance is a major hurdle in treatment of cancer. Components of the metastatic microenvironment have been shown to create a protective niche leading to treatment resistance. Therefore, we investigated whether the sympathetic nervous system (SNS) contribute to a chemoprotective niche that supports the growth of disseminated cancer cells.

Results
We first investigated the impact of chemotherapy on the architecture of sympathetic nerves in metastatic target organs. Chemotherapy remains a first line of treatment for Triple-Negative Breast Cancer (TNBC), a cancer known to be innervated by the SNS. Mice with MDA-MB-231^{HM} primary mammary TNBC tumours were treated with doxorubicin (1 mg/kg, iv, *vs*. saline vehicle), and anti-tyrosine hydroxylase (TH) immunostaining and multispectral imaging were used to quantify sympathetic innervation of metastatic target organs. Doxorubicin administration increased sympathetic nerve density in lung by 2.5-fold (average nerve area: 0.06 *vs.* 0.15%, $p = 0.002$) and liver by 1.5-fold (0.09 *vs.* 1.14%, $p = 0.043$) compared to vehicle. Moreover, doxorubicin treatment led to the swelling of varicosities that increased the average diameter of TH+ nerves in the lung (3.8 *vs.* 5.1 µm, $p < 0.0001$) and liver (3.5 *vs.* 4.8 µm, $p < 0.0001$) compared to vehicle.

To visualise the effect of disorganization of the SNS neural network on metastatic colonisation, we transduced MDA-MB-231^{HM} cancer cells to express five Lentiviral Gene Ontology (LeGO) vectors and selected 30 visually-distinct clonal populations using fluorescence-activated cell sorting. As there are currently no spatial imaging analysis techniques that can quantify distance relationship between sympathetic nerves and metastatic clones, we developed a custom **Sy**mpathetic **N**erve **A**nalysis **P**ipeline with **S**patial **E**valuation of the Metastatic Microenvironment (SyNAPSE) pipeline to resolve proximity relationships between sympathetic nerves and metastatic clones at the single-cell and cluster levels. In ongoing work, we are now using SyNAPSE to define how chemotherapy treatment impacts crosstalk between nerves and treatment-surviving clones in metastatic organs.

Conclusion
These findings provide the first evidence that anthracycline chemotherapy induces disorganization of the SNS neural network in organs that are colonized by metastases. The observed increase of sympathetic density in lung and liver tissue suggests a heightened sympathetic activity that could both activate adrenergic-mediated invasive programs in cancer cells, and could plausibly remodel the tumour microenvironment to favour the survival of metastases following chemotherapy treatment, thus contributing to treatment resistance.

Significance
The findings provide the framework for novel interventions for metastatic disease that target neural networks in the metastatic microenvironment.

AWARDEE BEST CENTRAL NERVOUS SYSTEM ABSTRACT

Title: DHGH3G34-mutant gliomas cells compose progenitors and GABAergic interneuron-like cells that form complex calcium networks and exert action potential bursts.

Gustavo Alencastro Veiga Cruzeiro, Sina Neyazi, Carlos Alberto Biagi Jr, Costanza Lo Cascio, Rebbeca Haase, Andrezza Nascimento, Stephen Charles Frederico, Katharina Sarnow, Varun Venkataramani, Ilon Liu, Kun Huang, Maria Pazyra-Murphy, Xin Tang, Rosalind Segal, Michelle Monje, Mariella Filbin
Dana Farber Cancer Institute, Harvard Medical School, Boston, MA

Introduction

High-grade gliomas are among the most lethal pediatric cancers. DHGH3G34-mutant tumors are characterized by driver mutations at glycine 34, occur predominantly in the hemispheres and account for over 30% of pediatric or adolescent cases. Interestingly, DHGH3G34-mutant gliomas exhibit a distinctive GABAergic interneuronal lineage (eIN-like) that remains not fully comprehended. In the normal brain, GABAergic interneurons shape the dynamics of neural networks by engaging in communication with other cells through the transmission of calcium and electrical signals. We questioned how eIN-like glioma cells are spatially organized in the tumor niche and whether they establish networks or exert electrical activity relevant to tumor growth and invasion.

Methods

Calcium Imaging: We combined the genetically encoded calcium indicator GCAMP7s and hDCXtdTomato promoter reporter construct.
Voltage Imaging: We used a genetically encoded voltage indicator ASAP3 for detecting changes in membrane potential.
Light Sheet Microscopy: Mouse DHGH3G34-mutant PDX brain were submitted to passive clearing method that involves shield, clearing, immunolabeling and index matching. The whole fixed PDX brain was mounted and imaged using the Zeiss Lightsheet 7 Microscope.

Results

To characterize eIN-like cells and determine their spatial organization within the DHGH3G34-mutant tumor milieu, we performed scRNAseq and immunocytochemistry experiments on DHGH3G34-mutant primary samples. We found that eIN-like cells highly express *DCX* (doublecortin) and form niches resembling structures seen in the 2nd trimester ganglionic eminences during corticogenesis. To determine whether eIN-like cells form putative communicating networks relevant to tumor growth, we developed a lentiviral tdTomato fluorescent reporter for *DCX* gene promoter activity, combined with GCAMP7s and performed calcium imaging. Interestingly, eIN-like cells show rhythmic calcium events at 62.5mHz frequency. Moreover, using voltage imaging we found that DHGH3G34-mutant cells showspontaneous action potential bursts. Importantly, network and electrical activity has been strongly linked to tumor growth and invasion. Furthermore, to determine the invasive pattern of a DHGH3G34-mutant mouse PDX model, we employed light sheet microscopy, imaged the whole brain, and found that gliomacells tangentially and radially populate the contralateral hemisphere.

Conclusion

Collectively, DHGH3G34-mutant are highly invasive tumors characterized by a cellular hierarchy showing an interneuronal lineage development, ranging from a self-renewing progenitor-like cell to a more differentiated cell resembling early immature GABAergic interneurons showing complex calcium events and spontaneous action potential bursts, along with quiescent astrocyte-like and mesenchymal-like cells.

Significance To The Cancer Neuroscience Field

In addition to the well-known concept that neuronal inputs promote glioma growth, our study shows that DHGH3G34-mutant resembling GABAergic interneurons form distinctive calcium networks and exert spontaneous electrical activity.

AWARDEE BEST PERIPHERAL NERVOUS SYSTEM ABSTRACT

Dissecting the role of neuronal mimicry in pancreatic neuroendocrine tumours

Zoey Wang[1,2], Areeba Qureshi[1], Andrea Trevisiol[1], Yacine Touahri[1], Nilakshi Kulathunga[1], Bojana Stefanovic[1,2], Carol Schuurmans[1,3], Housheng Hansen He[2,4], Iacovos Michael[1,2]

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Introduction

Pancreatic neuroendocrine tumours (PanNETs) are an understudied cancer type characterised by frequent metastasis, clinical recurrence, and high mortality rate. PanNETs originate from pancreatic islets, primarily β cells, and comprise two molecular subtypes: poorly invasive, relatively benign islet tumour (IT) and highly aggressive metastasis-like primary (MLP) tumour. The MLP subtype arises from IT through a switch in cell fate involving the acquisition of neuronal-like features, a process termed ‘neuronal mimicry’. Here, we examined potential roles of neuronal mimicry in PanNET progression, both through cancer cell-autonomous mechanisms and by promoting heterotypic interactions with tumour-infiltrating neurons.

Methods

Single-cell RNA sequencing of tumours derived from PanNET patients and transgenic mouse models was performed, and the enrichment of neuronal gene signatures was compared between IT and MLP-like tumours. Multi-electrode array (MEA) recordings of PanNET cells transfected with Channelrhodopsin-2 were also conducted to characterise the differences in electrical activity between the two subtypes. To examine cancer-neuron interactions, multiplex immunofluorescence imaging with markers for cancer cells and neurons was performed on mouse model-derived tumours, in addition to in vitro co-cultures of PanNET cells with murine dorsal root ganglia (DRG).

Results

Transcriptomic analyses of primary IT, primary MLP, and metastatic tumours revealed an upregulation of neuronal gene signatures during PanNET progression. Preliminary MEA recordings demonstrated spikes of longer duration in MLP-like cells compared to IT, in addition to a wider range of cell activation over time. Furthermore, quantitative immunofluorescence showed increased sympathetic innervation of the tumour core in advanced MLP lesions compared to IT. Finally, IT-like cancer cells developed neurite-like protrusions when co-cultured with DRG; these were more prominent with a higher DRG-to-cancer cell ratio in the co-culture, suggesting a dose-dependent effect.

Conclusion

Our results implicate neuronal mimicry and cancer-neuron interactions as potential factors contributing to PanNET progression. At the cell-extrinsic level, acquisition of neuronal-like features by cancer cells may potentiate crosstalk with neurons in the tumour microenvironment, in turn promoting tumour progression to more aggressive phenotypes. At the cell-intrinsic level, activation of neuronal genes may directly confer cancer cells a growth advantage. These will be investigated further by genetic and pharmacological approaches.

Significance to the cancer neuroscience field

This study begins to uncover the role of intrinsic neuronal-like features of PanNET cells and their interactions with the peripheral nervous system during PanNET progression. Results herein and from future studies may illuminate novel therapeutic avenues for PanNET by targeting neuronal gene programs and tumour innervation.

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Thursday Posters | Feb. 28th

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ADVANCED BIOLOGY

Thursday Abstract 17

Blockade of brain GFRAL signaling attenuates chemotherapy-induced fatigue and cognitive impairment in mice

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Introduction

Fatigue and cognitive impairments are commonly reported by cancer patients undergoing chemotherapy. The mechanisms of these neurotoxicities are still obscure. We hypothesize that they are mediated by mitokines, signaling molecules released by cells undergoing mitochondrial stress in response to chemotherapy. For the present study, we have focused on growth differentiation factor 15 (GDF15) which acts in the brain by activating its receptor, GDNF familyreceptor alpha-like (GFRAL).

Methods

Chemotherapy-induced fatigue was assessed by reduced voluntary wheel running. Cognitive impairment was assessed by altered performance in the Puzzle Box Test that measures executive function. We first checked whether commonly used chemotherapeutic drugs can induce GDF15. Mice were injected with cisplatin, paclitaxel, or bortezomib,and their effects on plasma levels of GDF15 were measured 24 h after (ELISA). Cisplatin was used in the following experiments in the form of one or two cycles of daily injections for five days with a rest of five days between cycles. To determine whether GFRAL mediates the effects of cisplatin on wheel running and performance in the puzzle box, mice were injected with a neutralizing antibody targeted to GFRAL (GFRALna) during or after cisplatin treatment.
Mitochondrial dysfunction was measured via Seahorse XF Analyzer Mito Stress Test. To determine whether activation ofthe GDF15/GFRAL axis recruits a central GDF15 compartment, *Gdf15* mRNA was measured by qRT-PCR in the brain parenchyma, meninges and choroid plexus.

Results

All the chemotherapeutic agents we tested induced statistically significant increases in circulating GDF15. GFRALna administered during cisplatin treatment (prevention) or after completion of cisplatin treatment (cure) abrogated the physical (wheel running), cognitive (Puzzle Box Test performance), and mitochondrial (maximal oxygen consumption) deficits caused by cisplatin treatment. Cisplatin did not induce *Gdf15* mRNA in the brain parenchyma butincreased its expression in the meninges and in the choroid plexus. The role of this GDF15 brain compartment in the neurotoxicities of cisplatin remains to be determined.

Conclusion

GDF15-GFRAL signaling represents a new target for the treatment of chemotherapy-induced fatigue and cognitive impairment.

Significance

Fatigue can be a dangerous feedback loop: fatigue worsens cancer progression, which begets more fatigue. Breaking this cycle may prove pivotal for restoring quality of life and bolstering survivorship in cancer patients. A morecomplete understanding of the mechanism underlying cancer-related fatigue will hopefully lead to more effective treatment options that do not interfere with the therapeutic efficacy of cancer therapy.

Thursday Abstract 18

Germline mutations and chemotherapy induce oligodendrocyte deficits and cognitive dysfunction in the Neurofibromatosis type 1 (NF1) cancer predisposition syndrome

Benjamin Emoefe Aghoghovwia, Anand Singh, Kechen Ban, Sama Alnassiry, Khushboo Irshad, Renae Bertrand, Hana El-Toukhy, Samer Sharafaldin and Yuan Pan.
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Introduction

Neurofibromatosis type 1 (NF1) is a cancer predisposition syndrome due to mutations in the *NF1* gene. The disease affects 1/3000 people worldwide and patients are predisposed to neoplasms such as optic pathway glioma and plexiform neurofibroma. NF1 is also a neurogenetic disease with symptoms including executive dysfunction, difficulties in learning, and memory impairments. Unfortunately, there is no cure for the disease yet. Chemotherapy remains the first-line treatment for NF1-associated low-grade glioma; while effective in reducing tumor burden, it does not reliably restore neuronal function. Besides chemotherapy, the germline *NF1* mutations also contribute to the neurological issues seen in NF1. There are over 3000 *NF1* mutations identified in patients associated with different clinical outcomes. In this study, we sought to determine how chemotherapy and *Nf1* germline mutations influence neurological function.

Methods

Genetically engineered mice of NF1: $Nf1^{+/neo}$ (engineered neomycin cassette into exon 31 of the *Nf1* gene), $Nf1^{+/R1809C}$ (NF1-patient derived germline mutation), and $Nf1^{+/R816X}$ (NF1-patient derived germline mutation) mice were used in this study. For cognitive tests, mice were tested on the novel object position recognition (NOPR), Y-maze, and puzzle box tests. For testing the effects of chemotherapy, 10-11 weeks old $Nf1^{+/neo}$ mice (both sexes) received intraperitoneal injection of 60 mg/kg/day carboplatin or PBS every other day for one week(making a total of 4 injections/mouse).

Transmission electron microscopy (TEM): samples were fixed with a solution containing 3% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.3), before processing (washing, post-fixation, dehydration, embedding, polymerization, sectioning, and staining). The stained samples were examined in a JEM 1010 transmission electron microscope, and digital images were obtained and analyzed for the g-ratio andthe percent of damaged myelin.

Immunohistochemistry: samples were fixed in 4% paraformaldehyde, cryoprotected, and frozen. 10μm thick cryostat sections were immunohistochemically labeled using antibodies against PDGFRa [oligodendrocyte precursor cells (OPCs)] and ASPA [oligodendrocytes (OLs)], followed by quantifying the density of OPCs and OLs.

Results

We found that carboplatin treatment of *Nf1*-heterozygous mice ($Nf1^{+/neo}$) impairs myelin sheaths and reduces the density of oligodendrocytes in the optic nerve. Using genetically engineered mice harboring NF1-patient-derived germline mutations ($Nf1^{+/R1809C}$ and $Nf1^{+/R816X}$), we found that these germline *Nf1* mutations induce loss of oligodendrocytes. Moreover, the $Nf1^{+/R1809C}$ mice exhibit cognitive impairment in spatial memory and executive function.

Conclusion

These findings indicate that both *Nf1* germline mutations and chemotherapy contribute to oligodendrocyte loss and neurological dysfunction in NF1.

Significance to the cancer neuroscience field

NF1 is a cancer predisposition syndrome with no cure. Our findings using genetically engineered *Nf1* mice provide valuable insights into the heterogeneous neurological symptoms associated with NF1, as well as the experimental platform to understand chemotherapy-induced neurological issues in affected patients.

Thursday Abstract 19

Understanding the differential effects of neurotransmitters on glioblastoma subtypes

Khushboo Irshad[1], Sanjay Singh[2], Khalil Ali Ahmad[1], Kechen Ban[1], Ben Aghoghovwia[1], Sama Alnassiry[1], Renae Bertrand[1], Aishani Gargapati[1], Nathan Bui[1], Rajasekaran Mahalingam[1], Truc Kuo[2], Frederick Lang[2], Yuan Pan[1,3]

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Introduction

Glioblastoma (GBM) are aggressive brain tumor associated with the worst patient prognosis. Neurons play critical roles in modulating GBM pathophysiology. It has been demonstrated that glutamatergic neurons form bona fide synapses with glioblastoma cells that are critical for tumor cell proliferation and migration. It is less clear whether the activity of other neuronal types influences GBM biology, especially among the different GBM molecular subtypes. Here, we examined the effects of various neurotransmitters on the growth of glioblastoma cells characterized as classical, proneural and mesenchymal subtypes.

Methods

Patient-derived GBM cell lines were exposed to varying concentrations of different neurotransmitters for 72 hours and assessed for cell viability using CCK-8 assay. We analyzed the transcriptomic data from TCGA (The Cancer Genome Atlas) GBM database for correlation of expression of neurotransmitter receptors with patient survival. Using the IVY-GAP database, we compared the expression of neurotransmitter receptors at the infiltrating/leading edge of GBM tumors as compared to the cellular tumor region to assess their possible involvement in GBM cell migration and micronenvironmentalinteractions.

Results

Consistent with previous findings, we observed an increase in cell viability in all GBM cell lines after being incubated with L-glutamate. The classical subtype has a stronger growth response to L- glutamate compared to the other two subtypes. Transcriptomic data from TCGA revealed the expression of *GRIK4* (glutamate ionotropic receptor kainate type subunit 4) as highest in the classical subtype as well as inversely correlated with worse patient survival in this GBM subtype, as opposed to proneural or mesenchymal patient groups. The CCK8 assay also suggests that acetylcholine and dopamine increase GBM cell growth. A subset of cholinergic and glutamatergic receptors (CHRM1 and GRM2) was found upregulated in the infiltrating and leading-edge regions of GBM tumors compared to the cellular tumor region, suggesting possible involvement in GBM-neuron interactions.

Conclusion

Our findings suggest that each GBM subtype may differentially leverage neuro-modulatory mechanisms to support their own growth. Ongoing studies are investigating the differential neuronal responses in GBM subtypes using pharmacological and genetic approaches.

Significance to the cancer neuroscience field

Our study sheds light on the subtype-dependent neuronal regulation of GBM pathophysiology, which may provide new therapeutic options for GBM patients in thefuture.

Thursday Abstract 20

Radiation insult mimics early-onset Alzheimer disease by dysregulating DNA methylation and gene expression in pyramidal cells

Tuba Aksoy[1,2], Pavel Sumazin2[2,4], Yue Lu[3], Mohammad Javad Najaf Panah[4], Miriam Zamorano[6],Thanh Lam[1,2], Wei Zhou[2], Ines Veselinovic[2], Joseph G Duman[5], M. Waleed Gaber[4], Cobi J Heijnen[7], David R Grosshans[2]

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Introduction

Cranial radiation is a crucial component of therapy for brain tumors[1] but is known to injure non-cancerous surrounding cells and issues as well. Surviving patients are at significant risk of developing progressive and irreversible radiation-induced neurocognitive deficits[3].

Methods

We investigated epigenetic changes as possible drivers of persistent cellular dysfuncton after radiation chose to profile cells in the prefrontal cortex and hippocampal CA1-3 regions because of their roles in executive function, attention, information encoding and retrieval. We evaluated long-term executive function in male and female mice after cranial irradiaton with 20Gy delivered in 4-Gy fractions over 5 consecutive days or sham radiation at 13 weeks of age. Seven weeks later (week-20), mice were given the puzzle-box and novel-object-place-recogniton tests. To explore epigenetic mechanisms as drivers of persistent cellular dysfuncton after radiaton exposure, we conducted an integrative analysis of single- nuclei sequencing (snRNA-Seq) and whole genome bisulfite sequencing (WGBS) profiles of brain regions to evaluate the influence of methylation on the transcriptome of PFC and hippocampal cells.

Results

Our behavioral tests revealed significant cognitive impairments in mice after cranial irradiaton to clinically relevant doses, particularly in spatial working memory and non-hippocampal–dependent domains, including executive function. These behavioral changes were accompanied by epigenetic changes in cell types within the non-neurogenic hippocampal subregions CA1-3, as well as in the PFC. Changes observed included alterations in pathways related to long-term potentiation, synaptic organization, ribosomal function, neuronal signaling, and cellular metabolism, all of which are crucial for normal cognitive functioning. Moreover, DNA methylation analysis indicated that alterations in DNA methylation profiles may underlie dysregulation of gene expression in specific cell types. We also identified a link between radiation-induced transcriptional dysregulation and genes related to AD. The consistent downregulation of TTR in irradiated hippocampal cells, along with the decreased expression of other AD-related genes raises questions about the potential long-term consequences of cranial radiation and its similarity to common neuro-degenerative diseases. Our findings show significant overlap between changes in irradiated hippocampus pyramidal cells and those in markers of early-onset AD but not markers of late- onset AD.

Significance

To our knowledge, this is the first such investigation snRNA-Seq and WGBS for radiation-induced brain damage. Cranial radiation therapy for brain tumors can lead to progressive cognitive deficits in survivors by mechanisms that are poorly understood. The findings of this study provide new insights into the molecular and cellular consequences of cranial radiation therapy in a mouse model.

Thursday Abstract 21

Synaptic gene expression profile across pediatric andadult brain tumors

Sonali Arora[1], Leyre Merino-Galan[2], Eric C. Holland[1], Myron Evans[2,3], Siobhan S.Pattwell[1,2,3*].

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Introduction

Brain tumors intricately interact with their microenvironment, where the dynamicinterplay between neurons and cancer cells is fundamental to brain cancer pathophysiology. Neuronal activity emerges as a key driver influencing glial malignancy growth through synaptic communication and paracrine signaling, withglioma cells acting postsynaptically. Beyond gliomas, diverse brain tumors exhibit altered synaptic protein expression, impacting receptor dynamics and influencing the tumor microenvironment and neuronal function. This underscores the critical need to understand the molecular and synaptic foundations of brain tumors. This study visualizes and compares synaptic processes and gene expression across braintumor types, contrasting findings with normal brain tissue.

Methods

We constructed a comprehensive Brain-UMAP reference, incorporating data from 702 adult gliomas, 802 pediatric tumors, and 1409 samples of a healthy normal brain (Arora et al.). Employing a rigorous approach, we conducted a differential gene expression analysis across three adult glioma subtypes, 21 pediatric tumor types, and healthy brain samples. A fold change cutoff of 50% and an adjusted p- value below 0.05 were applied to discern significant differentially regulated synaptic markers. Furthermore, GSVA analysis employing SynGO pathways was performed on the batch-corrected normalized gene counts, and the resulting scores were visually represented on the landscape.

Results

Distinct regions for each tumor type emerged in the landscape. Our observations reveal distinct synaptic pathway enrichments across various cancer subtypes. For instance, Ependymomas displayed an up-regulation of synaptic signaling via Nitric oxide, while Medulloblastoma exhibited increased expression in "Postsynaptic dense core vesicle exocytosis." Additionally, aggressive IDH-WT adult gliomas and pilocytic astrocytomas demonstrated an up-regulation in Synaptic target regulation, highlighting subtype-specific synaptic alterations.

Conclusion

This study enriches the cancer neuroscience field by offering a comprehensive overview of the involvement of synaptic components in diverse adult and pediatric brain tumors. Such insights are pivotal for unraveling the intricate mechanisms that govern brain tumor pathophysiology. Moreover, this understanding lays the foundation for developing targeted therapeutic strategies that disrupt the nuancedcommunication between tumor cells and their microenvironment. By doing so, thestudy holds the potential to propel advancements in effective interventions withinthe challenging landscape of brain cancers.

Significance to the cancer neuroscience field

Our resource enables researchers to explore and visualize gene- and pathway-leveldistinctions between tumor types. The tool aids in identifying dominating gene expression and pathway regulation patterns, providing a valuable resource for thecancer neuroscience field.

Thursday Abstract 22

Deciphering the role of BDNF-TrkB.T1 signaling on the glioma microenvironment

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Introduction

Gliomas are the most common type of brain tumor in both children and adults. Communication between cancer cells and surrounding cells is a fundamental component of brain cancer pathophysiology. The activity of neurons surrounding tumor microenvironment drives the proliferation and growth of glial malignancies through paracrine signaling, brain-derived neurotrophic factor (BDNF) being one of the key secreted factors. BDNF binds with high affinity to the tropomyosin receptor kinase B (TrkB). Recent work from our group has shown that the TrkB.T1 splice variant is upregulated in various human gliomas and that overexpressing TrkB.T1 enhances the tumor aggressiveness *in vivo* and is associated with the downregulation of genes involved in facilitating tumor cell recognition and elimination *in vitro.* However, the role of TrkB.T1 splice variant in this bidirectional communication between glioma cells and the surrounding cells remains unknown. Our aim was to assess if increased levels of TrkB.T1 observed in gliomas harbor a role in modulating immune responses.

Methods

To explore these mechanisms *in vitro*, 3T3 cells were transduced with pLJM1-lentivirus to overexpress TrkB.T1. Additionally, we used two glioma stem cell lines that express high or low levels of TrkB.T1, and cells were treated with regular media or serum-starved and treated with BDNF. Immunoprecipitation and affinity purification-mass spectrometry were used to identify TrkB.T1 interactors in 3T3 cells, and the conditioned media was subjected to a cytokine multiplex analysis. In addition, we explored the immune cell heterogeneity associated with increased levels of TrkB.T1 *in vivo* using a glioma mouse model engineered with RCAS/tv-a technology, as well as using single cell sequencing datasets from glioblastomaand low-grade glioma patients.

Results

The results show that TrkB.T1 significantly increases key chemokine levels and that it has a unique set of binding partners involved in neutrophil activation and MHC class I-mediated immune response. We also observed a significant decrease in the number of overall immune cells when TrkB.T1 is overexpressed *in vivo*, in accordance with single cell sequencing results from glioma patients clustered by high, medium, or low *NTRK2* expression.

Conclusion

This study identifies BDNF-TrkB.T1 axis as a key player in the communication dynamics between gliomacells and their microenvironment.

Significance

This study marks a significant contribution to the field of cancer neuroscience by advancing our understanding of neutrophin receptors in glioma-immune cell interactions, providing novel insights into the molecular mechanisms governing brain tumor pathophysiology and paving the way for innovative strategies to modulate disease progression.

Thursday Abstract 23

Cellular and functional remodeling of human cerebral organoids by glioblastoma stem-like cells.

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Introduction

Glioblastoma (GBM) is the most malignant primary brain tumor in adults. The clinical outcome of GBM patients is uniformly fatal, as their median survival despite best available clinical interventions is 14.6–22 months. The burgeoning field of cancer neuroscience focuses on deciphering the role of normal neural cells in brain tumor growth. Human induced pluripotent stem cell derived cerebral organoids (COs) as representative of normal human neural tissue is an exciting new frontier for brain tumor research. COs together with GBM-derived glioma stem-like cells (GSCs) present a unique model system to study brain tumor cells and normal human brain cells simultaneously.

Methods

By utilizing human induced-pluripotent stem cells derived cerebral organoids (COs) co-cultured with GSCs (RFP-luciferase tagged MDA-GSCs, n=3 representing each glioblastoma molecular sub-type), we investigated the effects of GSCs on CO-derived neural cells and their activity. COs and CO+MDA-GSCs were subjected to single-cell RNA-sequencing (scRNA-seq). As per typical micro-anatomy of COs, neural stem/progenitor cells are interiorly distributed whereas differentiated neurons are positioned on the surfaces, enabling measurement of neuronal action potential on surfaces of COs by micro-electrode- arrays (MEA).

Results

Compared with COs, co-cultured samples had significantly higher percentage of non- telencephalon neural progenitor cells (ntNPCs) (CO_vs._CO+MDA-GSC(luciferase^{-ve}): 15.6% vs. 47.2%- 63.4%) while the percentages of cortical NPC (cNPCs) and intermediate progenitors (IPs) was lower (CO_vs._CO+MDA-GSC(luciferase^{-ve}): 39.2% vs. 12.0%-20.5%). These alterations in NPC/IPs abundance were accompanied by decreased proportions of cortical excitatory neurons (CO_vs._CO+MDA- GSC(Luciferase^{-ve}): 33.2% vs. 7.9%-23.4%). Among luciferase^{+ve} MDA-GSCs-derived cells in co-culture samples, mesenchymal MDA-GSCs had higher abundance of ntNPCs versus cNPCs (30.4% versus 63.6%) whereas, among classical and proneural MDA-GSCs ntNPCs were 11.45-22.8 times higher. Interestingly, 14.9% of proneural MDA-GSCs derived cells displayed inhibitory neuron signatures. After 3 weeks, the mean firing rate measured by MEA was significantly lower in co-cultures compared to COs (CO_vs._CO+MDA-GSC: 0.052 Hz vs. 0.0081 Hz p-value 0.000219), suggesting functional remodeling.

Conclusions

Our results show that cellular remodeling of normal cells resulted in an increased percentageof ntNPCs whereas that of MDA-GSCs varied across molecular sub-type. This increase in ntNPCs coupled with decreased abundance of neurons may result in lowered neuronal activities.

Significance to the cancer neuroscience field

Here we report that in CO+MDA-GSC systems presence of tumor results in cellular and functional reprogramming of normal neural cells. A better understanding of these interactions between normal and tumor cells can potentially be leveraged to manage brain tumor growth and its impact on normal brain.

Thursday Abstract 24

Individualized MRI Neuromodulation Targeted Towards Decreasing Visuospatial, Selective Attention, andWorking Memory Deficits Following Cancer Chemotherapy.

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Introduction

This neuromodulation study is designed to reverse or decrease attention and memory deficits asa sequalae of cancer chemotherapy. Our goal is to strengthen selective extero- intero-ceptive attention (SEIA), motor planning (MP) and working memory (WM) by targeting networks that regulate visuospatial perception (VP) as a function of motion direction and coherence discrimination through our individualized fMRI neuromodulation, termed iNM **(U.S. Patent No.16/954,256**). fMRI measures the magnitude and spatial extent of oxy-(O_2)-to-deoxy- genated hemoglobin [Hb]. iNM: 1. is a non-invasive, precision-medicine intervention with 1mm anatomical and functional precision; and 2. is guided by reinforcing or inhibiting the HbO2 intensity and extent of each patient'sunique brain network, as opposed to relying on complete self-regulation of the HbO2 intensity. Here we wantedto assess the feasibility of iNM to strengthen the magnitude of the signal in VP, SEIA, WM, and MP networks.

Methods

Eight subjects (n=8) underwent iNM intervention and control-No iNM. Each participant's individualized visuospatial (VP) network was targeted for iNM. Two analyses were conducted: an encoding model via a GLM and a decoding model via an SVM. The encoding model determined the HbO2 magnitude area under the curve (AUC) for each network's area. We then predicted the stimulus from the brain activationmaps. The goal of encoding and decoding the data is that it presents a window of opportunity to validate the results and allude to causal inference.

Results

The increase in the AUCs' HbO2 magnitude under iNM across directions and coherences range from: 1. 48-76% in the SEIA; 2. 26-59% in the MP; 3. 20-47% in the WM; and 4. 100% for strong coherencesbut a decrease for weak coherences in the VP network. SVM resulted in statistically significant greater classification accuracies under iNM compared to control ($p<0.001$).

Conclusion

iNM enhances VP, SA, and WM networks as shown via encoding and decoding modeling. Theareas strengthened via iNM allow us to make a causal inference of the mechanisms induced via this intervention and thus, serve as clinical biomarkers.

Significance to the cancer neuroscience field

Cancer chemotherapy can result in transient or permanent, self-reported or observed cognitive impairment in 35-75% of patients (Das et al., Curr Neuropharmacol, 2020). In can affect various functions of cognition, such as attention, visuospatial, visuomotor, or visual memory (Raffaand Tallarida, J Clin Pharm Ther, 2012). Here, we aimed to assess an intervention that can modulate multiple aspects of cognition at once with the hope to increase iNM efficiency in the MRI environment.

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Thursday Abstract 25

Spatially precise *in situ* transcriptomics in intact biological systems

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Introduction

Methods for highly multiplexed RNA imaging are limited in their spatial resolution and their ability to image thick biological specimens. This imposes limitations on mapping transcripts throughout extended, detailed cellular morphologies, and on localizing transcripts tonanoscale and subcellular compartments. As patterns of gene expression are structured across extended length scales, from tissue scale to nanoscale, there is a need for methods that can measure gene expression in its native context, as gene expression constrains the available mechanisms for cellular behaviors and dynamics.

Methods

To address this need, we developed a suite of technologies that unite multiplexed RNAimaging with improved sample preparation methods for tissue imaging. First, we adapted expansion microscopy, which physically expands biological specimens, for use with targeted *insitu* sequencing, enabling the efficient detection of a targeted set of transcripts by enzymatic amplification and imaging-based decoding of oligonucleotide probes. Second, we modified the targeted ExSeq chemistry for the study of intact self-organizingmulticellular systems. We adapted our approach for non-expanded specimens and developed the ability to sequentially perform immunostaining followed by targeted *in situ* sequencing, enabling multimodal characterization of morphology and gene expression in the same cell.

Results

Targeted ExSeq enabled the generation of nanoscale-resolution maps of RNA transcripts throughout dendrites and spines in neurons of the mouse hippocampus, revealing subcellular RNA localization patterns across multiple cell types, layer-specific cell type organization acrossthe mouse visual cortex, and the organization and position-dependent states of tumor and immune cells in a human metastatic breast cancer biopsy. Using mouse whole-mount preimplantation embryos as a model self-organizing system,we deployed our method to study the emergence of spatially organized cell types during preimplantation development, finding that morula-stage blastomeres show early biasing toward trophectoderm and inner cell mass subtypes, which correlate with cellular morphology.

Conclusion

Targeted ExSeq can be used for highly multiplexed mapping of RNA transcripts from nanoscale to system scale in intact cells and tissues, linking gene expression to spatial context.

Significance to the cancer neuroscience field

Cancer neuroscience studies interactions between tumors and the nervous system in their native biological context. Many aspects of these interactions are structured at the nanoscale, such as paracrine signaling and synaptic communication. Targeted ExSeq holds promise for the multiscale mapping of these interactions in intact tissues or *in vitro* models ofdisease, such as organoids, contributing to a better understanding of pathological processes, potentially yielding insight for therapeutic approaches.

Thursday Abstract 26

Same-slide fully automated spatial multiomics profiling of immune cells in the tumor microenvironmentthrough integration of RNAscope™ and sequential immunofluorescence on COMET™ platform.

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Introduction

Spatial biology methods have shed light on the complexity of the tumor microenvironment (TME) at the single- cell level, revealing common and rare cell types and their intercellular interactions in a spatial context (Bressan, 2023). Hyperplex immunofluorescence allows the identification of multiple biomarkers on the same sample, enabling immune cell profiling within the TME. However, revealing in-depth TME activation status requires mapping of cytokines/chemokines, currently achievable by RNA *in situ* hybridization techniques on a separate serial section. The ability to spatially profile both RNA and protein markers on the same slide is neededto facilitate investigations of specific cell populations such as immune cells and their activation states (Palla, 2022).

Methods

The panel is designed to identify key components of the TME such as macrophages, dendritic cells, fibroblasts, and tumor-infiltrating lymphocytes (TILs). RNA probes are selected to provide further information about the activation state of TILs by targeting key biomarkers like co-inhibitory receptors and transcription factors, together with secreted molecules such as cytokines and proteases. The simultaneous detection of RNA and proteins provides extensive insights into the TME molecular landscape, revealing co-expression patterns and relationships between RNA and proteins within individual cells. The signature of an immunosuppressive microenvironment is also studied, focusing on TIL activation states and immune checkpoint expression.

Results

Here, we show a novel multiomics approach (Migliozzi, 2019) that integrates RNAscope™ and sequential immunofluorescence (seqIF™) to simultaneously identify RNA and protein targets on the same tissue slide. The analysis is fully automated on COMET™, an advanced tissue staining and imaging platform with precise temperature control and full workflow automation, ensuring optimal efficiency and reproducibility. Our integrated protocol combines up to three cycles of RNA detection with seqIF™, where two markers are identifiedin each cycle, for a final dataset including 12-plex RNA and 20-plex protein multiomics panel.

Conclusions

Our results affirm the successful implementation of the combined RNAscope™ and seqIF™ protocols on the COMET™ platform to allow same-slide spatial multiomics analysis. Preserving spatial context and intercellular relationships, this technology will help to unravel complex cellular interactions among distinct cell populations, thus opening new avenues for personalized medicine and the identification of therapeutic targets.

Significance

This unique multiomics approach will play a crucial role in elucidating the molecular landscape of brain tumors.It will provide a more holistic view of biological pathways, aiding in tumor classification, biomarker signature, and the development of targeted therapies.

Thursday Abstract 27

Investigating the role of enteric neurons in colorectal cancer progression and metastasisusing hPSC-derived models

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Introduction

Nerves play a critical role in regulating many physiological processes that affect human health and disease, including the initiation and progression of tumorigenesis. Gastrointestinal cancers,including colorectal cancer (CRC), are a leading cause of global cancer-related mortality. These tumors are highly innervated by the enteric nervous system (ENS). Perineural invasion (PNI), present in one-third of CRC cases, is an independent predictor of poor prognosis (Hu, 2020 & Albo, 2011 & Liebig, 2009). The ENS has been implicated in CRC, but the exact mechanisms by which the ENS influences CRC progression and metastasis has remained elusive.

Methods

To define the neuronal signals that influence CRC behavior, we conducted a high-throughput screening (HTS) of a neuromodulator library and identified pathways that regulate CRC migration. We found that small molecular modulators of serotonergic and adrenergic signaling,which are integral to ENS function, suppress CRC migration. To further characterize these pathways are probe CRC-ENS crosstalk, we established a direct coculture model using human pluripotent stem cell (hPSC)-derived enteric neurons (EN) and a primary CRC cell line, to investigate cellular interactions and determine impacts on CRC. Using this system, we performed extensive phenotypical assays including transwell assays, neurite outgrowth assay, scratch assay coupled with conditioned media to define the role of neuronal interaction in CRC behavior. Furthermore, this system allowed us to assess the effect of CRC cell on EN activity using calcium imaging and neurotransmitter release assays.

Results

Our HTS identified neuronal signaling pathways that are previously documented to have antitumor effects in various cancers, warranting further investigations to delineate their mechanisms reducing CRC metastasis.
Our co-culture assays revealed substantial interactionsbetween CRC and EN, which leads to increased migration of CRC and altered EN activity.
Enhanced CRC migration in coculture, underscored the potential role of EN in the tumorinvasion and metastasis.

Conclusion

This study establishes an experimental system to study the crosstalk between the ENS and CRC.Our results uncover a previously unexplored dimension in CRC pathogenesis and potential development of novel neuromodulator therapeutics for CRC. Targeting this novel axis may offerinnovative avenues for the development of precision medicine to ultimately improve patients with CRC.

Significance To The Cancer Neuroscience Field

This proposal introduces innovative approaches to model and study neurochemical interactionsbetween CRC and EN, addressing a longstanding gap in understanding CRC-ENS crosstalk.
Leveraging hPSCs provides access to authentic EN models that accurately recapitulate the cellular and molecular heterogeneity of the human ENS. This research serves as a foundation tofacilitate futures studies of the complex cellular and molecular mechanisms underlying CRC-ENS interaction.

Thursday Abstract 28

Modeling tumor-neuron crosstalk via multielectrode array and considerations for de-risking cancer therapy

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Introduction

Brain tumors, including glioblastomas, interact with healthy parenchymal tissue, both directly via synaptic integration and indirectly via inflammation and modulation of the microenvironment. Modeling this crosstalk *in vitro* is important to understand mechanisms and influence on cancer progression, as well as for therapeutic development. Here, we present examples using microelectrode arrays (MEA) to assess functional effects of glioma cells on neural cultures and an impedance-based method to evaluate the efficacy and safety of cancer cell therapies.

Methods

For neural experiments, primary cortical neurons were co-cultured on CytoView MEA plates directly or via transwell inserts with tumor cells from rodents or human patients. Neural activity was recorded on the Maestro Pro MEA platform (Axion Biosystems). To evaluate on-target, off-tumor effects, human iPSC-derived neurons and cardiomyocytes were co-cultured on CytoView MEA plates with HER2-CAR T cells at increasing effector:target (E:T) ratios. Viability and functional activity measurements were taken via the Maestro. For cytotoxicity assays, cancer cells were cultured in CytoView-Z plates, then CAR T cells were added at increasing E:T ratios. Resistance was monitored via continuous impedance measurements on the Maestro over 48-72 hours to calculate % Cytotoxicity and Kill Time 50 (KT50).

Results

When cultured directly with healthy neurons, primary tumor cells differentially affect neural activity depending on tumor functional connectivity. Tumors with high functional connectivity induce significant hyperexcitability, while less functionally connected tissues have relatively little effect, suggesting integrated tumors remodel neural circuits and activity *in vivo* (Krishna et al., 2023). Tumor cells cultured indirectly with neurons induce similar hyperexcitability, indicated by increased network burst frequency, serving as a model of glioma-induced epilepsy (Mortazavi et al., 2022). Impedance-based cell analysis was used to assess both potency of CAR T cells and safety regarding on-target off-tumor effects. Targeted CAR T cells effectively killed cancer cells in an E:T ratio-dependent manner. HER2 CAR T cells also resulted in cardiomyocyte toxicity, a common consideration for CAR T cell therapy, while neurons were unaffected.

Conclusion

Functional analysis of neural activity via MEA and cell viability via impedance-based cell analysis provides label-free characterization of tumor-neuron interactions and evaluation of potential therapeutic approaches.

Significance to the cancer neuroscience field

Tumor-neuron crosstalk affects prognosis and symptom presentation, as well as tumor growth and progression, representing a promising target for cancer therapy. Modeling functional tumor-neuron activity and evaluating cell therapy potency and safety can serve as an approach to de-risk potential therapeutics during discovery and development.